
JMIRx Med

Overlay journal for preprints with post-review manuscript marketplace
Volume 6 (2025) ISSN 2563-6316 Editor in Chief: Edward Meinert, MA (Oxon), MSc, MBA, MPA, PhD,
CEng, FBCS, EUR ING

Contents

Peer-Review Reports

Peer Review of "Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis" (e69895) Anonymous.	4
Peer Review for "Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review" (e69705) Anonymous.	6
Peer Review of "Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis" (e69896) Dina Elsalamony.	8
Peer Review of "Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study" (e71529) Ali Ahmed.	11
Peer Review of "The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study" (e70808) Sanjeev Kumar Thalari.	12
Peer Review of "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study" (e70142) Bilkisu Nwankwo.	14
Peer Review of "Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study" (e71531) Anonymous.	16
Peer Review of "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study" (e70144) Md Islam.	18
Peer Review of "Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis" (e70039) Anonymous.	21

Peer Review of “Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis” (e70041)
 Anonymous. 23

Peer Review of “Safety and Efficacy of Chimeric Antigen Receptor T-Cell Therapy for Recurrent Glioblastoma: An Augmented Meta-Analysis of Phase 1 Clinical Trials (Preprint)” (e71293)
 Vanessa Fairhurst, Randa Mahmoud, Toba Olatoye, Sylvester Sakilay. 131

Authors’ Response To Peer Reviewss

Authors’ Response to Peer Reviews of “Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review” (e68769)
 Feryal Kurdi, Yahya Kurdi, Igor Reshetov. 25

Author’s Response to Peer Reviews of “Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis” (e69307)
 Bernard Friedenson. 27

Authors’ Response to Peer Reviews of “The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study” (e70059)
 Ajit Kerketta, Raghavendra A N. 31

Authors’ Response to Peer Reviews of “Mothers’ Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study” (e70145)
 Tahazid Tamannur, Sadhan Das, Arifatun Nesa, Foijun Nahar, Nadia Nowshin, Tasnim Binty, Shafiul Shakil, Shuvojit Kundu, Md Siddik, Shafkat Rafsun, Umme Habiba, Zaki Farhana, Hafiza Sultana, Anton Kamil, Mohammad Rahman. 33

Authors’ Response to Peer Reviews of “Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study” (e71528)
 Abdul Tayoun. 38

Author’s Response to Peer Reviews of “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis” (e69894)
 Hojjat Borhany. 41

Original Papers

Mothers’ Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study (e59379)
 Tahazid Tamannur, Sadhan Das, Arifatun Nesa, Foijun Nahar, Nadia Nowshin, Tasnim Binty, Shafiul Shakil, Shuvojit Kundu, Md Siddik, Shafkat Rafsun, Umme Habiba, Zaki Farhana, Hafiza Sultana, Anton Kamil, Mohammad Rahman. 46

Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis (e50712)
 Bernard Friedenson. 59

The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: A Qualitative Study (e48346)
 Ajit Kerketta, Raghavendra A N. 90

Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study
([e57597](#))
Abdul Tayoun..... 105

Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and
Batch Studies With Statistical Analysis ([e50458](#))
Hojjat Borhany..... 115

Protocol

Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a
Scoping Review ([e66213](#))
Feryal Kurdi, Yahya Kurdi, Igor Reshetov..... 100

Peer Review of “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis”

Anonymous

Related Articles:

Companion article: <https://www.biorxiv.org/content/10.1101/2023.06.21.545938v2>

Companion article: <https://med.jmirx.org/2025/1/e69894>

Companion article: <https://med.jmirx.org/2025/1/e50458>

(*JMIRx Med* 2025;6:e69895) doi:[10.2196/69895](https://doi.org/10.2196/69895)

KEYWORDS

multistep fermentation; specific methane production; anaerobic digestion; kinetics study; biochar; first-order; modified Gompertz; mass balance; waste management; environment sustainability

This is a peer-review report for “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis”

Round 1 Review

The present manuscript [1] deals with the study of the valorization of organic fractions of municipal solid waste through the production of volatile fatty acids and biogas. The article is interesting; in my opinion, it should be revised.

Comments

1. The presentation of the manuscript is very poor; the figures are not in the same format.
 2. Some of the recent works should be discussed and cited in the Introduction section: [2-6].
 3. The novelty of the work should be highlighted.
 4. Full stops should be removed from all subheadings.
 5. The Results and Discussion should be written in detail with proper subheadings.
 6. There are some typo errors; they should be rectified.
-

Conflicts of Interest

None declared.

References

1. Borhany H. Converting organic municipal solid waste into volatile fatty acids and biogas: experimental pilot and batch studies with statistical analysis. *JMIRx Med* 2025;6:e50458. [doi: [10.2196/50458](https://doi.org/10.2196/50458)]
 2. Jung S, Shetti NP, Reddy KR, et al. Synthesis of different biofuels from livestock waste materials and their potential as sustainable feedstocks – a review. *Energy Conversion Manage* 2021 May 15;236:114038. [doi: [10.1016/j.enconman.2021.114038](https://doi.org/10.1016/j.enconman.2021.114038)]
 3. Srivastava RK, Shetti NP, Reddy KR, Aminabhavi TM. Sustainable energy from waste organic matters via efficient microbial processes. *Sci Total Environ* 2020 Jun 20;722:137927. [doi: [10.1016/j.scitotenv.2020.137927](https://doi.org/10.1016/j.scitotenv.2020.137927)] [Medline: [32208271](https://pubmed.ncbi.nlm.nih.gov/32208271/)]
 4. Sampath P, Brijesh, Reddy KR, et al. Biohydrogen production from organic waste – a review. *Chem Eng Technol* 2020 Jul;43(7):1240-1248. [doi: [10.1002/ceat.201900400](https://doi.org/10.1002/ceat.201900400)]
 5. Velvizhi G, Goswami C, Shetti NP, Ahmad E, Kishore Pant K, Aminabhavi TM. Valorisation of lignocellulosic biomass to value-added products: paving the pathway towards low-carbon footprint. *Fuel (Lond)* 2022 Apr 1;313:122678. [doi: [10.1016/j.fuel.2021.122678](https://doi.org/10.1016/j.fuel.2021.122678)]
 6. Monga D, Shetti NP, Basu S, et al. Engineered biochar: a way forward to environmental remediation. *Fuel (Lond)* 2022 Mar 1;311:122510. [doi: [10.1016/j.fuel.2021.122510](https://doi.org/10.1016/j.fuel.2021.122510)]
-

Edited by T Leung; submitted 10.12.24; this is a non-peer-reviewed article; accepted 10.12.24; published 04.02.25.

Please cite as:

Anonymous

Peer Review of “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis”

JMIRx Med 2025;6:e69895

URL: <https://xmed.jmir.org/2025/1/e69895>

doi: [10.2196/69895](https://doi.org/10.2196/69895)

© Anonymous. Originally published in JMIRx Med (<https://med.jmirx.org>), 4.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review for “Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review”

Anonymous

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.07.30.24311256v1>

Companion article: <https://med.jmirx.org/2025/1/e68769>

Companion article: <https://med.jmirx.org/2025/1/e66213>

(*JMIRx Med* 2025;6:e69705) doi:[10.2196/69705](https://doi.org/10.2196/69705)

KEYWORDS

indocyanine green; ICG; sentinel lymph node; breast cancer; breast; fluorescence; axillary lymph node mapping; NIR; surgical planning; near-infrared

This is the peer-review report for “Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review.”

Round 1 Review

General Comments

This paper [1] summarized the application value and existing problems of indocyanine green (ICG) in sentinel lymph node (SLN) biopsy of early breast cancer, which has positive significance for improving the accuracy of clinical SLN detection. This study has certain clinical value.

Specific Comments

Major Comments

1. Due to the high hardware requirements for the clinical application of ICG, the number of relevant studies in the search is relatively small. It is hoped that the author can search the recent relevant literature to improve the credibility of this review.
2. It is hoped that the author will analyze and compare the advantages and disadvantages of ICG and traditional SLN biopsy methods, so as to guide clinicians to adopt appropriate methods for appropriate patients.

Conflicts of Interest

None declared.

Reference

1. Kurdi F, Kurdi Y, Reshetov IV. Applications of indocyanine green in breast cancer for sentinel lymph node mapping: protocol for a scoping review. *JMIRx Med* 2024;5:e66213. [doi: [10.2196/66213](https://doi.org/10.2196/66213)]
-

Abbreviations

ICG: indocyanine green

SLN: sentinel lymph node

Edited by S Tungjitviboonkun; submitted 05.12.24; this is a non-peer-reviewed article; accepted 05.12.24; published 06.01.25.

Please cite as:

Anonymous

Peer Review for “Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review”

JMIRx Med 2025;6:e69705

URL: <https://xmed.jmir.org/2025/1/e69705>

doi: [10.2196/69705](https://doi.org/10.2196/69705)

© Anonymous. Originally published in JMIRx Med (<https://med.jmirx.org>), 6.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis”

Dina Elsalamony, MSc

Department of Biotechnology, Institute of Graduate Studies & Research, University of Alexandria, Alexandria, Egypt

Related Articles:

Companion article: <https://www.biorxiv.org/content/10.1101/2023.06.21.545938v2>

Companion article: <https://med.jmirx.org/2025/1/e69894>

Companion article: <https://med.jmirx.org/2025/1/e50458>

(*JMIRx Med* 2025;6:e69896) doi:[10.2196/69896](https://doi.org/10.2196/69896)

KEYWORDS

multistep fermentation; specific methane production; anaerobic digestion; kinetics study; biochar; first-order; modified Gompertz; mass balance; waste management; environment sustainability

This is a peer-review report for “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis.”

Round 1 Review

General Comments

Generally, the manuscript [1] should be strictly improved in English language writing and corrected for all grammatical errors throughout the whole manuscript. The author has to use a uniform style of the English language, either American or British English. Further English assistance is particularly required. Many missing articles and a lot of grammatical and punctuation errors must be corrected in the manuscript as in the corrected abstract.

Specific Comments

This paper shows an important aspect of multiple fermentation steps for the complete utilization of municipal solid waste and conversion to useful products, which is highly recommended for circular economic sustainability worldwide. However, it needs some major revision and arrangement to allow for a better presentation of this valuable work.

Major Comments

Title

1. “Valorization of Organic Fraction of Municipal Solid Waste Through Production of Volatile Fatty Acids (VFAs) and Biogas” is a long title that should be shortened to be more concise with no abbreviations—more indicative. Suggested title: “Valorization of Organic Municipal Solid Waste for Volatile Fatty Acids and Biogas Production.”

Abstract Section

2. Generally speaking, it must be more concise and specific.
3. Please clearly mention the take-home message and the main findings of the research.
4. The abstract is too long and lacks the main methodology and main experimental techniques that were carried out in this work. The author may add some hints about the main methods used before mentioning the main results.

Manuscript

5. Keywords: Words must be modified to be more informative and representative of the research interest and differ from the word in the manuscript title. Maybe add “Multi Step of Fermentation Process” or “Waste Management and Environment Sustainability.”
6. Arrangement of the experimental work in the manuscript may be needed in the Results and Discussion accordingly.
7. There is a lack of figures to describe the main parameter optimization steps well. Please reformulate to describe some data using figures with error bars.
8. The SD and table footnotes with the number of replicates should be noted underneath all of the given tables.
9. A mechanistic in-detail discussion is required, not just comparing your results with the previous work; justify better.
10. In research articles, do not include any table comparing literature results; the author can discuss the main findings in the text itself, as in Table 5.
11. The Conclusions section is missing in the manuscript to summarize and point out the novelty and the main findings from the research.

12. Generally speaking, in academic writing, (1) abstracts do not include abbreviations, (2) avoid articles in the title (the, a, an), and (3) avoid keywords that exist in the title.

13. As a rule of thumb, no dots in titles or subtitles as in the Experimental section, Anaerobic Pilot Units, etc.

14. Multiple references should be merged, not written separately, as in “29, 30” and “23, 27”; the author may use the merge reference option in reference software.

15. The author may add numbers for all titles and subtitles accordingly all over the manuscript.

Minor Comments

16. The author should avoid general and well-known information, and be selective in the recent references used. May add one small paragraph to the Biological Waste Management and Environment Sustainability section.

17. The author should clarify the main aim of the work clearly in the last paragraph of the Introduction.

18. Do not use our, we, or us in academic writing.

19. The author may mention novel applications of VFA and biogas. Mention different novel sources of biogas production.

20. The author should mention the gas chromatography type, gas injection rate, column dimensions, and the used carrier gas in the main document.

21. The author did not mention that flushing with nitrogen or carbon dioxide took place in anaerobic digestion while feeding reactors and how the anaerobic conditions were maintained; please mention it clearly or add the references used for the methodology.

22. Organize titles all over the manuscript.

23. Generally, the subtitles are too generic; modify them to be more indicative and precise.

24. “unless Saturday and Sunday” in line 208 is not important information; the suggested word “daily” is enough.

25. “Unite”: Please correct.

26. Remove the grid lines in the figures.

27. The author has to mention the range used for the chemical oxygen demand method, and the original reference should be cited appropriately.

28. “As can be seen”: This statement is repetitive more than once in the Discussion, in lines 301, 315, and 423.

29. Figure 3 caption: mesophilic fermentation: Please specify which stage because both of the sequential steps were called mesophilic fermentation in Figure 1.

30. What is the rationale for comparing 3 days to 4.5 days for all the used systems; the author may justify why 4.5 days is better to complete with this hydraulic retention time in the rest of the experiments or describe the one variable at a time optimization method that is used to determine the significant factors and the insignificant one; mention them clearly. Also, use in the Discussion the terms “significant” and “insignificant” according to the obtained *P* value.

31. The author has to mention tables and figures in the text in their appropriate place.

Round 2 Review

This paper is greatly enhanced compared to the previous copy, and the author followed the previous comments precisely.

I recommend its publication. Thanks for allowing me to review this interesting work.

General Note

The Word file is the correct revised one, but the PDF seems to be the old version.

Conflicts of Interest

None declared.

Reference

1. Borhany H. Converting organic municipal solid waste into volatile fatty acids and biogas: experimental pilot and batch studies with statistical analysis. *JMIRx Med* 2025;6:e50458. [doi: [10.2196/50458](https://doi.org/10.2196/50458)]

Abbreviations

VFA: volatile fatty acid

Edited by T Leung; submitted 10.12.24; this is a non-peer-reviewed article; accepted 10.12.24; published 04.02.25.

Please cite as:

Elsalamony D

Peer Review of “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis”

JMIRx Med 2025;6:e69896

URL: <https://xmed.jmir.org/2025/1/e69896>

doi: [10.2196/69896](https://doi.org/10.2196/69896)

© Dina Elsalamony. Originally published in JMIRx Med (<https://med.jmirx.org>), 4.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study”

Ali Ahmed, MPhil, PhD, PharmD

Division of Infectious Diseases and Global Public Health, School of Medicine, University of California, San Diego, La Jolla, CA, United States

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.02.03.24302286v1>

Companion article: <https://med.jmirx.org/2025/1/e71528>

Companion article: <https://med.jmirx.org/2025/1/e57597>

(*JMIRx Med* 2025;6:e71529) doi:[10.2196/71529](https://doi.org/10.2196/71529)

KEYWORDS

periodic health examination; PHE; preventive health services; routine health checkups; Jordan; cross-sectional study

This is a peer-review report for “Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study.”

2. Why was a convenience sampling technique employed?
3. “All collected data are treated with strict confidentiality.” Some language corrections are required.

Round 1 Review

Specific Comments

Major Comments

1. In this manuscript [1], write in detail about the data collection procedure.

Minor Comments

There are a lot of formatting issues; many things seem copied and pasted.

Conflicts of Interest

None declared.

Reference

1. Tayoun AA. Determinants of periodic health examination uptake: insights from a Jordanian cross-sectional study. *JMIRx Med* 2025;6:e57597. [doi: [10.2196/57597](https://doi.org/10.2196/57597)]

Edited by T Leung; submitted 20.01.25; this is a non-peer-reviewed article; accepted 20.01.25; published 05.02.25.

Please cite as:

Ahmed A

Peer Review of “Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study”

JMIRx Med 2025;6:e71529

URL: <https://xmed.jmirx.org/2025/1/e71529>

doi: [10.2196/71529](https://doi.org/10.2196/71529)

© Ali Ahmed. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 5.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study”

Sanjeev Kumar Thalari, MBA, MSc, PhD

Department of Management Studies & Research Center, CMR Institute of Technology, Bangalore, India

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2023.04.12.23288461v1>

Companion article: <https://med.jmirx.org/2025/1/e70059>

Companion article: <https://med.jmirx.org/2025/1/e48346>

(*JMIRx Med* 2025;6:e70808) doi:[10.2196/70808](https://doi.org/10.2196/70808)

KEYWORDS

rural alimentation; community health workers; motivation; retention; rural health; rural nutrition; workforce

This is the peer-review report for “The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study.”

Round 1 Review

General Comments

This paper [1] has given the impression that the researcher has done thorough homework before starting the research and it is evident in the paper. Case methodology and thematic analysis are a few of the approaches that depict the quality of the paper. Overall, as a reviewer, it is my opinion that the research paper is of quality.

Specific Comments

1. A few more factors like government initiatives should be included in studying the impact on the motivation and retention of community health workers.

Major Comments

1. I feel that the analysis also can include education as a parameter.
2. The thematic analysis is one of the strengths of this research and is appreciated.

Minor Comments

1. Common wording should be used in every section of the paper, like qualitative case research methodology and qualitative case research.

Conflicts of Interest

None declared.

Reference

1. Kerketta A, A N R. The impact of rural alimentation on the motivation and retention of Indigenous community health workers in India: qualitative study. *JMIRx Med* 2025;6:e48346. [doi: [10.2196/48346](https://doi.org/10.2196/48346)]

Edited by A Schwartz; submitted 02.01.25; this is a non-peer-reviewed article; accepted 02.01.25; published 23.01.25.

Please cite as:

Kumar Thalari S

Peer Review of “The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study”

JMIRx Med 2025;6:e70808

URL: <https://xmed.jmir.org/2025/1/e70808>

doi: [10.2196/70808](https://doi.org/10.2196/70808)

© Sanjeev Kumar Thalari. Originally published in JMIRx Med (<https://med.jmirx.org>), 23.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “Mothers’ Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study”

Bilkisu Nwankwo, MSc

Department of Community Medicine, College of Medicine, Kaduna State University, Kaduna, Nigeria

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.04.05.24305403v1>

Companion article: <https://med.jmirx.org/2025/1/e70145>

Companion article: <https://med.jmirx.org/2025/1/e59379>

(*JMIRx Med* 2025;6:e70142) doi:[10.2196/70142](https://doi.org/10.2196/70142)

KEYWORDS

mother's knowledge and practices; oral hygiene; child oral health; bangladesh

This is the peer-review report for “Mothers’ Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study.”

Round 1 Review

Specific Comments

There were a lot of grammatical issues and typographical errors. The manuscript [1] needs to be edited for grammar and syntax. It is also obvious that the manuscript was not proofread adequately.

Major Comments

Abstract

- A word is missing in the first sentence. Authors should proofread the manuscript.
- Keywords: Dhaka is a more appropriate keyword than Bangladesh.
- Under the Results in the abstract, respondents should be referred to as such and not as samples.

Introduction

- The global prevalence of oral diseases was stated, but authors did not capture the prevalence in the study area/country and so have not shown that oral disease is a problem. Even the global prevalence that was stated was only that of dental caries among the seven conditions that make up oral diseases as stated by the authors.
- The objective stated here (last sentence) comes off like the authors are assessing the knowledge and practices of oral hygiene with regard to themselves and not their children as stated in the topic.

Methods

- Was it permission that was given by the institutional review board or an ethical clearance?
- This section is quite disorganized. There is a logical flow expected in this section.
- Why was a nonprobability sampling technique (convenient sampling) used for this study? The sampling technique was not explained at all. This will make replicating this study difficult.
- I have an issue with the scoring system and the grading. Is there a reference for it? I particularly have an issue with “moderately average.” It is not a standard term.
- The exclusion criteria are not the opposite of the inclusion criteria as stated by the authors. Exclusion criteria are those already included in the study but that are ineligible for one reason or the other.

Results

- In the text above Table 1, authors wrote that most respondents (39.3%) had a monthly family income of “21,000 - 40,000 taka per month.” This figure (39.3%) is just over one-third of the respondents and not a majority.
- Table 1: What is the meaning of graduation and above? Is it graduated secondary school or graduated college?
- “Respectively” should be added at the end of the following sentence. “Out of 400 mothers, more than 90% knew the importance of brushing teeth while 82.3% and 80.8% of them knew the recommended frequency and appropriate time for brushing teeth.”

Discussion

- The second sentence: the study is not evaluating parent’s knowledge and practices but that of mothers.
- Grammatical errors and missing words

Reference List

- Some of the references were not cited correctly. Authors should adhere to the Vancouver referencing style.

Conflicts of Interest

None declared.

Reference

1. Tamannur T, Das SK, Nesa A, et al. Mothers' knowledge of and practices toward oral hygiene of children aged 5-9 years in Bangladesh: cross-sectional study. *JMIRx Med* 2025;6:e59379. [doi: [10.2196/59379](https://doi.org/10.2196/59379)]

Edited by T Leung; submitted 16.12.24; this is a non-peer-reviewed article; accepted 06.01.25; published 03.02.25.

Please cite as:

Nwankwo B

Peer Review of "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study"

JMIRx Med 2025;6:e70142

URL: <https://xmed.jmir.org/2025/1/e70142>

doi: [10.2196/70142](https://doi.org/10.2196/70142)

© Bilkisu Nwankwo. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 3.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study”

Anonymous

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.02.03.24302286v1>

Companion article: <https://med.jmirx.org/2025/1/e71528>

Companion article: <https://med.jmirx.org/2025/1/e57597>

(*JMIRx Med* 2025;6:e71531) doi:[10.2196/71531](https://doi.org/10.2196/71531)

KEYWORDS

periodic health examination; PHE; preventive health services; routine health checkups; Jordan; cross-sectional study

This is a peer-review report for “Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study.”

Round 1 Review

The following items were noted in this paper [1].

- Periodic health examination (PHE) uptake: Only 27.1% of participants underwent a PHE in the last 2 years.
- Predictors: Significant predictors include recent visits to a primary health care facility, monthly income, and knowledge about PHEs and preventive health measures.
- Nonsignificant factors: Gender, marital status, smoking status, and BMI did not show a significant association with PHE uptake.

Strengths

1. Comprehensive analysis: The study employs a robust methodology, combining descriptive, inferential, and multivariate statistical techniques to provide a thorough understanding of PHE uptake.
2. Significant predictors identified: Key factors influencing PHE uptake were identified, offering valuable insights for health care providers and policy makers.
3. First of its kind in Jordan: This study fills a gap in existing knowledge by being the first to investigate PHE uptake in Jordan.

Negative Points and Areas for Improvement

Cross-Sectional Design

- Limitation: The study’s design limits the ability to establish causality.
- Improvement: Future research could benefit from a longitudinal approach to better establish causal relationships between the identified predictors and PHE uptake.

Convenience Sampling

- Limitation: This method may introduce selection bias, and the online survey format may lead to measurement bias.
- Improvement: Employing a more randomized and stratified sampling method could enhance the representativeness and validity of the findings.

Limited Generalizability

- Limitation: Results may not be generalizable to populations outside of Jordan or those not included in the sample.
- Improvement: Expanding the study to include diverse populations and different geographic regions would provide a more comprehensive understanding of PHE uptake.

Survey Instrument

- Limitation: The questionnaire’s comprehensiveness and relevance to the Jordanian context might not have been fully ensured.
- Improvement: Pretesting the survey with a larger and more varied group, followed by adjustments based on feedback, could improve its applicability and accuracy.

Behavioral Factors

- Limitation: The study did not find a relationship between behavioral factors and PHE uptake, which contradicts findings in other contexts.
- Improvement: A more detailed investigation into cultural and societal influences on health behaviors in Jordan is needed to clarify these results.

English Language and Clarity

- Limitation: The manuscript contains some grammatical errors and awkward phrasings, which can detract from its readability.
- Improvement: A thorough review and editing for language and clarity by a native English speaker or professional editor would enhance the manuscript’s quality.

Conflicts of Interest

None declared.

Reference

1. Tayoun AA. Determinants of periodic health examination uptake: insights from a Jordanian cross-sectional study. *JMIRx Med* 2025;6:e57597. [doi: [10.2196/57597](https://doi.org/10.2196/57597)]
-

Abbreviations

PHE: periodic health examination

Edited by T Leung; submitted 20.01.25; this is a non-peer-reviewed article; accepted 20.01.25; published 05.02.25.

Please cite as:

Anonymous

Peer Review of "Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study"

JMIRx Med 2025;6:e71531

URL: <https://xmed.jmir.org/2025/1/e71531>

doi: [10.2196/71531](https://doi.org/10.2196/71531)

© Reviewer DD Anonymous. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 5.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “Mothers’ Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study”

Md Hafizul Islam

Institute of Nutrition and Food Science, University of Dhaka, Dhaka, Bangladesh

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.04.05.24305403v1>

Companion article: <https://med.jmirx.org/2025/1/e70145>

Companion article: <https://med.jmirx.org/2025/1/e59379>

(*JMIRx Med* 2025;6:e70144) doi:[10.2196/70144](https://doi.org/10.2196/70144)

KEYWORDS

mothers’ knowledge and practices; oral hygiene; child oral health; Bangladesh

This is the peer-review report for “Mothers’ Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study.”

Round 1 Review

This is an interesting piece of research [1], which highlights mothers’ knowledge and practices regarding their children’s oral health in Dhaka City. However, several issues made the study scientifically questionable. The major issues are as follows. The study included mothers from two hospitals in Dhaka City, but the title of the study does not mention this. The sample selection from the mothers visiting the hospitals might not represent general mothers from the whole of Dhaka. Thus, this study might not be generalizable to all mothers in Dhaka City.

Introduction

Revise the last paragraph of the Introduction to highlight the study gap in Bangladesh and clearly state the objective of the study. Use the formal word “mother” and avoid the word “moms.”

Methods

Study Setting and Participants

Give clear reasoning as to why you selected study participants from the hospitals. The last line is confusing. It is not clear whether the participants filled out the questionnaire on their own or they were interviewed by the enumerators.

Sampling Technique

Please mention the nonresponse bias for the convenient sampling. Give a short description of the pretesting mentioning the number of samples, period, and location for it.

Measurement of Knowledge and Practice Score

Give the 15 knowledge-related questions and 13 practice-related questions in the supplementary file. Mention if these questions are your own or if you used any valid tools or questions adopted from the relevant previous studies. Give adequate information regarding the scoring system of the variables, mentioning the highest possible aggregated score and examples of two questions (one for knowledge and one for practice).

Statistical Analyses

The authors mentioned that they used the Mann-Whitney *U* test and the Kruskal-Wallis test. However, they did not mention the underlying assumptions of the tests. Moreover, the Results section also shows the χ^2 test but is not mentioned in the Methods section. Furthermore, the last line of the Results of the abstract shows the Pearson correlation coefficient, but nothing is mentioned in the Methods or Results section of the entire manuscript.

Results

Table 1

It is confusing as the text description of Table 1 and the title of Table 1 are different. It is recommended to use two separate tables: one for socioeconomic variables and another for the frequency distribution of the knowledge level among socioeconomic variables. Mention the knowledge- and practice-related raw scores first and then the cross-tab results. There is a major mistake in the results of Tables 1 and 2. The frequency distribution for educational status, occupation, family type, number of family members, and monthly income in Tables 1 and 2 are the same. However, the *P* values are different. How is this possible? Please check the results.

Discussion

It is confusing whether the practice was for the children or how a mother takes care of their children's dental health. Mention the implications of your findings rather than just comparing the findings with previous studies. State the limitation of the study, especially the bias regarding convenient sampling. Provide a section on the public health significance of the study findings in Bangladesh.

Conclusion

The Conclusion section of the study is poorly written and not focused on the findings of the study. Revise the Conclusion section to highlight your study findings.

Round 2 Review

The authors impressively amended the initial version of the manuscript based on the reviewers' comments. However, several issues remain unaddressed.

1. The authors should include the city in the title of the study. You can revise the title to "Knowledge and practices towards oral hygiene of children aged 5 - 9 years old: a cross-sectional study among mothers visited tertiary level hospitals in Dhaka, Bangladesh."
2. Use the full form when it appears first and then use the abbreviation afterward. For example, "KP" in the abstract.
3. Please mention this statistical test in the Methods section of the abstract. You did not mention the χ^2 test and Pearson correlation.
4. It is recommended to make the recommendation simple and easy to understand for the readers. Avoid duplication of the same term.
5. In the sample size calculation, you used $P=.58$ and $P=.57$. Please clarify why you used those prevalences. Cite the relevant study here.
6. Before the heading for the sociodemographic variables in the Methods section, you mention outcome measures. However, the sociodemographic variables are not your outcome variables according to your objectives. You can remove the term outcome measures from here.
7. You mentioned that you used 13 questions for the assessment of practices. Thus, according to your scoring approach, there should be a score of 1-13, but here, it is 1-11.
8. Please mention the name of the software and version you used for the statistical analysis.
9. Revise the sentence before Table 1. You can make it two sentences. One for family income and another for occupation.
10. There is no chi-square-related data in Table 1. Please remove the footnotes from Table 1.
11. In Figure 1, it is recommended to keep the values to one decimal point for 1a and 1b.
12. Please revise the sentence before Table 3 to give a clear meaning.
13. You can remove the percentage symbol from the value and give it in the vertical axis title.
14. Please give the correlation results in the main manuscript or as a supplementary table.
15. The authors overlooked the association of knowledge and practice with income and family size. Please give more details on those two points in the Discussion section.

Conflicts of Interest

None declared.

Reference

1. Tamannur T, Das SK, Nesa A, et al. Mothers' knowledge of and practices toward oral hygiene of children aged 5-9 years in Bangladesh: cross-sectional study. *JMIRx Med* 2025;6:e59379. [doi: [10.2196/59379](https://doi.org/10.2196/59379)]

Edited by T Leung; submitted 16.12.24; this is a non-peer-reviewed article; accepted 16.12.24; published 03.02.25.

Please cite as:

Islam MH

Peer Review of "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study"

JMIRx Med 2025;6:e70144

URL: <https://xmed.jmir.org/2025/1/e70144>

doi: [10.2196/70144](https://doi.org/10.2196/70144)

© Md Hafizul Islam. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 3.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*,

is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis”

Anonymous

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2023.07.03.23292185v1>

Companion article: <https://med.jmirx.org/2025/1/e69307>

Companion article: <https://med.jmirx.org/2025/1/e50712>

(*JMIRx Med* 2025;6:e70039) doi:[10.2196/70039](https://doi.org/10.2196/70039)

KEYWORDS

breast; cancer; oncology; ovarian; virus; viral; Epstein-Barr; herpes; bioinformatics; chromosome; gene; genetic; genetics; chromosomal; DNA; genomic; BRCA; metastasis; biology

This is the peer-review report for “Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis.”

Round 1 Review

Review Report With Major Revisions for the Paper

Title: “Herpesvirus infections eliminate safeguards against breast cancer and its metastasis: comparable to hereditary breast cancers”

Summary

The paper [1] hypothesizes that Epstein-Barr virus (EBV) infections promote breast cancer by disabling cancer safeguards. It is a bioinformatics analysis of public information from about 2100 breast cancers. The study finds that breast and ovarian cancer breakpoints cluster around EBV-associated cancer breakpoints, suggesting a significant role of EBV in promoting these cancers. The paper also identifies similarities in the molecular and cellular disruptions caused by EBV with those found in hereditary breast cancers.

Major Revisions Needed

Clarification of Hypotheses and Objectives

The hypothesis, while intriguing, needs clearer articulation. Specifically, the connection between EBV and breast cancer needs more explicit theoretical underpinning. Clarify the objectives and expected outcomes of the study at the outset.

Methodological Rigor and Data Sources

While the bioinformatics approach is robust, it would benefit from a more detailed description of the methods and algorithms used. Additionally, the selection criteria for the breast cancer data should be justified more thoroughly to avoid selection bias.

Statistical Analysis

The statistical methods used need more comprehensive detailing. For complex analyses, ensure the statistical assumptions and any transformations of data are clearly explained. Include more information on the statistical tests used for hypothesis testing and the justification for their use.

Comparative Analysis

The comparison between hereditary breast cancers and those potentially caused by EBV is insightful. However, a more detailed comparative analysis would strengthen the argument. This could include molecular or genetic profiling comparisons.

Discussion on Contradictory or Supporting Evidence

The discussion section should address not only the supporting evidence but also any contradictory findings in the literature. This balance is crucial for a nuanced understanding of the subject.

Implications and Future Research Directions

The implications of these findings are profound but need clearer articulation. Discuss the potential impact on breast cancer treatment and prevention strategies. Also, outline future research directions, particularly in clinical or experimental studies, to confirm these bioinformatics findings.

References

Please add more background information about breast cancer (please cite: 1. Cao Y, Efetov S, He M, et al. Updated clinical perspectives and challenges of chimeric antigen receptor-T cell therapy in colorectal cancer and invasive breast cancer. *Arch Immunol Ther Exp (Warsz)*. Aug 11, 2023;71(1):19. [doi: 10.1007/s00005-023-00684-x] [Medline: 37566162]; and 2. Liu Y, Lu S, Sun Y, et al. Deciphering the role of QPCTL in glioma progression and cancer immunotherapy. *Front Immunol*.

Mar 29, 2023;14:1166377. [doi: 10.3389/fimmu.2023.1166377]
[Medline: 37063864]).

revisions to enhance its methodological rigor, clarity, and comprehensiveness. Addressing these concerns will significantly strengthen the manuscript's impact and contribution to the field.

Concluding Remarks

The paper presents a novel and potentially significant hypothesis linking EBV to breast cancer. However, it requires major

Conflicts of Interest

None declared.

Reference

1. Friedenson B. Identifying safeguards disabled by Epstein-Barr virus infections in genomes from patients with breast cancer: chromosomal bioinformatics analysis. JMIRx Med 2025;6:e50712. [doi: [10.2196/50712](https://doi.org/10.2196/50712)]

Abbreviations

EBV: Epstein-Barr virus

Edited by A Schwartz; submitted 13.12.24; this is a non-peer-reviewed article; accepted 13.12.24; published 29.01.25.

Please cite as:

Anonymous

Peer Review of "Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis"

JMIRx Med 2025;6:e70039

URL: <https://xmed.jmir.org/2025/1/e70039>

doi: [10.2196/70039](https://doi.org/10.2196/70039)

© Anonymous. Originally published in JMIRx Med (<https://med.jmirx.org>), 29.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis”

Anonymous

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2023.07.03.23292185v1>

Companion article: <https://med.jmirx.org/2025/1/e69307>

Companion article: <https://med.jmirx.org/2025/1/e50712>

(*JMIRx Med* 2025;6:e70041) doi:[10.2196/70041](https://doi.org/10.2196/70041)

KEYWORDS

breast; cancer; oncology; ovarian; virus; viral; Epstein-Barr; herpes; bioinformatics; chromosome; gene; genetic; chromosomal; DNA; genomic; BRCA; metastasis; biology

This is the peer-review report for “Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis.”

Round 1 Review

Dear Author,

After a thorough review of the paper titled “Herpesvirus infections eliminate safeguards against breast cancer and its metastasis: comparable to hereditary breast cancers” [1] by Bernard Friedenson, here is the negative feedback and evaluation, along with a recommendation for the inclusion of a specific article in the discussion section.

Negative Feedback and Evaluation

Clarity and Scope

The paper ambitiously attempts to link Epstein-Barr virus (EBV) infections to breast cancer development and metastasis. While the hypothesis is intriguing, the narrative sometimes lacks clarity and could benefit from a more focused scope. The vast amount of data and the complex mechanisms presented can be overwhelming and occasionally detract from the main message.

Methodological Concerns

The reliance on bioinformatics analyses and previously published datasets raises questions about the direct experimental validation of the proposed mechanisms. Although the computational approach is valid, the absence of direct experimental evidence or validation in breast cancer samples limits the strength of the conclusions.

Interpretation of Data

The interpretation of viral homology and its impact on cancer development is speculative in several sections. The connections

made between EBV infections, chromosomal breakpoints, and cancerous mutations rely heavily on correlative data without sufficient causal evidence. A more cautious interpretation of the results, highlighting the need for further experimental validation, would strengthen the manuscript.

Consideration of Alternate Hypotheses

The paper could benefit from a more balanced discussion of alternative hypotheses explaining the observed data. For instance, the role of other environmental, genetic, or lifestyle factors in breast cancer development is not adequately considered. Acknowledging and discussing these potential confounders would provide a more comprehensive understanding of the complex etiology of breast cancer.

References and Current Literature

While the paper cites a significant amount of relevant literature, it sometimes overlooks recent studies that could either support or challenge the proposed hypotheses. Incorporating a more current and diverse range of references would enhance the paper’s relevance and credibility.

Recommendation for Discussion Inclusion

To broaden the discussion and contextualize the findings within the broader research landscape, it is recommended to include the following article in the discussion section.

Al-Awaida W, Al-Ameer HJ, Sharab A, Akasheh RT. Modulation of wheatgrass (*Triticum aestivum* Linn) toxicity against breast cancer cell lines by simulated microgravity. *Curr Res Toxicol.* Sep 19, 2023;5:100127. [doi: 10.1016/j.crtox.2023.100127] [Medline: 37767028]

Incorporating this article could provide valuable insights into innovative approaches for studying cancer therapies. Specifically, the effects of simulated microgravity on the efficacy of natural compounds like wheatgrass against breast

cancer could open up new avenues for research on the environmental and physical conditions affecting cancer treatment outcomes. Discussing this study would enrich the manuscript by introducing the concept of microgravity as a novel factor influencing cancer cell behavior and therapy resistance, thereby offering a broader perspective on cancer research methodologies and therapeutic strategies.

Round 2 Review

General Comments

This paper tests the idea that EBV infections can help cause breast cancer by weakening the body's defenses against cancer. The study uses bioinformatics to compare chromosome breakpoints in breast cancer to those in cancers known to be caused by EBV. The results show that EBV might play a role in breast cancer by damaging important cell functions.

Specific Comments

Major Comments

The methods section needs more details about how the datasets were chosen and combined.

The discussion should explain more about how EBV might cause the chromosome breaks and rearrangements seen in breast cancer.

More data or references are needed to support the idea that EBV helps breast cancer spread to other parts of the body.

Minor Comments

Adding more references would strengthen the sections that talk about how EBV affects breast cancer.

Figures and tables should be clearly mentioned in the text to help readers follow the data.

Some parts of the manuscript need clearer writing and better organization, especially where complex bioinformatics results are explained.

The abstract should be revised to clearly highlight the main findings and why they are important.

Make sure all abbreviations are defined when they are first used to help readers understand the text better.

Conflicts of Interest

None declared.

Reference

1. Friedenson B. Identifying safeguards disabled by Epstein-Barr virus infections in genomes from patients with breast cancer: chromosomal bioinformatics analysis. *JMIRx Med* 2025;6:e50712. [doi: [10.2196/50712](https://doi.org/10.2196/50712)]

Abbreviations

EBV: Epstein-Barr virus

Edited by A Schwartz; submitted 13.12.24; this is a non-peer-reviewed article; accepted 13.12.24; published 29.01.25.

Please cite as:

Anonymous

Peer Review of "Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis"

JMIRx Med 2025;6:e70041

URL: <https://xmed.jmir.org/2025/1/e70041>

doi: [10.2196/70041](https://doi.org/10.2196/70041)

© Anonymous. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 29.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Authors' Response to Peer Reviews of "Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review"

Feryal Kurdi*, MD; Yahya Kurdi*, MD; Igor Vladimirovich Reshetov, MD, PhD

Department of Oncology, Radiotherapy and Plastic and Reconstructive Surgery, Sechenov University, Bolshaya Pirogovskaya, 6c1, Moscow, Russian Federation

*these authors contributed equally

Corresponding Author:

Feryal Kurdi, MD

Department of Oncology, Radiotherapy and Plastic and Reconstructive Surgery, Sechenov University, Bolshaya Pirogovskaya, 6c1, Moscow, Russian Federation

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.07.30.24311256v1>

Companion article: <https://med.jmirx.org/2025/1/e69705>

Companion article: <https://med.jmirx.org/2025/1/e66213>

(*JMIRx Med* 2025;6:e68769) doi:10.2196/68769

KEYWORDS

indocyanine green; ICG; sentinel lymph node; breast cancer; breast; fluorescence; axillary lymph node mapping; NIR; surgical planning; near-infrared

This is the authors' response to peer-review reports for "Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review."

is relatively small. It is hoped that the author can search the recent, relevant literature to improve the credibility of this review.

Round 1 Review

Anonymous [1]

General Comments

This paper [2] summarized the application value and existing problems of indocyanine green (ICG) in sentinel lymph node (SLN) biopsy of early breast cancer, which has positive significance for improving the accuracy of clinical SLN detection. This study has certain clinical value.

Response: Thank you for your thoughtful comments and feedback on our paper. Below are my responses to your points.

Specific Comments

Major Comments

1. Due to the high hardware requirements for the clinical application of ICG, the number of relevant studies in the search

Response: This paper is a protocol for a scoping review, serving as a roadmap for the search strategy and inclusion criteria that we will follow. As such, it outlines our plan rather than reporting the outcomes of the literature search. As noted in Multimedia Appendix 1, we will conduct a comprehensive search across multiple databases to ensure the inclusion of all relevant, recent studies.

2. It is hoped that the author will analyze and compare the advantages and disadvantages of ICG and traditional SLN biopsy methods, so as to guide clinicians to adopt appropriate methods for appropriate patients.

Response: As indicated in Multimedia Appendix 1, this comparison is a core objective of our review. We hope these clarifications address your concerns.

References

1. Anonymous. Peer review of "Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review. *JMIRx Med* 2024;5:e69705. [doi: [10.2196/69705](https://doi.org/10.2196/69705)]

2. Kurdi F, Kurdi Y, Reshetov IV. Applications of indocyanine green in breast cancer for sentinel lymph node mapping: protocol for a scoping review. *JMIRx Med* 2024;5:e66213. [doi: [10.2196/66213](https://doi.org/10.2196/66213)]

Abbreviations

ICG: indocyanine green

SLN: sentinel lymph node

Edited by S Tungjitviboonkun; submitted 13.11.24; this is a non-peer-reviewed article; accepted 13.11.24; published 06.01.25.

Please cite as:

Kurdi F, Kurdi Y, Reshetov IV

Authors' Response to Peer Reviews of "Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review"

JMIRx Med 2025;6:e68769

URL: <https://xmed.jmir.org/2025/1/e68769>

doi: [10.2196/68769](https://doi.org/10.2196/68769)

© Feryal Kurdi, Yahya Kurdi, Igor Vladimirovich Reshetov. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 6.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Author's Response to Peer Reviews of "Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis"

Bernard Friedenson, PhD

Department of Biochemistry and Medical Genetics, Cancer Center, University of Illinois Chicago, 900 s Ashland, Chicago, IL, United States

Corresponding Author:

Bernard Friedenson, PhD

Department of Biochemistry and Medical Genetics, Cancer Center, University of Illinois Chicago, 900 s Ashland, Chicago, IL, United States

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2023.07.03.23292185v1>

Companion article: <https://med.jmirx.org/2025/1/e70039>

Companion article: <https://med.jmirx.org/2025/1/e70041>

Companion article: <https://med.jmirx.org/2025/1/e50712>

(*JMIRx Med* 2025;6:e69307) doi:[10.2196/69307](https://doi.org/10.2196/69307)

KEYWORDS

breast; cancer; oncology; ovarian; virus; viral; Epstein-Barr; herpes; bioinformatics; chromosome; gene; genetic; chromosomal; DNA; genomic; BRCA; metastasis; biology

This is the author's response to peer-review reports for "Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis."

Round 1 Review

Anonymous [1]

Review Report With Major Revisions for the Paper

Title: "Herpesvirus infections eliminate safeguards against breast cancer and its metastasis: comparable to hereditary breast cancers"

Summary

The paper [2] hypothesizes that Epstein-Barr virus (EBV) infections promote breast cancer by disabling cancer safeguards. It is a bioinformatics analysis of public information from about 2100 breast cancers. The study finds that breast and ovarian cancer breakpoints cluster around EBV-associated cancer breakpoints, suggesting a significant role of EBV in promoting these cancers. The paper also identifies similarities in the molecular and cellular disruptions caused by EBV with those found in hereditary breast cancers.

Major Revisions Needed

Clarification of Hypotheses and Objectives

The hypothesis, while intriguing, needs clearer articulation. Specifically, the connection between EBV and breast cancer needs more explicit theoretical underpinning. Clarify the objectives and expected outcomes of the study at the outset.

Response: The objectives and expected outcomes of the study were clarified at the outset in the Abstract and Introduction.

Methodological Rigor and Data Sources

While the bioinformatics approach is robust, it would benefit from a more detailed description of the methods and algorithms used. Additionally, the selection criteria for the breast cancer data should be justified more thoroughly to avoid selection bias.

Response: A more detailed description of the methods and algorithms used has been added in the Methods section (page 6).

Statistical Analysis

The statistical methods used need more comprehensive detailing. For complex analyses, ensure the statistical assumptions and any transformations of data are clearly explained. Include more information on the statistical tests used for hypothesis testing and the justification for their use.

Response: I included more information on the statistical tests, the justification, and limitations of their use (page 7).

Comparative Analysis

The comparison between hereditary breast cancers and those potentially caused by EBV is insightful. However, a more detailed comparative analysis would strengthen the argument. This could include molecular or genetic profiling comparisons.

Response: I added a more detailed comparative analysis with results in Figure 2H and Table S2, as described on page 10.

Discussion on Contradictory or Supporting Evidence

The discussion section should address not only the supporting evidence but also any contradictory findings in the literature. This balance is crucial for a nuanced understanding of the subject.

Response: The paper's hypothesis more clearly accounts for the absence of demonstrable EBV infection in breast cancer, explaining contradictory results. The other contradictory result posits an imperfect palindrome on chromosome 11. This result is tested on page 13.

Implications and Future Research Directions

The implications of these findings are profound but need clearer articulation. Discuss the potential impact on breast cancer treatment and prevention strategies. Also, outline future research directions, particularly in clinical or experimental studies to confirm these bioinformatics findings.

Response: I articulated the implications of these finding more clearly with their impact on breast cancer treatment and prevention strategies. I also outlined future research directions with clinical or experimental studies to confirm the bioinformatics findings (Discussion, page 16).

References

Please add more background information about breast cancer (please cite: 1. Cao Y, Efetov S, He M, et al. Updated clinical perspectives and challenges of chimeric antigen receptor-T cell therapy in colorectal cancer and invasive breast cancer. Arch Immunol Ther Exp (Warsz). Aug 11, 2023;71(1):19. [doi: 10.1007/s00005-023-00684-x] [Medline: 37566162]; and 2. Liu Y, Lu S, Sun Y, et al. Deciphering the role of QPCTL in glioma progression and cancer immunotherapy. Front Immunol. Mar 29, 2023;14:1166377. [doi: 10.3389/fimmu.2023.1166377] [Medline: 37063864]).

Response: I added these references.

Concluding Remarks

The paper presents a novel and potentially significant hypothesis linking EBV to breast cancer. However, it requires major revisions to enhance its methodological rigor, clarity, and comprehensiveness. Addressing these concerns will significantly strengthen the manuscript's impact and contribution to the field.

Anonymous [3]

Dear Author,

After a thorough review of the paper titled "Herpesvirus infections eliminate safeguards against breast cancer and its

metastasis: comparable to hereditary breast cancers" by Bernard Friedenson, here is the negative feedback and evaluation, along with a recommendation for the inclusion of a specific article in the discussion section.

Negative Feedback and Evaluation

Clarity and Scope

The paper ambitiously attempts to link Epstein-Barr virus (EBV) infections to breast cancer development and metastasis. While the hypothesis is intriguing, the narrative sometimes lacks clarity and could benefit from a more focused scope. The vast amount of data and the complex mechanisms presented can be overwhelming and occasionally detract from the main message.

Response: I focused the scope in this revision in the Abstract and Introduction.

Methodological Concerns

The reliance on bioinformatics analyses and previously published datasets raises questions about the direct experimental validation of the proposed mechanisms. Although the computational approach is valid, the absence of direct experimental evidence or validation in breast cancer samples limits the strength of the conclusions.

Response: I explained in the Discussion section that direct experimental evidence or validation has already been done. EBV-infected human mammary epithelial cells produce breast cancer in immunosuppressed mice (page 17).

Interpretation of Data

The interpretation of viral homology and its impact on cancer development is speculative in several sections. The connections made between EBV infections, chromosomal breakpoints, and cancerous mutations rely heavily on correlative data without sufficient causal evidence. A more cautious interpretation of the results, highlighting the need for further experimental validation, would strengthen the manuscript.

Response: I added more evidence (Figure 2H and Table S2) to the association of EBV infection and cancer development and took greater care throughout to interpret the results more cautiously.

Consideration of Alternate Hypotheses

The paper could benefit from a more balanced discussion of alternative hypotheses explaining the observed data. For instance, the role of other environmental, genetic, or lifestyle factors in breast cancer development is not adequately considered. Acknowledging and discussing these potential confounders would provide a more comprehensive understanding of the complex etiology of breast cancer.

Response: I explained how EBV relates to alternate hypotheses and exacerbates the effects of other known breast cancer risk factors (page 16).

References and Current Literature

While the paper cites a significant amount of relevant literature, it sometimes overlooks recent studies that could either support or challenge the proposed hypotheses. Incorporating a more

current and diverse range of references would enhance the paper's relevance and credibility.

Response: I included more information from more current and diverse ranges of references.

Recommendation for Discussion Inclusion

To broaden the discussion and contextualize the findings within the broader research landscape, it is recommended to include the following article in the discussion section.

Al-Awaida W, Al-Ameer HJ, Sharab A, Akasheh RT. Modulation of wheatgrass (*Triticum aestivum* Linn) toxicity against breast cancer cell lines by simulated microgravity. *Curr Res Toxicol.* Sep 19, 2023;5:100127. [doi: 10.1016/j.crtox.2023.100127] [Medline: 37767028]

Incorporating this article could provide valuable insights into innovative approaches for studying cancer therapies. Specifically, the effects of simulated microgravity on the efficacy of natural compounds like wheatgrass against breast cancer could open up new avenues for research on the environmental and physical conditions affecting cancer treatment outcomes. Discussing this study would enrich the manuscript by introducing the concept of microgravity as a novel factor influencing cancer cell behavior and therapy resistance, thereby offering a broader perspective on cancer research methodologies and therapeutic strategies.

Response: I could not find a way to apply and cite this interesting work since it was so far afield from the manuscript.

Round 2 Review

Anonymous [3]

General Comments

This paper tests the idea that EBV infections can help cause breast cancer by weakening the body's defenses against cancer. The study uses bioinformatics to compare chromosome breakpoints in breast cancer to those in cancers known to be caused by EBV. The results show that EBV might play a role in breast cancer by damaging important cell functions.

Specific Comments

Major Comments

The methods section needs more details about how the datasets were chosen and combined.

Response: More details on how the datasets were chosen have been added.

The discussion should explain more about how EBV might cause the chromosome breaks and rearrangements seen in breast cancer.

Response: The discussion includes an expanded explanation about how EBV might cause the chromosome breaks and rearrangements seen in breast cancer.

More data or references are needed to support the idea that EBV helps breast cancer spread to other parts of the body.

Response: A new Figure 7 and more data have been added. Additional references have also been added, and the metastasis topic has been clarified and expanded.

Minor Comments

Adding more references would strengthen the sections that talk about how EBV affects breast cancer.

Response: Many more references have been added.

Figures and tables should be clearly mentioned in the text to help readers follow the data.

Response: Figures and tables are now more prominently mentioned in the text.

Some parts of the manuscript need clearer writing and better organization, especially where complex bioinformatics results are explained.

Response: I revised the manuscript with clearer writing and better organization, especially where complex bioinformatics results are explained.

The abstract should be revised to clearly highlight the main findings and why they are important.

Response: I revised the Abstract to highlight the main findings and why they are important.

Make sure all abbreviations are defined when they are first used to help readers understand the text better.

Response: I went through the manuscript to be sure all abbreviations were defined. I also added a glossary containing abbreviations, gene names, and viruses.

References

1. Anonymous. Peer review of "Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis". *JMIRx Med* 2025;6:e70039. [doi: [10.2196/70039](https://doi.org/10.2196/70039)]
2. Friedenson B. Identifying safeguards disabled by Epstein-Barr virus infections in genomes from patients with breast cancer: chromosomal bioinformatics analysis. *JMIRx Med* 2025;6:e50712. [doi: [10.2196/50712](https://doi.org/10.2196/50712)]
3. Anonymous. Peer review of "Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis". *JMIRx Med* 2025;6:e70041. [doi: [10.2196/70041](https://doi.org/10.2196/70041)]

Abbreviations

EBV: Epstein-Barr virus

Edited by A Schwartz; submitted 26.11.24; this is a non-peer-reviewed article; accepted 26.11.24; published 29.01.25.

Please cite as:

Friedenson B

Author's Response to Peer Reviews of "Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis"

JMIRx Med 2025;6:e69307

URL: <https://xmed.jmir.org/2025/1/e69307>

doi: [10.2196/69307](https://doi.org/10.2196/69307)

© Bernard Friedenson. Originally published in JMIRx Med (<https://med.jmirx.org>), 29.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Authors' Response to Peer Reviews of "The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study"

Ajit Kerketta*, MHA; Raghavendra A N*, PhD

CHRIST (Deemed to be University), Hosur Road, Bhavani Nagar, Bengaluru, India

* all authors contributed equally

Corresponding Author:

Ajit Kerketta, MHA

CHRIST (Deemed to be University), Hosur Road, Bhavani Nagar, Bengaluru, India

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2023.04.12.23288461v1>

Companion article: <https://med.jmirx.org/2025/1/e70808>

Companion article: <https://med.jmirx.org/2025/1/e48346>

(*JMIRx Med* 2025;6:e70059) doi:[10.2196/70059](https://doi.org/10.2196/70059)

KEYWORDS

rural alimentation; community health workers; motivation; retention; rural health; rural nutrition; workforce

This is the authors' response to peer-review reports for "The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study."

Round 1 Review [1]

General Comments

This paper [2] has given the impression that the researcher has done thorough homework before starting the research and it is evident in the paper. Case methodology and thematic analysis are a few of the approaches that depict the quality of the paper. Overall, as a reviewer, it is my opinion that the research paper is of quality.

Specific Comments

1. A few more factors like government initiatives should be included in studying the impact on the motivation and retention of community health workers.

Response: Factors such as government initiatives and policies have been additionally incorporated into the Discussion section.

Major Comments

1. I feel that the analysis also can include education as a parameter.

2. The thematic analysis is one of the strengths of this research and is appreciated.

Response: Due to time constraints, education could not be included as a sample parameter.

Minor Comments

1. Common wording should be used in every section of the paper, like qualitative case research methodology and qualitative case research.

Response: The term "qualitative case research" has been consistently used throughout the study.

References

1. Kumar Thalari S. Peer review of "The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study". *JMIRx Med* 2025;6:e70808. [doi: [10.2196/70808](https://doi.org/10.2196/70808)]
2. Kerketta A, A N R. The impact of rural alimentation on the motivation and retention of Indigenous community health workers in India: qualitative study. *JMIRx Med* 2025;6:e48346. [doi: [10.2196/48346](https://doi.org/10.2196/48346)]

Edited by A Schwartz; submitted 13.12.24; this is a non-peer-reviewed article; accepted 13.12.24; published 23.01.25.

Please cite as:

Kerketta A, A N R

Authors' Response to Peer Reviews of "The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: Qualitative Study"

JMIRx Med 2025;6:e70059

URL: <https://xmed.jmir.org/2025/1/e70059>

doi: [10.2196/70059](https://doi.org/10.2196/70059)

© Ajit Kerketta, Raghavendra A N. Originally published in JMIRx Med (<https://med.jmirx.org>), 23.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Authors' Response to Peer Reviews of "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study"

Tahazid Tamannur¹, MPH; Sadhan Kumar Das¹, MPH; Arifatun Nesa², MPH; Fojjun Nahar¹, MPH; Nadia Nowshin¹, MPH; Tasnim Haque Binty¹, MPH; Shafiul Azam Shakil², MPH; Shuvojit Kumar Kundu³, MPH; Md Abu Bakkar Siddik⁴, MPH; Shafkat Mahmud Rafsun⁵, MPH; Umme Habiba⁶, MPH; Zaki Farhana⁷, MS; Hafiza Sultana¹, MPhil; Anton Abdulbasah Kamil⁸, PhD; Mohammad Meshbahur Rahman⁹, MS

¹Department of Health Education, National Institute of Preventive and Social Medicine, Dhaka, Bangladesh

²Department of Public Health and Hospital Administration, National Institute of Preventive and Social Medicine, Mohakhali, Dhaka, Bangladesh

³Directorate General of Health Services, Ministry of Health & Family Welfare, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh

⁴School of the Environment, Nanjing University, Nanjing, China

⁵Dental Speciality Center, Dhaka, Bangladesh

⁶BRAC James P Grant School of Public Health, BRAC University, Dhaka, Bangladesh

⁷Credit Information Bureau, Bangladesh Bank, Dhaka, Bangladesh

⁸Department of Business Administration, Istanbul Gelisim University, Istanbul, Turkey

⁹Department of Biostatistics, National Institute of Preventive and Social Medicine, Dhaka, Bangladesh

Corresponding Author:

Mohammad Meshbahur Rahman, MS

Department of Biostatistics, National Institute of Preventive and Social Medicine, Dhaka, Bangladesh

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.04.05.24305403v1>

Companion article: <https://med.jmirx.org/2025/1/e70142>

Companion article: <https://med.jmirx.org/2025/1/e70144>

Companion article: <https://med.jmirx.org/2025/1/e59379>

(*JMIRx Med* 2025;6:e70145) doi:[10.2196/70145](https://doi.org/10.2196/70145)

KEYWORDS

mothers' knowledge and practices; oral hygiene; child oral health; Bangladesh

This is the authors' response to peer-review reports for "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study."

Round 1 Review

Reviewer BZ [1]

This is an interesting piece of research [2], which highlights mothers' knowledge and practices regarding their children's oral health in Dhaka City. However, several issues made the study scientifically questionable. The major issues are as follows. The study included mothers from two hospitals in Dhaka City, but the title of the study does not mention this. The sample selection from the mothers visiting the hospitals might not represent general mothers from the whole of Dhaka. Thus, this study might not be generalizable to all mothers in Dhaka City.

Response: The authors are grateful to the reviewers for critically reviewing our manuscript. We agree with the comments. Respondents of this study were the mothers visiting the tertiary-level hospitals of Dhaka City. Generally, the respondents visiting hospitals belonged to all administrative wards (small regions of Dhaka), and it is convenient to get the mothers with children aged 5 - 9 years to interview. That is why we chose tertiary-level hospitals to reach the respondents. However, we revised our manuscript title and omitted "Dhaka" from the title. The new title is "Knowledge and practices towards oral hygiene of children aged 5 - 9 years old: a cross-sectional study among mothers visited tertiary level hospitals."

Introduction

Revise the last paragraph of the Introduction to highlight the study gap in Bangladesh and clearly state the objective of the

study. Use the formal word “mother” and avoid the word “moms.”

Response: We appreciate the reviewer for this comment. We revised the Introduction of our study and replaced the word “Moms” with mother.

Methods

Study Setting and Participants

Give clear reasoning as to why you selected study participants from the hospitals. The last line is confusing. It is not clear whether the participants filled out the questionnaire on their own or they were interviewed by the enumerators.

Response: We are thankful to the reviewer for this comment. Respondents of this study were the mothers visiting the tertiary-level hospitals of Dhaka City. Generally, the respondents who visited hospitals belonged to all administrative wards (small regions of Dhaka), and it was convenient to get this group of mothers with children aged 5 - 9 years to interview. That is why we chose tertiary-level hospitals to reach the respondents. However, we revised our manuscript title and omitted “Dhaka” from the title. We interviewed the respondents, and the sentence was revised in our revised manuscript.

Sampling Technique

Please mention the nonresponse bias for the convenient sampling. Give a short description of the pretesting mentioning the number of samples, period, and location for it.

Response: We are again thankful to the reviewer. While we had a 5% nonresponse rate in our final survey, we found less than 5% (2 of 50 mothers refused to be involved in the study) as the nonresponse rate during pretesting of our study. The description of the pretest has been given in our revised manuscript. In our main survey, the nonresponse rate was 2%.

Measurement of Knowledge and Practice Score

Give the 15 knowledge-related questions and 13 practice-related questions in the supplementary file. Mention if these questions are your own or if you used any valid tools or questions adopted from the relevant previous studies. Give adequate information regarding the scoring system of the variables, mentioning the highest possible aggregated score and examples of two questions (one for knowledge and one for practice).

Response: We again appreciate the reviewer. The knowledge and practice questions have been added to the supplementary file (Supplementary Table S1 and Table S2). Both knowledge and practice questions were adopted from reviewing the literature and revised according to our selection criteria. The summation scoring technique was used in computation, and their descriptive statistics, including percentiles, were observed. Then, both the knowledge and practice scores were classified according to percentile, which is evident in the existing literature (reference added). The range for the knowledge and practice scores was 1-15 and 1-11, respectively. In the main text, the section has been revised accordingly.

Statistical Analyses

The authors mentioned that they used the Mann-Whitney U test and the Kruskal-Wallis test. However, they did not mention the underlying assumptions of the tests. Moreover, the Results section also shows the χ^2 test but is not mentioned in the Methods section. Furthermore, the last line of the Results of the abstract shows the Pearson correlation coefficient, but nothing is mentioned in the Methods or Results section of the entire manuscript.

Response: We apologize for the mistake. Necessary assumptions were checked before performing statistical analysis. The Statistical Analysis section has been revised and mentions the χ^2 test and Pearson correlation coefficient. All the necessary corrections raised by the editor and reviewers have been addressed.

Results

Table 1

It is confusing as the text description of Table 1 and the title of Table 1 are different. It is recommended to use two separate tables: one for socioeconomic variables and another for the frequency distribution of the knowledge level among socioeconomic variables. Mention the knowledge- and practice-related raw scores first and then the cross-tab results. There is a major mistake in the results of Tables 1 and 2. The frequency distribution for educational status, occupation, family type, number of family members, and monthly income in Tables 1 and 2 are the same. However, the P values are different. How is this possible? Please check the results.

Response: Please accept our apology for the error that happened unconsciously. The frequency distribution for educational status, occupation, family type, number of family members, and monthly income in Tables 1 and 2 has been rechecked and revised. In addition, Table 1 has been separated into two tables (Tables 1 and 2) and presented accordingly.

Discussion

It is confusing whether the practice was for the children or how a mother takes care of their children’s dental health. Mention the implications of your findings rather than just comparing the findings with previous studies. State the limitation of the study, especially the bias regarding convenient sampling. Provide a section on the public health significance of the study findings in Bangladesh.

Response: We sincerely appreciate the reviewer for these comments. The Discussion of the manuscript has been revised accordingly. The limitations have been revised in the Discussion section.

Conclusion

The Conclusion section of the study is poorly written and not focused on the findings of the study. Revise the Conclusion section to highlight your study findings.

Response: Thank you again. The Conclusion of the manuscript has been revised accordingly.

Reviewer AJ [3]**Specific Comments**

There were a lot of grammatical issues and typographical errors. The manuscript needs to be edited for grammar and syntax. It is also obvious that the manuscript was not proofread adequately.

Major Comments**Abstract**

- A word is missing in the first sentence. Authors should proofread the manuscript.
- Keywords: Dhaka is a more appropriate keyword than Bangladesh.
- Under the Results in the abstract, respondents should be referred to as such and not as samples.

Introduction

- The global prevalence of oral diseases was stated, but authors did not capture the prevalence in the study area/country and so have not shown that oral disease is a problem. Even the global prevalence that was stated was only that of dental caries among the seven conditions that make up oral diseases as stated by the authors.
- The objective stated here (last sentence) comes off like the authors are assessing the knowledge and practices of oral hygiene with regard to themselves and not their children as stated in the topic.

Methods

- Was it permission that was given by the institutional review board or an ethical clearance?
- This section is quite disorganized. There is a logical flow expected in this section.
- Why was a nonprobability sampling technique (convenient sampling) used for this study? The sampling technique was not explained at all. This will make replicating this study difficult.
- I have an issue with the scoring system and the grading. Is there a reference for it? I particularly have an issue with “moderately average.” It is not a standard term.
- The exclusion criteria are not the opposite of the inclusion criteria as stated by the authors. Exclusion criteria are those already included in the study but that are ineligible for one reason or the other.

Results

- In the text above Table 1, authors wrote that most respondents (39.3%) had a monthly family income of “21,000 - 40,000 taka per month.” This figure (39.3%) is just over one-third of the respondents and not a majority.
- Table 1: What is the meaning of graduation and above? Is it graduated secondary school or graduated college?
- “Respectively” should be added at the end of the following sentence. “Out of 400 mothers, more than 90% knew the importance of brushing teeth while 82.3% and 80.8% of them knew the recommended frequency and appropriate time for brushing teeth.”

Discussion

- The second sentence: the study is not evaluating parent’s knowledge and practices but that of mothers.
- Grammatical errors and missing words

Reference List

- Some of the references were not cited correctly. Authors should adhere to the Vancouver referencing style.

Round 2 Review**Reviewer BZ**

The authors impressively amended the initial version of the manuscript based on the reviewers’ comments. However, several issues remain unaddressed.

1. The authors should include the city in the title of the study. You can revise the title to “Knowledge and practices towards oral hygiene of children aged 5 - 9 years old: a cross-sectional study among mothers visited tertiary level hospitals in Dhaka, Bangladesh.”

Response: Thanks for this suggestion. We revised the title of the manuscript accordingly as “Knowledge and practices towards oral hygiene of children aged 5 - 9 years old: a cross-sectional study among mothers visited tertiary level hospitals in Dhaka, Bangladesh.”

2. Use the full form when it appears first and then use the abbreviation afterward. For example, “KP” in the abstract.

Response: Thanks again for this suggestion. We revised the title of the manuscript accordingly.

3. Please mention this statistical test in the Methods section of the abstract. You did not mention the χ^2 test and Pearson correlation.

Response: Revised the Methods section of the manuscript accordingly as “Statistical analysis including the χ^2 test and Pearson correlation test were performed. The Mann–Whitney *U* test and Kruskal–Wallis one-way ANOVA test were performed to show average knowledge and practice variations among different socio-demographics groups.”

4. It is recommended to make the recommendation simple and easy to understand for the readers. Avoid duplication of the same term.

Response: Revised the Recommendation section accordingly.

5. In the sample size calculation, you used $P=.58$ and $P=.57$. Please clarify why you used those prevalences. Cite the relevant study here.

Response: The Sample Size Calculation section has been revised accordingly as “A convenient sampling technique was followed for this study. During literature search, no study was found that assessed knowledge and practice towards children’s oral hygiene among Bangladeshi mothers. But, a very few studies found in other country with similar socio-demography (eg, India). Mohandas et al, 2021 in his study entitled ‘Knowledge and practice of rural mothers on oral hygiene for

children' showed the prevalence of knowledge and practice were 58% and 57% respectively [4]. The sample size was calculated using the below equation.

$$n = (z^2 pq) / d^2 \dots\dots\dots (1)$$

"the sample size for the mother's knowledge when $P=0.58$ was

$$n = ([1.96]^2 \times 0.58 \times (1 - 0.58)) / [0.05]^2 = 375$$

"Similarly, the sample size for mother's practice level when $P=0.57$ was

$$n = ([1.96]^2 \times 0.57 \times (1 - 0.57)) / [0.05]^2 = 377$$

"Therefore, we initially chose a maximum of 377 as the required sample size. Considering a maximum 5% non-response rate (based on pre-testing), we rounded up this figure and selected 400 as the approximate sample size in the study."

6. Before the heading for the sociodemographic variables in the Methods section, you mention outcome measures. However, the sociodemographic variables are not your outcome variables according to your objectives. You can remove the term outcome measures from here.

Response: The heading "Outcome measure" has been removed from the revised manuscript.

7. You mentioned that you used 13 questions for the assessment of practices. Thus, according to your scoring approach, there should be a score of 1-13, but here, it is 1-11.

Response: Thank you again. We revised the error. The change is "The range for knowledge and practice score was 1 to 15, and 1 to 13 respectively."

8. Please mention the name of the software and version you used for the statistical analysis.

Response: Thank you again. We added the statistical software name with the version as "All the data management and statistical analyses were carried out through IBM SPSS Statistics 25.0."

9. Revise the sentence before Table 1. You can make it two sentences. One for family income and another for occupation.

Response: We revised the sentence accordingly as "Majority of the respondents (39.3%) had the monthly family income of 21000 - 40000 (\$206.19-\$392.73) Taka per month. About 13.3% mothers were involved in any paid worked activities (Table 1)."

10. There is no chi-square-related data in Table 1. Please remove the footnotes from Table 1.

Response: Removed the errors.

11. In Figure 1, it is recommended to keep the values to one decimal point for 1a and 1b.

Response: Thank you for this suggestion. We removed Figures 1c and 1d in our revised manuscript.

12. Please revise the sentence before Table 3 to give a clear meaning.

Response: We revised the sentence accordingly as "The educational status ($P=0.002$) and income ($P=0.044$) were significantly associated with mothers' oral hygiene practices (Table 3)."

13. You can remove the percentage symbol from the value and give it in the vertical axis title.

Response: Removed accordingly.

14. Please give the correlation results in the main manuscript or as a supplementary table.

Response: The correlation results have been given as the supplementary result. Please see Supplementary Result S6.

15. The authors overlooked the association of knowledge and practice with income and family size. Please give more details on those two points in the Discussion section.

Response: The variable family income has been addressed in the Discussion. Please see page 17 (before the Strengths and Limitation section). Family income has been discussed briefly in the Principal Findings section.

Conflicts of Interest

None declared.

References

- Islam MH. Peer review of "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study". JMIRx Med 2025;6:e70144. [doi: [10.2196/70144](https://doi.org/10.2196/70144)]
- Tamannur T, Das SK, Nesa A, et al. Mothers' knowledge of and practices toward oral hygiene of children aged 5-9 years in Bangladesh: cross-sectional study. JMIRx Med 2025;6:e59379. [doi: [10.2196/59379](https://doi.org/10.2196/59379)]
- Nwankwo B. Peer review of "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study". JMIRx Med 2025;6:e70142. [doi: [10.2196/70142](https://doi.org/10.2196/70142)]
- Mohandass B, Chaudhary H, Pal GK, Kaur S. Knowledge and practice of rural mothers on oral hygiene for children. Indian J Continuing Nurs Education 2021;22(1):39-43. [doi: [10.4103/IJCN.IJCN_7_20](https://doi.org/10.4103/IJCN.IJCN_7_20)]

Edited by T Leung; submitted 16.12.24; this is a non-peer-reviewed article; accepted 16.12.24; published 03.02.25.

Please cite as:

Tamannur T, Das SK, Nesa A, Nahar F, Nowshin N, Binty TH, Shakil SA, Kundu SK, Siddik MAB, Rafsun SM, Habiba U, Farhana Z, Sultana H, Kamil AA, Rahman MM

Authors' Response to Peer Reviews of "Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study"

JMIRx Med 2025;6:e70145

URL: <https://xmed.jmir.org/2025/1/e70145>

doi: [10.2196/70145](https://doi.org/10.2196/70145)

© Tahazid Tamannur, Sadhan Kumar Das, Arifatun Nesa, Foijun Nahar, Nadia Nowshin, Tasnim Haque Binty, Shafiul Azam Shakil, Shuvojit Kumar Kundu, Md Abu Bakkar Siddik, Shafkat Mahmud Rafsun, Umme Habiba, Zaki Farhana, Hafiza Sultana, Anton Abdulbasah Kamil, Mohammad Meshbahur Rahman. Originally published in JMIRx Med (<https://med.jmirx.org>), 3.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Authors' Response to Peer Reviews of "Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study"

Abdul Aziz Tayoun, MPH

School of Medicine, Department of Family and Community Medicine, Jordan University, Qween Rania Street, Amman, Jordan

Corresponding Author:

Abdul Aziz Tayoun, MPH

School of Medicine, Department of Family and Community Medicine, Jordan University, Qween Rania Street, Amman, Jordan

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.02.03.24302286v1>

Companion article: <https://med.jmirx.org/2025/1/e71529>

Companion article: <https://med.jmirx.org/2025/1/e71531>

Companion article: <https://med.jmirx.org/2025/1/e57597>

(*JMIRx Med* 2025;6:e71528) doi:[10.2196/71528](https://doi.org/10.2196/71528)

KEYWORDS

periodic health examination; PHE; preventive health services; routine health checkups; Jordan; cross-sectional study

This is the author's response to peer-review reports for "Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study."

Round 1 Review

Anonymous [1]

The following items were noted in this paper [2].

- *Periodic health examination (PHE) uptake: Only 27.1% of participants underwent a PHE in the last 2 years.*
- *Predictors: Significant predictors include recent visits to a primary health care facility, monthly income, and knowledge about PHEs and preventive health measures.*
- *Nonsignificant factors: Gender, marital status, smoking status, and BMI did not show a significant association with PHE uptake.*

Strengths

- *Comprehensive analysis: The study employs a robust methodology, combining descriptive, inferential, and multivariate statistical techniques to provide a thorough understanding of PHE uptake.*
- *Significant predictors identified: Key factors influencing PHE uptake were identified, offering valuable insights for health care providers and policy makers.*
- *First of its kind in Jordan: This study fills a gap in existing knowledge by being the first to investigate PHE uptake in Jordan.*

Negative Points and Areas for Improvement

Cross-Sectional Design

- *Limitation: The study's design limits the ability to establish causality.*
- *Improvement: Future research could benefit from a longitudinal approach to better establish causal relationships between the identified predictors and PHE uptake.*

Response: We acknowledge the limitation of the cross-sectional design in establishing causality and have highlighted this in the Discussion section, suggesting future longitudinal studies.

Convenience Sampling

- *Limitation: This method may introduce selection bias, and the online survey format may lead to measurement bias.*
- *Improvement: Employing a more randomized and stratified sampling method could enhance the representativeness and validity of the findings.*

Response: We have clarified the rationale for using convenience sampling due to resource constraints and have suggested more randomized methods for future studies.

Limited Generalizability

- *Limitation: Results may not be generalizable to populations outside of Jordan or those not included in the sample.*
- *Improvement: Expanding the study to include diverse populations and different geographic regions would provide a more comprehensive understanding of PHE uptake.*

Response: We understand the concern regarding generalizability. However, as the study aimed to estimate PHE uptake and its determinants specifically in Jordan, the focus on this population was intentional. For future research, we recommend conducting multinational studies, particularly in Arab countries, or performing systematic reviews or meta-analyses to obtain results that can be generalized beyond Jordan.

Survey Instrument

- *Limitation: The questionnaire's comprehensiveness and relevance to the Jordanian context might not have been fully ensured.*
- *Improvement: Pretesting the survey with a larger and more varied group, followed by adjustments based on feedback, could improve its applicability and accuracy.*

Response: We have taken steps to improve the relevance and comprehensiveness of the questionnaire by pretesting it and incorporating feedback.

Behavioral Factors

- *Limitation: The study did not find a relationship between behavioral factors and PHE uptake, which contradicts findings in other contexts.*
- *Improvement: A more detailed investigation into cultural and societal influences on health behaviors in Jordan is needed to clarify these results.*

Response: We agree that further investigation into cultural and societal influences on health behaviors in Jordan is needed and have discussed this in the manuscript.

English Language and Clarity

- *Limitation: The manuscript contains some grammatical errors and awkward phrasings, which can detract from its readability.*

- *Improvement: A thorough review and editing for language and clarity by a native English speaker or professional editor would enhance the manuscript's quality.*

Response: The manuscript has undergone a thorough review and editing process to enhance its readability and clarity.

Thank you for these excellent comments. We have thoroughly reviewed and integrated your suggestions into the main manuscript.

Reviewer AV [3]

Specific Comments

Major Comments

1. *In this manuscript, write in detail about the data collection procedure.*

Response: The data collection process was reviewed in detail. Please refer to the Methodology section and note that the questionnaire has been added as an appendix (see Multimedia Appendix 1).

2. *Why was a convenience sampling technique employed?*

Response: A convenience sampling technique was employed due to resource constraints, as the study was not funded and was conducted by a single author. This has been mentioned in the Methodology section.

3. *"All collected data are treated with strict confidentiality." Some language corrections are required.*

Response: We have rephrased the Ethical Consideration section to improve clarity and accuracy.

Minor Comments

There are a lot of formatting issues; many things seem copied and pasted.

Response: We have addressed the formatting issues to ensure consistency and clarity throughout the document.

Conflicts of Interest

None declared.

References

1. Anonymous. Peer review of "Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study". JMIRx Med 2025;6:e71531. [doi: [10.2196/71531](https://doi.org/10.2196/71531)]
2. Tayoun AA. Determinants of periodic health examination uptake: insights from a Jordanian cross-sectional study. JMIRx Med 2025;6:e57597. [doi: [10.2196/57597](https://doi.org/10.2196/57597)]
3. Ahmed A. Peer review of "Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study". JMIRx Med 2025;6:e71529. [doi: [10.2196/71529](https://doi.org/10.2196/71529)]

Abbreviations

PHE: periodic health examination

Edited by T Leung; submitted 20.01.25; this is a non-peer-reviewed article; accepted 20.01.25; published 05.02.25.

Please cite as:

Tayoun AA

Authors' Response to Peer Reviews of "Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study"

JMIRx Med 2025;6:e71528

URL: <https://xmed.jmir.org/2025/1/e71528>

doi: [10.2196/71528](https://doi.org/10.2196/71528)

© Abdul Aziz Tayoun. Originally published in JMIRx Med (<https://med.jmirx.org>), 5.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Author's Response to Peer Reviews of "Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis"

Hojjat Borhany, MSc

Faculty of Environmental Science, Department of Environmental Science, Informatic, and Statistics, University of Ca' Foscari Venice, Mestre (VE), Italy

Corresponding Author:

Hojjat Borhany, MSc

Faculty of Environmental Science, Department of Environmental Science, Informatic, and Statistics, University of Ca' Foscari Venice, Mestre (VE), Italy

Related Articles:

Companion article: <https://www.biorxiv.org/content/10.1101/2023.06.21.545938v2>

Companion article: <https://med.jmirx.org/2025/1/e69895>

Companion article: <https://med.jmirx.org/2025/1/e69896>

Companion article: <https://med.jmirx.org/2025/1/e50458>

(*JMIRx Med* 2025;6:e69894) doi:[10.2196/69894](https://doi.org/10.2196/69894)

KEYWORDS

multistep fermentation; specific methane production; anaerobic digestion; kinetics study; biochar; first-order; modified Gompertz; mass balance; waste management; environment sustainability

This is the author's response to peer-review reports of "Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis."

Round 1 Review

Anonymous [1]

The present manuscript [2] deals with the study of the valorization of organic fractions of municipal solid waste through the production of volatile fatty acids (VFAs) and biogas. The article is interesting; in my opinion, it should be revised.

Comments

1. The presentation of the manuscript is very poor; the figures are not in the same format.

Response: The remaining figures, which included the box plots of VFA concentration, VFA/soluble chemical oxygen demand (SCOD) ratio, scheme of line, VFA and SCOD concentration, VFA weight ratio distribution, capital cost and yearly income, and biomethane content, were kept and reformulated to have the same shape. The figures outlining the kinetics study were deleted.

2. Some of the recent works should be discussed and cited in the Introduction section: [3-7].

Response: Some of the recent relevant works and studies were discussed and cited in the Introduction section as follows:

- Inyang M, Gao B, Pullammanappallil P, Ding W, Zimmerman AR. Biochar from anaerobically digested sugarcane bagasse. *Bioresour Technol.* Nov 2010;101(22):8868-8872. [doi: 10.1016/j.biortech.2010.06.088] [Medline: 20634061]
- Jung S, Shetti NP, Reddy KR, et al. Synthesis of different biofuels from livestock waste materials and their potential as sustainable feedstocks – a review. *Energy Conversion Manage.* May 15, 2021;236:114038. [doi: 10.1016/j.enconman.2021.114038]
- Sampath P, Brijesh, Reddy KR, et al. Biohydrogen production from organic waste – a review. *Chem Eng Technol.* Jul 2020;43(7):1240-1248. [doi: 10.1002/ceat.201900400]
- Algahashm S, Qian S, Hua Y, et al. Properties of biochar from anaerobically digested food waste and its potential use in phosphorus recovery and soil amendment. *Sustainability.* Dec 10, 2018;10(12):4692. [doi:10.3390/su10124692]

3. The novelty of the work should be highlighted.

Response: We noted at the end of the Introduction and at the beginning of the Discussion that this study is novel in that it presents a strong framework for evaluating a proposal for the

financial and technical valorization of organic municipal solid waste using statistical analysis, process kinetics, mass balance, and experimental testing. Furthermore, as compared to single-step anaerobic digestion, our data showed a notably high improvement in profitability and a corresponding decrease in the payback period. In order to further close the cycle circuit and prolong the product life, we also proposed the integration of two potential future units.

4. *Full stops should be removed from all subheadings.*

Response: They are all removed.

5. *The Results and Discussion should be written in detail with proper subheadings.*

Response: The Results section was rewritten and divided into subheadings to mirror their counterparts in the Methods, and the Discussion section has the added subheadings Principal Results, Comparison With Previous Works, and Conclusion and Limitations according to the required information in the guidelines of JMIR Publications.

6. *There are some typo errors; they should be rectified.*

Response: They were corrected.

Reviewer GA [8]

General Comments

Generally, the manuscript should be strictly improved in English language writing and corrected for all grammatical errors throughout the whole manuscript. The author has to use a uniform style of the English language, either American or British English. Further English assistance is particularly required. Many missing articles and a lot of grammatical and punctuation errors must be corrected in the manuscript as in the corrected abstract.

Response: The abstract was prepared in an organized format and corrected for its language. We also employed English assistance. The manuscript's English was improved, and its style was harmonized with American English.

Specific Comments

This paper shows an important aspect of multiple fermentation steps for the complete utilization of municipal solid waste and conversion to useful products, which is highly recommended for circular economic sustainability worldwide. However, it needs some major revision and arrangement to allow for a better presentation of this valuable work.

Major Comments

Title

1. *“Valorization of Organic Fraction of Municipal Solid Waste Through Production of Volatile Fatty Acids (VFAs) and Biogas” is a long title that should be shortened to be more concise with no abbreviations—more indicative. Suggested title: “Valorization of Organic Municipal Solid Waste for Volatile Fatty Acids and Biogas Production.”*

Response: It was adopted according to the guidelines for the descriptive title of the original paper: “Issue/Intervention in

Demographic/Disease/Condition: Method/Study Design”; “Conversion of Organic Municipal Solid Waste to Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies with Statistical Analysis.”

Abstract Section

2. *General language; it must be more concise and specific.*

Response: I did search for all the general language in the manuscript and tried to provide concise information on the matter.

3. *Please clearly mention the take-home message and the main findings of the research.*

Response: The research's primary conclusions include the development of a reliable technique for evaluating the recovery proposal for the conversion of organic solid waste into valuable products and assessing both its technical and financial viability. Furthermore, our proposal outperforms the conventional approaches in terms of economics.

4. *The abstract is too long and lacks the main methodology and main experimental techniques that were carried out in this work. The author may add some hints about the main methods used before mentioning the main results.*

Response: Subheadings for the background, objective, method, findings, and conclusion were added to the revised abstract. There are fewer words in the abstract overall than the 450-word limit. Additionally, some pointers regarding experimental techniques such as gas chromatography are provided, along with the kind of statistical test used to verify the significance and efficacy of the suggested process amendment. We also mentioned the use of mass flow models for the process's economic evaluation and the various kinetics models that can be used to describe biogas production.

Manuscript

5. *Keywords: Words must be modified to be more informative and representative of the research interest and differ from the word in the manuscript title. Maybe add “Multi Step of Fermentation Process” or “Waste Management and Environment Sustainability.”*

Response: We updated the keywords to include “Multistep Fermentation,” “Environment Sustainability,” “Waste management,” “Specific Methane Production,” “Anaerobic Digestion,” “Kinetics Study,” “Biochar,” “First-Order,” “Modified Gompertz,” and “Mass Balance.”

6. *Arrangement of the experimental work in the manuscript may be needed in the Results and Discussion accordingly.*

Response: It was completed in a way that would make it easier for specialists in the field to follow the stages, and a Discussion section was included to compare the findings with earlier research, highlight the key conclusions, and clarify the research's limitations.

7. *There is a lack of figures to describe the main parameter optimization steps well. Please reformulate to describe some data using figures with error bars.*

Response: Our optimization procedure focused on reducing the payback period by decreasing the cost and increasing the profit from bioproducts. This was achieved through pilot tests for examining the effectiveness of the hydraulic retention time (HRT) manipulation and pretreatment in increasing the VFA yield and the integration of our process knowledge of using the fine-tuned feedstock/inoculum ratio as well as biochar addition to obtain the biogas in a cost-effective process. Detailed information and calculations regarding the mass flow analysis are available in the supplementary documents in the Excel spreadsheet named “Mass Balance.”. For figures, we provided the VFA concentrations and distribution for two HRTs and a *t* test to confirm the significance of the results. Further, for biogas production, we provide results from a kinetics study showing an 8-fold increase in the hydrolysis rate and a 100% decrease in the lag phase. This brought about a small anaerobic digester working at a high organic loading rate, leading to a reasonably priced process.

8. *The SD and table footnotes with the number of replicates should be noted underneath all of the given tables.*

Response: For all data that was accompanied by an SD, the number of replicates was reported beneath all the given tables.

9. *A mechanistic in-detail discussion is required, not just comparing your results with the previous work; justify better.*

Response: The comparisons of results from similar studies were done mechanistically and in detail.

For example:

- “Because of the extra pretreatment unit in our study, our VFA yield was significantly higher than the study by valentino et al ”
- “The higher hydrolysis rate was due to the destruction of the solids structure caused by bacterial enzymes and a hot alkaline solution. Additionally, we provided a higher active biomass per feedstock using a fine-tuned FS/IN ratio of 0.3 (VS basis), which was noticeably lower than the quantities (1 and 0.5) reported in similar studies ”
- “due to the added fresh WS with higher digestible content and better nutrient balance than the fermented solids, the SMP value by valentino et al was higher.”
- “The higher practicability than the 2 steps of bioethanol and biogas production as a result of sterilization and high bioethanol concentration requirements.”
- “Our proposal is more favorable since it does not limit the VFA weight ratio distribution and does shifts the recovery route toward higher market-valued products like VFAs than single step AF + AD by Papa et al”

10. *In research articles, do not include any table comparing literature results; the author can discuss the main findings in the text itself, as in Table 5.*

Response: All the data in the tables comparing results were deleted, and we discussed them in the text.

11. *The Conclusions section is missing in the manuscript to summarize and point out the novelty and the main findings from the research.*

Response: The Conclusion was included in the manuscript and presents the main findings as follows: “To conclude, we presented a robust framework to assess a proposal for the valorization of organic waste through experimental tests, statistical analysis, and process kinetics, along with mass and energy flow analysis. The findings support considerably higher profitability and, as a result, a shorter payback period for multistep reclamation than the current single anaerobic digestion. Further, our results encourage the circular economy perspective on the conversion of OMSW into biogas and VFAs, with the pros of fewer residual solids due to reusing them in a pyrolysis line.”

12. *Generally speaking, in academic writing, (1) abstracts do not include abbreviations, (2) avoid articles in the title (the, a, an), and (3) avoid keywords that exist in the title.*

Response: (1) Based on JMIR House Style and Guidelines, the usage of abbreviations and acronyms in the abstract section is not forbidden. Further, all author-invented abbreviations were omitted. We also stop using “AD” as an abbreviation for anaerobic digestion since it may make it ambiguous with “AD” (the reference year). In fact, keeping the number of words in the abstract within the limits is really impossible without using some of them. (2) It was avoided. (3) It was avoided to be as informative as possible.

13. *As a rule of thumb, no dots in titles or subtitles as in the Experimental section, Anerobic Pilot Unities, etc.*

Response: The dots were removed.

14. *Multiple references should be merged, not written separately, as in “29, 30” and “23, 27”; the author may use the merge reference option in reference software.*

Response: It was corrected.

15. *The author may add numbers for all titles and subtitles accordingly all over the manuscript.*

Response: Based on the JMIR guidelines for the author, it is not allowed to use numbering for headings and subheadings.

Minor Comments

16. *The author should avoid general and well-known information, and be selective in the recent references used. May add one small paragraph to the Biological Waste Management and Environment Sustainability section.*

Response: The small paragraph already discussed the current state of municipal organic waste production and treatment in the European Union. We extended it and incorporated all other information regarding environmental sustainability from some relevant sources suggested by the peer reviewer.

17. *The author should clarify the main aim of the work clearly in the last paragraph of the Introduction.*

Response: The main aim of this study was an assessment of multistep pretreatment acidogenic fermentation, followed by anaerobic digestion of municipal organic waste in comparison with the existing method of single anaerobic digestion in terms of financial profit and technical feasibility.

18. *Do not use our, we, or us in academic writing.*

Response: Based on the journal guidelines, there are no issues with using we and us in the article submitted to JMIR Publications; nevertheless, I do my best to avoid overusing these words in my manuscripts.

19. *The author may mention novel applications of VFA and biogas. Mention different novel sources of biogas production.*

Response: It was already mentioned in the study that biogas and VFA typically were used for energy production and biopolymer synthesis, respectively. Moreover, other sources of biogas typically were from nonbiological processes, which were beyond our scope since we focused on carbon-neutral microbiological processes.

20. *The author should mention the gas chromatography type, gas injection rate, column dimensions, and the used carrier gas in the main document.*

Response: It was included in the Methods section.

21. *The author did not mention that flushing with nitrogen or carbon dioxide took place in anaerobic digestion while feeding reactors and how the anaerobic conditions were maintained; please mention it clearly or add the references used for the methodology.*

Response: The anaerobic condition was ensured in bottles just by sealing them after filling without any flushing with nitrogen or carbon dioxide since we had known that the oxygen transfer at the surface of the waste stream was impossible as it contained high total solids and SCOD. This type of procedure was adopted in our lab and has been conducted for years.

22. *Organize titles all over the manuscript.*

23. *Generally, the subtitles are too generic; modify them to be more indicative and precise.*

Response: The subtitles were modified to be more indicative and precise.

24. *“unless Saturday and Sunday” in line 208 is not important information; the suggested word “daily” is enough.*

Response: It was corrected.

25. *“Unite”: Please correct.*

Response: All units are corrected.

26. *Remove the grid lines in the figures.*

Response: They were removed.

27. *The author has to mention the range used for the chemical oxygen demand method, and the original reference should be cited appropriately.*

Response: The method for determination of soluble and solid chemical oxygen demand of the waste stream was according to the Standard Methods for Water and Wastewater. We also clearly discussed in the Methods section a proper limit of detection and reference.

28. *“As can be seen”: This statement is repetitive more than once in the Discussion, in lines 301, 315, and 423.*

Response: Line 301 was corrected. Line 315 was corrected to be informative and avoid repetition. Line 423 was rectified in English language, and the repetitive statements were removed.

29. *Figure 3 caption: Mesophilic fermentation: Please specify which stage because both of the sequential steps were called mesophilic fermentation in Figure 1.*

Response: In fact, Figure 3 depicts the weight ratio distribution from the second step named mesophilic acidogenic fermentation. Surprisingly, the VFA could only be obtained from the second stage. Additionally, we modified the caption to read “VFAs weight ratio distribution for mesophilic acidogenic fermentation” and made a clear reference to Figure 1, which depicts the processes of pretreatment, acidogenic fermentation followed by mesophilic anaerobic digestion. In terms of pH and HRT, the two later procedures differ from one another substantially.

30. *What is the rationale for comparing 3 days to 4.5 days for all the used systems; the author may justify why 4.5 days is better to complete with this HRT in the rest of the experiments or describe the one variable at a time optimization method that is used to determine the significant factors and the insignificant one; mention them clearly. Also, use in the Discussion the terms “significant” and “insignificant” according to the obtained P value.*

Response: The values for the two HRTs to increase the VFA concentration in the outlet were selected based on our experience and process knowledge. According to this information, exceeding the HRT value of more than 3 - 5 days can bring the process into an anaerobic digestion step. As a result, the VFAs with high-added value markets are converted to biogas. Hence, the two HRTs of 3 days and 4.5 days were tried in the pilot test, knowing that the VFA concentration would either increase or decrease linearly in this local region of operation.

31. *The author has to mention tables and figures in the text in their appropriate place.*

Response: They were mentioned where they were referred to.

References

1. Anonymous. Peer review of “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis”. JMIRx Med 2025;6:e69895. [doi: [10.2196/69895](https://doi.org/10.2196/69895)]
2. Borhany H. Converting organic municipal solid waste into volatile fatty acids and biogas: experimental pilot and batch studies with statistical analysis. JMIRx Med 2025;6:e50458. [doi: [10.2196/50458](https://doi.org/10.2196/50458)]
3. Jung S, Shetti NP, Reddy KR, et al. Synthesis of different biofuels from livestock waste materials and their potential as sustainable feedstocks – a review. Energy Convers Manage 2021 May 15;236:114038. [doi: [10.1016/j.enconman.2021.114038](https://doi.org/10.1016/j.enconman.2021.114038)]

4. Srivastava RK, Shetti NP, Reddy KR, Aminabhavi TM. Sustainable energy from waste organic matters via efficient microbial processes. *Sci Total Environ* 2020 Jun 20;722:137927. [doi: [10.1016/j.scitotenv.2020.137927](https://doi.org/10.1016/j.scitotenv.2020.137927)] [Medline: [32208271](https://pubmed.ncbi.nlm.nih.gov/32208271/)]
5. Sampath P, Brijesh, Reddy KR, et al. Biohydrogen production from organic waste – a review. *Chem Eng Technol* 2020 Jul;43(7):1240-1248. [doi: [10.1002/ceat.201900400](https://doi.org/10.1002/ceat.201900400)]
6. Velvizhi G, Goswami C, Shetti NP, Ahmad E, Kishore Pant K, Aminabhavi TM. Valorisation of lignocellulosic biomass to value-added products: paving the pathway towards low-carbon footprint. *Fuel (Lond)* 2022 Apr 1;313:122678. [doi: [10.1016/j.fuel.2021.122678](https://doi.org/10.1016/j.fuel.2021.122678)]
7. Monga D, Shetti NP, Basu S, et al. Engineered biochar: a way forward to environmental remediation. *Fuel (Lond)* 2022 Mar 1;311:122510. [doi: [10.1016/j.fuel.2021.122510](https://doi.org/10.1016/j.fuel.2021.122510)]
8. Elsalamony D. Peer review of “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis”. *JMIRx Med* 2025;6:e69896. [doi: [10.2196/69896](https://doi.org/10.2196/69896)]

Abbreviations

HRT: hydraulic retention time

SCOD: soluble chemical oxygen demand

VFA: volatile fatty acid

Edited by T Leung; submitted 10.12.24; this is a non-peer-reviewed article; accepted 10.12.24; published 04.02.25.

Please cite as:

Borhany H

Author's Response to Peer Reviews of “Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis”

JMIRx Med 2025;6:e69894

URL: <https://xmed.jmir.org/2025/1/e69894>

doi: [10.2196/69894](https://doi.org/10.2196/69894)

© Hojjat Borhany. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 4.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study

Tahazid Tamannur¹, MPH; Sadhan Kumar Das¹, MPH; Arifatun Nesa², MPH; Fojjun Nahar¹, MPH; Nadia Nowshin¹, MPH; Tasnim Haque Binty¹, MPH; Shafiul Azam Shakil², MPH; Shuvojit Kumar Kundu³, MPH; Md Abu Bakkar Siddik⁴, MPH; Shafkat Mahmud Rafsun⁵, MPH; Umme Habiba⁶, MPH; Zaki Farhana⁷, MS; Hafiza Sultana¹, MPhil; Anton Abdulbasah Kamil⁸, PhD; Mohammad Meshbahur Rahman⁹, MS

¹Department of Health Education, National Institute of Preventive and Social Medicine, Dhaka, Bangladesh

²Department of Public Health and Hospital Administration, National Institute of Preventive and Social Medicine, Mohakhali, Dhaka, Bangladesh

³Directorate General of Health Services, Ministry of Health & Family Welfare, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh

⁴School of the Environment, Nanjing University, Nanjing, China

⁵Dental Speciality Center, Dhaka, Bangladesh

⁶BRAC James P Grant School of Public Health, BRAC University, Dhaka, Bangladesh

⁷Credit Information Bureau, Bangladesh Bank, Dhaka, Bangladesh

⁸Department of Business Administration, Istanbul Gelisim University, Istanbul, Turkey

⁹Department of Biostatistics, National Institute of Preventive and Social Medicine, Dhaka, Bangladesh

Corresponding Author:

Mohammad Meshbahur Rahman, MS

Department of Biostatistics, National Institute of Preventive and Social Medicine, Dhaka, Bangladesh

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.04.05.24305403v1>

Companion article: <https://med.jmirx.org/2025/1/e70142>

Companion article: <https://med.jmirx.org/2025/1/e70144>

Companion article: <https://med.jmirx.org/2025/1/e70145>

Abstract

Background: Healthy oral hygiene is crucial for overall health and well-being. Parents' dental care knowledge and practices affect their children's oral health.

Objective: This study examined mothers' knowledge and practices regarding their children's oral hygiene through a cross-sectional survey.

Methods: This cross-sectional survey was conducted from January 1 to December 31, 2022, in Dhaka, Bangladesh. Mothers' knowledge and practices regarding their children's oral hygiene were assessed through a semistructured questionnaire. Statistical analyses, including the χ^2 test and Pearson correlation test, were performed. The Mann-Whitney *U* and Kruskal-Wallis 1-way ANOVA tests were also used to show the average variations in knowledge and practices among different sociodemographic groups.

Results: Of 400 participants, the mean age of mothers was 30.94 (SD 5.15) years, and 388 (97%) were of the Muslim faith, 347 (86.8%) were housewives, and 272 (68%) came from nuclear families. A total of 165 (41.3%) participants showed good knowledge of their children's oral hygiene, followed by 86 (21.5%) showing moderately average knowledge, 75 (18.8%) showing average knowledge, and 74 (18.5%) showing poor knowledge. A total of 182 (45.5%) mothers had children with good oral hygiene practices, followed by mothers with children who had average (n=78, 19.5%), moderately average (n=75, 18.8%), and poor (n=65, 16.3%) oral hygiene practices. The mother's knowledge level was significantly associated with age ($P=.01$), education ($P<.001$), family size ($P=.03$), and monthly income ($P<.001$). On the other hand, educational status ($P=.002$) and income ($P=.04$) were significantly associated with the mother's practices regarding their children's oral hygiene. Nonparametric analysis revealed that mothers who were older (mean knowledge score: 12.13, 95% CI 10.73-13.54 vs 11.21, 95% CI 10.85-11.58; $P=.01$), with a

bachelor's degree or higher (mean knowledge score: 12.93, 95% CI 12.55 - 13.31 vs 9.66, 95% CI 8.95 - 10.37; $P < .001$), who were working mothers (mean knowledge score: 12.30, 95% CI 11.72 - 12.89 vs 11.45, 95% CI 11.17 - 11.73; $P = .03$), and who had a higher family income (mean knowledge score: 12.49, 95% CI 12.0 - 12.98 vs 10.92, 95% CI 10.48 - 11.36; $P < .001$) demonstrated significantly higher levels of oral health knowledge. Conversely, good oral hygiene practices were significantly associated with higher maternal education (mean practice score: 6.88, 95% CI 6.54 - 7.22 vs 6.01, 95% CI 5.63 - 6.40; $P < .001$) and family income (mean practice score: 6.77, 95% CI 6.40 - 7.14 vs 5.96, 95% CI 5.68 - 6.24; $P = .002$). The mother's knowledge was also significantly and positively correlated (Pearson correlation coefficient $r = 0.301$; $P < .001$) with their children's oral hygiene practices, shown by both the Pearson chi-square ($\chi^2 = 25.2$; $P < .001$) test and correlation coefficient.

Conclusions: The mothers' knowledge and their children's oral hygiene practices were inadequate. The mother's age, education level, family size, and monthly income significantly influenced their knowledge level. Children's oral hygiene habits were significantly associated with family income and the mother's educational status. This underscores the need for educational programs, accessible dental care services, oral health education in the curriculum, media and technology involvement in oral health educational campaigns, and proper research and monitoring.

(*JMIRx Med* 2025;6:e59379) doi:[10.2196/59379](https://doi.org/10.2196/59379)

KEYWORDS

mothers' knowledge and practices; oral hygiene; child oral health; Bangladesh

Introduction

According to the World Health Organization, dental caries, periodontal disease, tooth loss, mouth cancer, oro-dental trauma, noma, and congenital defects including cleft lip and palate are classified as oral diseases. Oral health issues are prevalent in low-income nations owing to poor socio-educational-economic circumstances [1]. In terms of general health and well-being, there is a significant connection between oral health and overall health [2,3]. It impacts individuals' capacity to do tasks, communicate, and engage in social interactions. Thus, it has an impact on both the physical and psychological aspects of an individual [4]. Most common oral health problems and conditions can be readily avoided by establishing suitable oral hygiene routines, such as twice daily brushing with the best toothbrush, using fluoride-containing toothpaste, and using the proper brushing technique [5]. Other preventive measures include eating a balanced diet low in free sugar, going to the dentist regularly for exams, and receiving treatment for illnesses when they are still in the early stages [6]. It can be minimized by practicing good oral hygiene habits, such as brushing and flossing teeth and visiting the dentist frequently [7].

Worldwide, over 2 billion individuals have dental caries in their permanent teeth, while 514 million children have dental caries in their primary teeth [8]. Early childhood caries (ECC) in children have been linked mostly to poor dental hygiene. Infants and toddlers with significant plaque accumulation were more likely to experience severe ECC and caries from birth to toddlerhood [9]. ECC has several causes, including excessive sugar intake, poor dental hygiene, inadequate fluoride exposure, and enamel abnormalities [10]. So, the development of caries and the acquisition of infection are substantially influenced by diet and feeding habits.

The children in Bangladesh have various infections and disorders [11-13]. Poor oral health is another prevalent health problem among them, which is still neglected [13]. As parents are the major caregivers, their involvement is crucial in the maintenance and development of excellent oral health in

children, such as teaching healthy eating and drinking habits [14]. In addition, several factors impact the dental health of children, including the mother's level of education, the mother's work situation, and her understanding of oral hygiene [15]. The adoption of good oral health practices in children is influenced by the parents', and particularly the mother's, oral health knowledge, attitudes, and awareness [16]. An Indian study found that the oral hygiene quality of children aged 12 years was shown to be significantly influenced by their mother's oral hygiene knowledge [17]. Children with high rates of dental caries and low rates of fillings were found to have parents with inadequate oral health literacy, according to another study [18]. As a result, it is essential for parents, and particularly mothers, to have awareness about oral health. Scholars argued that a mother's knowledge about oral health and the consequence of adequate dental hygiene has a beneficial impact on their children's dental well-being and adherence to dental care practices [19,20].

Research on dental caries awareness among parents in Pakistan has found low levels of knowledge about oral hygiene standards [21]. A study conducted in India on the oral health status of children aged 3 - 6 years and their mother's oral health-related knowledge, attitude, and practices found most mothers had a medium level of knowledge, an average level of attitude, and a high level of practices regarding oral health [22]. Another study in Malaysia on parental knowledge and practices in preschool children's oral health found that the majority had good knowledge [23]. Numerous studies have been conducted globally regarding parents' or mothers' oral hygiene knowledge and practices, but these have been insufficient, particularly among mothers in Bangladesh. There is a lack of research investigating the extent to which mothers are aware of and follow oral hygiene practices. Hence, this study aimed to assess mothers' level of oral hygiene knowledge and practices regarding their 5- to 9-year-old children.

Methods

This study followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines to prepare the manuscript, and the STROBE checklist is provided in [Multimedia Appendix 1](#).

Ethical Considerations

Permission to conduct this study was given by the institutional review board of the National Institute of Preventive and Social Medicine (NIPSOM), Bangladesh (Ref NIPSOM/IRB/2017/09). The Shaheed Suhrawardy Medical College Hospital and Dhaka Dental College Hospital provided the necessary documentation. Both written and verbal consent were taken before initiating the interview. Participants received an overview of the study's goals, and those who consented were eventually included. No compensation was given to the participants, and data anonymity was strictly maintained.

Study Setting and Participants

This cross-sectional study was conducted from January 1 to December 31, 2022, in two tertiary-level hospitals in Dhaka South named Shaheed Suhrawardy Medical College Hospital and Dhaka Dental College Hospital in Dhaka City. Mothers of children aged 5-9 years who visited these tertiary hospitals were interviewed through a semistructured questionnaire.

Study Pretesting

To observe the overall scenario including questionnaire information, possible sampling techniques, and approximate nonresponse rate in the study, we first performed a pretest of the study. The pretesting was conducted among 50 mothers of children aged 5 - 9 years in the Sapporo Dental College & Hospital located at Dhaka North.

Sampling Technique and Sample Size

A convenience sampling technique was followed for this study. During the literature search, no study was found that assessed the knowledge and practices toward children's oral hygiene among Bangladeshi mothers. However, a study was found from India with a similar sociodemography. Mohandass et al [20] showed that the prevalence of adequate knowledge and practices were 58% and 57%, respectively. The sample size for this study was calculated using the below equation.

$$(1)n=z^2pq/d^2$$

The sample size when $P=.58$ for the mother's knowledge was:

$$n=1.962 \times 0.58 \times (1-0.58) / 0.052^2 = 375$$

Similarly, the sample size when $P=.57$ for the mother's practice level was:

$$n=1.962 \times 0.57 \times (1-0.57) / 0.052^2 = 377$$

Therefore, we initially chose a maximum of 377 as the required sample size. Considering a maximum 5% nonresponse rate (based on pretesting), we rounded up this figure and selected 400 as an approximate sample size for the study.

Selection Criteria

The inclusion criteria for this study were mothers of Bangladeshi nationality who were living in Dhaka for at least 1 year, mothers of children aged 5 - 9 years, and mothers who provided consent and agreed to participate in the study. The exclusion criteria for the study were mothers who were not Bangladeshi but currently living in Dhaka, mothers of children older than 10 years or younger than 5 years, and mothers younger than 21 years or older than 48 years.

Sociodemographic Variables

Respondents' sociodemographic variables such as age (21-48 years), religion (Muslim, non-Muslim), educational status (up to primary, secondary, higher secondary, and bachelor's degree or higher), occupational status (housewife, working), family type (nuclear, joint), family size (<5 persons, ≥5 persons), and monthly family income (≤20,000 BDT, 20,001 - 40,000 BDT, ≥40,001 BDT; a currency exchange rate of 101.85 BDT=US \$1 was used) were the independent variables in this study.

Measurement of Knowledge and Practice

The study used 15 variables to assess mothers' knowledge and 13 to assess their children's practices related to oral hygiene ([Multimedia Appendices 2 and 3](#)). Both knowledge and practice questions were adopted from the existing literature and revised according to our selection criteria. The summation scoring technique was used in computation, and the descriptive statistics, including percentiles, were observed. The range for the knowledge and practice scores were 1-15 and 1-13, respectively. According to the percentile approach, knowledge was classified into four levels: poor (<25% percentile cut point: ≤9.999), moderately average (25% - 49% percentile cut point: 10.0 - 11.99), average (50% - 74% percentile cut point: 12.0 - 12.99), and good knowledge (≥75% percentile cut point: ≥13.0) [24]. Practices were also classified into four levels: poor (<25% percentile cut point: ≤4.99), moderately average (25% - 49% percentile cut point: 5.0 - 5.999), average (50% - 74% percentile cut point: 6.0 - 6.99), and good practices (≥75% percentile cut point: ≥7.0). For all cases, the cut points were statistically evident [25,26].

Data Quality Control

To ensure the reliability and validity of the study findings, we observed the reliability analysis for both knowledge and practice variables, yielding a Cronbach α ; the reliability coefficient values for the variables related to knowledge and practice were found to be 0.78 and 0.81, indicating acceptable internal consistency.

Statistical Analysis

Descriptive statistics were performed to present participants' sociodemographic characteristics and mean knowledge and practice scores. The Pearson χ^2 test and Pearson correlation coefficient were used as a bivariate analysis. Since both knowledge and practice scores did not follow normality, we performed the Mann-Whitney U test and Kruskal-Wallis 1-way ANOVA test to show the mean knowledge and practice score variations between two (eg, housewife vs working mother) and more than two groups (eg, different age groups), respectively.

Necessary assumptions were checked before performing the statistical analysis. All the data management and statistical analyses were carried out through SPSS Statistics 27.0 (IBM Corp). The *P* value was observed for all the cases at a 5% level, and 95% was considered as the CI [27-29].

Results

Sociodemographic Characteristics of the Respondents

The majority of the respondents (n=209, 52.3%) were within the 21 - 30 years age group, followed by 44% (n=176) in the

31 - 40 years age group. Most (n=57, 39.3%) respondents had a secondary level of education. Most were Muslims (n=388, 97%) and housewives (n=347, 86.8%). Many of the respondents (n=157, 39.3%) had a monthly family income of 20,001 - 40,000 BDT (US \$206.19-\$392.73) per month. About 13.3% (n=53) of mothers were working (Table 1).

Table . Distribution of sociodemographic characteristics of the respondents (N=400).

Characteristics	Respondents, n (%)
Age group (years)	
21 - 30	209 (52.3)
31 - 40	176 (44.0)
41 - 48	15 (3.8)
Religion	
Muslim	388 (97.0)
Non-Muslim	12 (3.0)
Educational status	
Up to primary	76 (19.0)
Secondary	157 (39.3)
Higher secondary	68 (17.0)
Bachelor's degree or higher	99 (24.8)
Occupation	
Housewife	347 (86.8)
Working	53 (13.3)
Family type	
Nuclear	272 (68.0)
Joint	128 (32.0)
Number of family members	
<5 persons	193 (48.3)
≥5 persons	207 (51.8)
Monthly family income (BDT) ^a	
≤20,000	143 (35.8)
20,001 - 40,000	157 (39.3)
≥40,001	100 (25.0)

^aA currency exchange rate of 101.85 BDT=US \$1 was used.

Knowledge Among Mothers Regarding Their Children's Oral Hygiene

Multimedia Appendix 4 shows the mothers' knowledge scores regarding their children's oral hygiene. Among the 400 mothers, more than 90% (n=360) knew the importance of brushing teeth, while 82.3% (n=329) and 80.8% (n=323) knew the recommended frequency and appropriate time for brushing teeth, respectively. Surprisingly, only 29.5% (n=118) and 38.5%

(n=154) knew the duration for brushing teeth and that fluoride protects against caries, respectively. However, most of the respondents knew about the "importance of cleaning tongue" (n=365, 91.3%), "gingival disease common cause of gum bleeding" (n=286, 71.5%), "brushing and flossing protect against bleeding gum" (n=243, 60.8%), "yellow coating plaque" (n=362, 90.5%), "sugary item cause caries" (n=387, 96.8%), "soft drinks cause caries" (n=295, 73.8%), and "regular brushing protects against caries" (n=380, 95%).

Mothers' Practices Regarding Their Children's Oral Hygiene

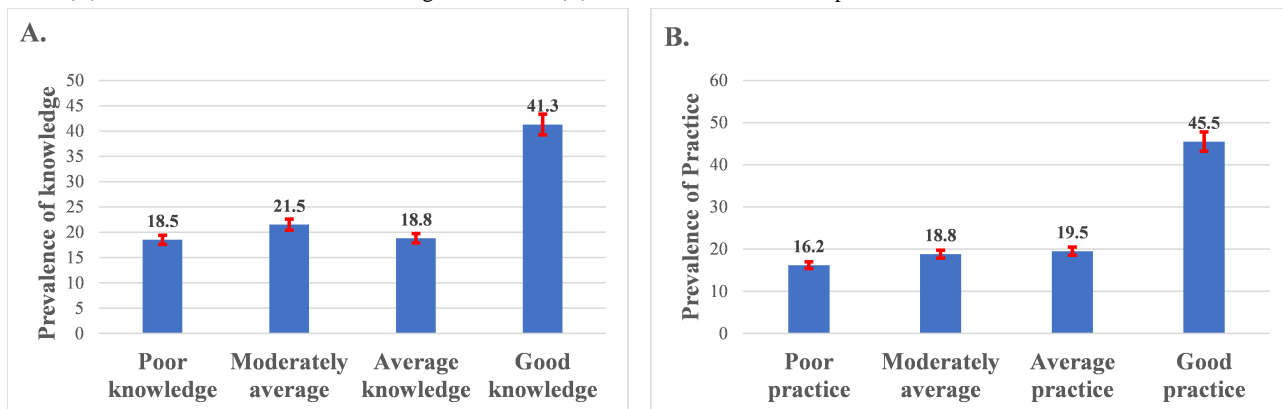
Multimedia Appendix 5 shows the individual distribution of mothers' practices regarding their children's oral hygiene. Most (n=381, 95.3%) of the mothers reported that their child brushed their teeth regularly, 99% (n=396) of children used a toothbrush, 62% (n=248) changed their toothbrush every 3 - 4 months or if the bristles were frayed, 97.8% (n=391) used their toothpaste, and 77.8% (n=311) rinsed their mouth after eating. Surprisingly, 44.3% (n=177) of children brushed their teeth twice daily, 42% (n=168) cleaned their tongues, and 2.8% (n=11) used floss.

Only 12.5% (n=50) were given sugary items with meals, and 0.3% (n=1) were taken to dentists every 6 months.

Overall Knowledge and Practice Levels of the Respondents

Figure 1 depicts the level of knowledge and practices of mothers regarding their children's oral hygiene and the association with the mother's educational status. Only 41.3% (n=165) had good knowledge, while 18.5% (n=74) had poor knowledge (Figure 1A). Similarly, only 45.5% (n=182) of the mothers showed good practices, while 16.2% (n=65) showed poor practice levels (Figure 1B).

Figure 1. (A) Distribution of the overall knowledge of mothers. (B) Distribution of the overall practices of mothers.



Sociodemographic Variations in the Mother's Knowledge Level Regarding Their Children's Oral Hygiene

A total of 66.7% (10/15) of mothers aged 41-48 years had good knowledge regarding their children's oral hygiene. The Pearson

χ^2 association test revealed that mothers' knowledge levels were significantly associated with age ($P=.01$), education ($P<.001$), family size ($P=.03$), and monthly income ($P<.001$; Table 2).

Table . Association of mothers' knowledge with sociodemographic characteristics.

Characteristics	Poor knowledge, n (%)	Moderately average, n (%)	Average knowledge, n (%)	Good knowledge, n (%)	P value ^a
Age group (years)					.01
21 - 30 (n=209)	43 (20.6)	55 (26.3)	39 (18.7)	72 (34.4)	
31 - 40 (n=176)	28 (15.9)	29 (16.5)	36 (20.5)	83 (47.2)	
41 - 48 (n=15)	3 (20.0)	2 (13.3)	0 (0.0)	10 (66.7)	
Religion					.22
Muslim (n=388)	72 (18.6)	86 (22.2)	72 (18.6)	158 (40.7)	
Non-Muslim (n=12)	2 (16.7)	0 (0.0)	3 (25.0)	7 (58.3)	
Educational status					<.001
Up to primary (n=76)	32 (42.1)	22 (28.9)	8 (10.5)	14 (18.4)	
Secondary (n=157)	29 (18.5)	46 (29.3)	33 (21.0)	49 (31.2)	
Higher secondary (n=68)	8 (11.8)	8 (11.8)	16 (23.5)	36 (52.9)	
Bachelor's degree or higher (n=99)	5 (5.1)	10 (10.1)	18 (18.2)	66 (66.7)	
Occupation					.10
Housewife (n=347)	68 (19.6)	77 (22.2)	67 (19.3)	135 (38.9)	
Working (n=53)	6 (11.3)	9 (17.0)	8 (15.1)	30 (56.6)	
Family type					.06
Nuclear (n=272)	46 (16.9)	52 (19.1)	59 (21.7)	115 (42.3)	
Joint (n=128)	28 (21.9)	34 (26.6)	16 (12.5)	50 (39.1)	
Number of family members					.03
<5 persons (n=193)	27 (14.0)	36 (18.7)	39 (20.2)	91 (47.2)	
≥5 persons (n=207)	47 (22.7)	50 (24.2)	36 (17.4)	74 (35.7)	
Monthly family income (BDT) ^b					<.001
≤20,000 (n=143)	38 (26.6)	37 (25.9)	25 (17.5)	43 (30.1)	
20,001 - 40,000 (n=157)	26 (16.6)	33 (21.0)	37 (23.6)	61 (38.9)	
≥41,001 (n=100)	10 (10.0)	16 (16.0)	13 (13.0)	61 (61.0)	

^a χ^2 /Fisher exact test.

^bA currency exchange rate of 101.85 BDT=US \$1 was used.

Sociodemographic Variation of the Mother's Practice Level Regarding Their Children's Oral Hygiene

Table 3 represents the association between mothers' sociodemographic characteristics and their practices regarding their children's oral hygiene. The analysis found that more than

half (n=8, 53.3%) of older-aged mothers had good practices, and 66.7% (n=60) of mothers with a bachelor's degree or higher showed good practices regarding their children's oral hygiene. The educational status ($P=.002$) and income ($P=.04$) were significantly associated with the mothers' practices regarding their children's oral hygiene (Table 3).

Table . Association between sociodemographic characteristics and practice level regarding their children's oral hygiene.

Characteristics	Poor practice, n (%)	Moderately average, n (%)	Average practice, n (%)	Good practice, n (%)	P value ^a
Age group (years)					.34
21 - 30 (n=209)	34 (16.3)	44 (21.1)	46 (22.0)	85 (40.7)	
31 - 40 (n=176)	30 (17.0)	27 (15.3)	30 (17.0)	89 (50.6)	
41 - 48 (n=15)	1 (6.7)	4 (26.7)	2 (13.3)	8 (53.3)	
Religion of the respondents					.42
Muslim (n=388)	65 (16.8)	73 (18.8)	76 (19.6)	174 (44.8)	
Non-Muslim (n=12)	0 (0.0)	2 (16.7)	2 (16.7)	8 (66.7)	
Educational status of the respondent					.002
Up to primary (n=76)	15 (19.7)	19 (25.0)	6 (7.9)	36 (47.4)	
Secondary (n=157)	27 (17.2)	34 (21.7)	41 (26.1)	55 (35.0)	
Higher secondary (n=68)	12 (17.6)	12 (17.6)	13 (19.1)	31 (45.6)	
Bachelor's degree or higher (n=99)	11 (11.1)	10 (10.1)	18 (18.2)	60 (60.6)	
Occupation of the respondent					.24
Housewife (n=347)	60 (17.3)	68 (19.6)	65 (18.7)	154 (44.4)	
Working (n=53)	5 (9.4)	7 (13.2)	13 (24.5)	28 (52.8)	
Family type of the respondent					.98
Nuclear (n=272)	43 (15.8)	51 (18.8)	54 (19.9)	124 (45.6)	
Joint (n=128)	22 (17.2)	24 (18.8)	24 (18.8)	58 (45.3)	
Number of family members					.93
<5 persons (n=193)	30 (15.5)	38 (19.7)	36 (18.7)	89 (46.1)	
≥5 persons (n=207)	35 (16.9)	37 (17.9)	42 (20.3)	93 (44.9)	
Monthly family income (BDT) ^b					.04
≤20,000 (n=143)	30 (21.0)	30 (21.0)	28 (19.6)	55 (38.5)	
20,001 - 40,000 (n=157)	22 (14.0)	31 (19.7)	35 (22.3)	69 (43.9)	
≥41,001 (n=100)	13 (13.0)	14 (14.0)	15 (15.0)	58 (58.0)	

^a χ^2 /Fisher exact test significant level.

^bA currency exchange rate of 101.85 BDT=US \$1 is applicable.

Variation in Knowledge and Practices of the Respondents

A significant difference in respondents' knowledge and practices with sociodemographic characteristics was observed (Table 4). The analysis found that the knowledge was comparatively higher among mothers of higher age groups compared to lower age

groups (mean knowledge score: 12.13, 95% CI 10.73-13.54 vs 11.23, 95% CI 10.85-11.58; $P=.01$). Similarly, both the knowledge and practice behaviors were significantly higher among mothers with higher education and income than their counterparts. In addition, working mothers and mothers with small families had significantly higher knowledge (Table 4).

Table . Knowledge and practice variation of mothers according to sociodemographic characteristics.

Characteristics	Knowledge score (range 1-15), mean (95% CI)	<i>P</i> value ^a	Practice score (range 1-13), mean (95% CI)	<i>P</i> value ^a
Age group (years)		.01		.21
21 - 30 (n=209)	11.21 (10.85 - 11.58)		6.13 (5.92 - 6.35)	
31 - 40 (n=176)	11.93 (11.56 - 12.29)		6.36 (6.09 - 6.62)	
41 - 48 (n=15)	12.13 (10.73 - 13.54)		6.8 (5.67 - 7.93)	
Religion		.22		.19
Muslim (n=388)	11.54 (11.28 - 11.80)		6.24 (6.07 - 6.41)	
Non-Muslim (n=12)	12.25 (10.53 - 13.97)		6.83 (6.08 - 7.59)	
Educational status		<.001		<.001
Up to primary (n=76)	9.66 (8.95 - 10.37)		6.01 (5.63 - 6.40)	
Secondary (n=157)	11.32 (10.97 - 11.67)		6.01 (5.75 - 6.27)	
Higher secondary (n=68)	12.26 (11.71 - 12.82)		6.19 (5.79 - 6.59)	
Bachelor's degree or higher (n=99)	12.93 (12.55 - 13.31)		6.88 (6.54 - 7.22)	
Occupation		.03		.12
Housewife (n=347)	11.45 (11.17 - 11.73)		6.21 (6.02 - 6.39)	
Working (n=53)	12.30 (11.72 - 12.89)		6.59 (6.19 - 6.98)	
Family type		.13		.88
Nuclear (n=272)	11.7 (11.39 - 12.00)		6.25 (6.05 - 6.45)	
Joint (n=128)	11.28 (10.81 - 11.75)		6.28 (5.98 - 6.59)	
Number of family members		<.001		.95
<5 persons (n=193)	11.96 (11.6 - 12.32)		6.27 (6.03 - 6.51)	
≥5 persons (n=207)	11.19 (10.84 - 11.55)		6.25 (6.01 - 6.48)	
Monthly family income (BDT) ^b		<.001		.002
≤20,000 (n=143)	10.92 (10.48 - 11.36)		5.96 (5.68 - 6.24)	
20,001 - 40,000 (n=157)	11.56 (11.17 - 11.95)		6.20 (5.96 - 6.45)	
≥40,001 (n=100)	12.49 (12.0 - 12.98)		6.77 (6.40 - 7.14)	

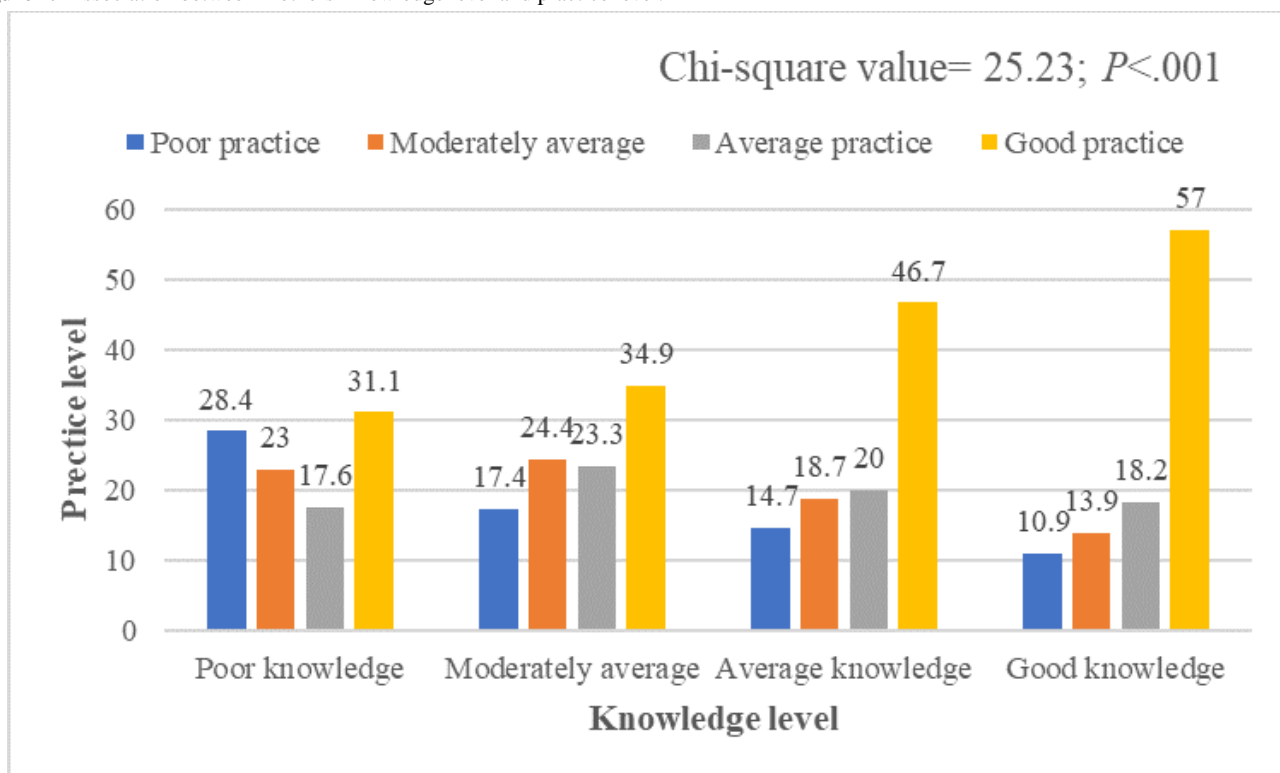
^aMann-Whitney *U* test and Kruskal-Wallis 1-way ANOVA test.

^bA currency exchange rate of 101.85 BDT=US \$1 is applicable.

Association Between Mothers' Oral Hygiene Knowledge and Practice Levels

Figure 2 represents the association between mothers' oral hygiene knowledge and practice levels. Over 50% of mothers

with good knowledge had good practice behaviors regarding their children's oral hygiene. The Pearson correlation coefficient analysis also found a significant and positive association ($r=0.301$; $P<.001$) between the knowledge and practice scores of the respondents (Multimedia Appendix 6).

Figure 2. Association between mothers' knowledge level and practice level.

Discussion

Principal Findings

Oral health is an integral component of overall health, and it is important in our everyday lives. This study intended to evaluate mothers' knowledge and practices regarding their children's oral hygiene. An increased knowledge level was observed among older mothers, those with higher education levels, working mothers, and mothers from higher income groups. Similarly, good practices regarding children's oral hygiene were associated with the mother's education level and economic status.

Comparison to Prior Work

To maintain oral health, brushing twice a day is standard [30]. The study found that most mothers know the standard brushing recommendation for their children. Many mothers also agreed that gingival disease was the most common cause of gum bleeding, and brushing and flossing could protect against bleeding gums. The findings align with the existing literature [31]. If one wants to protect themselves against any kind of dental sickness, brushing regularly is required [32]. Over 50% of the mothers in our study agreed with this statement, which is comparable to existing research findings [33]. In this study, less than half of the mothers had good knowledge regarding their children's oral hygiene, and nearly 1 in every 5 mothers had poor knowledge. The findings suggest that health education programs among mothers regarding their children's oral hygiene are needed. Various education and awareness programs, including television, social and mass media campaigns, and community-based educational interventions may improve mothers' knowledge regarding children's oral hygiene [34-36].

In this study, the mother's knowledge regarding their children's oral hygiene was significantly associated with their age, and mothers in the higher age group had comparatively higher knowledge than those in the lower age group. The finding is comparable to many studies that suggest oral health educational programs for younger mothers [34,37,38]. The mother's educational status and monthly family income were two important predictors for increasing their children's oral hygiene knowledge and practices. Parents with higher education were more aware of their children's dental health [39,40]. Our research results align with the existing literature that indicates that mothers who have attained a university degree possess superior knowledge about oral health in comparison to those with a lower level of education [41]. This might be rationalized by the deduction that women with a lower level of education may lack awareness about the consequences of probable risk factors linked to the progression of oral disorders. Consequently, health awareness and promotion play a vital role for mothers who have inadequate educational backgrounds [40,42,43]. Our results align with the existing research, which demonstrates that mothers with extensive knowledge tend to promote good oral health habits in their children [25].

Strengths and Limitations

This study aimed to identify the variables that impact oral hygiene habits among mothers and evaluate their level of knowledge and compliance with oral hygiene practices. The primary merit of this study is the results. We identified the variables that influence individuals' understanding and behaviors related to oral hygiene. We experienced a few limitations during this study. First, this was cross-sectional research, which lacks strength in cause-effect analysis. Second, the study was conducted among mothers visiting tertiary-level hospitals in

Dhaka. Therefore, there is a chance of nonresponse bias due to convenience sampling.

Future Directions

Maintaining good oral hygiene is crucial for every child's overall health; mothers, in particular, play a vital role in this regard. Based on our study findings, the following recommendations may help enhance maternal knowledge and improve children's oral hygiene practices.

Educational Workshops and School-Based Initiatives

Community-based educational programs including workshops and seminars may help educate mothers of different age groups [34,40]. These workshops should focus on the importance of oral hygiene, practical tips for maintaining children's oral health, and common misconceptions. Monthly informational sessions on oral hygiene practices facilitated by dental health professionals and community health centers could play an important role in improving children's oral hygiene practices. Various school-based initiatives, like partnering with schools to offer regular seminars and distributing informative materials to parents during parent-teacher meetings that emphasize the critical role of oral hygiene from an early age, could be implemented [37].

Incorporate Oral Health Education Into the Curriculum

Integration of basic oral health education into the curriculum of early childhood education programs, ensuring that children learn about oral hygiene from a young age, may help children improve their oral hygiene practices [39,44]. Various programs within schools that encourage parental involvement in learning about and practicing good oral hygiene, and providing resources and support for mothers to reinforce these practices at home may help children improve their oral hygiene practices [44].

Media and Technology Use

Launching social media campaigns targeting mothers; using platforms like Facebook, Instagram, and YouTube to disseminate information on children's oral hygiene; and featuring engaging content such as infographics, videos, and interactive question-and-answer sessions with dental professionals could also be influential initiatives [35].

Research and Monitoring

Support should also be provided for ongoing research to monitor the effectiveness of these initiatives and to identify new trends and needs related to children's oral hygiene [45]. Establishing feedback mechanisms, such as surveys and focus groups, can help gather insights from mothers on the effectiveness of current programs and identify areas for improvement.

Conclusion

This study revealed that mothers' knowledge and practices regarding their children's oral health were insufficient. The mother's age, education level, family size, and monthly income significantly influenced their knowledge level. Children's oral hygiene habits were significantly associated with family income and the mother's educational status. Women aged 41-48 years with a bachelor's degree or higher, from higher socioeconomic backgrounds, and with school-aged children demonstrated significantly higher levels of knowledge. Mothers with higher socioeconomic status and more education demonstrated a much higher level of dental hygiene practices for their children. The mother's knowledge regarding their children's oral hygiene had positive effects and significantly correlated with their children's oral hygiene practices. The findings of this study emphasize the need for educational and school-based initiatives, accessible dental care services, oral health education in the curriculum, media and technology involvement in oral health educational campaigns, and proper research and monitoring.

Acknowledgments

We acknowledge the Department of Biostatistics, National Institute of Preventive and Social Medicine for their technical support during the study. We are also grateful to all participants included in this study. We are thankful to the Supporo Dental College and Hospital for their administrative support during data collection.

Data Availability

The datasets generated or analyzed during this study were deposited onto figshare [46].

Authors' Contributions

Conceptualization: TT, MMR, HS

Formal analysis: TT, MMR

Investigation: TT, SKD, AN, FN, NN, THB, SAS, SKK, MABS, SMR, UH, ZF, MMR

Methodology: TT, MMR

Project administration: TT, MMR

Supervision: MMR

Funding acquisition: TT, SKD, FN, NN, THB, SAS, SKK, SMR, UH, ZF, AAK, MMR

Validation: HS, AAK, MMR

Visualization: TT, SKD, MMR

Writing - original draft: TT, SKD, MMR

Investigation: TT, SKD, AN, FN, NN, THB, SAS, SKK, MABS, SMR, UH, ZF, HS, AAK, MMR

Writing - review and editing: TT, SKD, AN, FN, NN, THB, SAS, SKK, MABS, SMR, UH, ZF, HS, AAK, MMR

Conflicts of Interest

None declared.

Multimedia Appendix 1

STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement: checklist of items that should be included in reports of cross-sectional studies.

[PDF File, 76 KB - [xmed_v6i1e59379_app1.pdf](#)]

Multimedia Appendix 2

List of variables used to assess mothers' knowledge regarding their children's oral hygiene.

[DOCX File, 14 KB - [xmed_v6i1e59379_app2.docx](#)]

Multimedia Appendix 3

List of variables used to assess mothers' practices regarding their children's oral hygiene.

[DOCX File, 14 KB - [xmed_v6i1e59379_app3.docx](#)]

Multimedia Appendix 4

Mothers' individual knowledge regarding their children's oral hygiene.

[DOCX File, 14 KB - [xmed_v6i1e59379_app4.docx](#)]

Multimedia Appendix 5

Mothers' individual practices regarding their children's oral hygiene.

[DOCX File, 14 KB - [xmed_v6i1e59379_app5.docx](#)]

Multimedia Appendix 6

Correlation between knowledge and practice scores.

[DOCX File, 13 KB - [xmed_v6i1e59379_app6.docx](#)]

References

1. Global oral health status report: towards universal health coverage for oral health by 2030. World Health Organization. 2022 Nov 18. URL: <https://www.who.int/publications/i/item/9789240061484> [accessed 2025-01-13]
2. Alama MA, Abdullah US, Mofiz M, Aktar S, Karim M. Oral health status among the under five children attending at OPD of Dhaka Dental College Hospital. Update Dent Coll J 2016 Apr 7;5(2):9-17. [doi: [10.3329/updcj.v5i2.27263](https://doi.org/10.3329/updcj.v5i2.27263)]
3. Haque MF, Rahman MM, Alif SM, et al. Estimation and prediction of doubling time for COVID-19 epidemic in Bangladesh: a study of first 14 month's daily confirmed new cases and deaths. Global Biosecurity 2021 Apr 26;3(1). [doi: [10.31646/gbio.91](https://doi.org/10.31646/gbio.91)]
4. Shah PM, Jeevanadan G. Oral hygiene maintenance in children - a survey on parental awareness. Int J Pharm Res 2017;9(3). [doi: [10.31838/ijpr/2020.12.01.311](https://doi.org/10.31838/ijpr/2020.12.01.311)]
5. Toothbrushes. American Dental Association. 2019. URL: <https://www.ada.org/resources/research/science-and-research-institute/oral-health-topics/toothbrushes> [accessed 2022-11-27]
6. Lawal FB, Fagbule OF, Akinloye SJ, Lawal TA, Oke GA. Impact of oral hygiene habits on oral health-related quality of life of in-school adolescents in Ibadan, Nigeria. Front Oral Health 2022 Sep 9;3:979674. [doi: [10.3389/froh.2022.979674](https://doi.org/10.3389/froh.2022.979674)] [Medline: [36338573](https://pubmed.ncbi.nlm.nih.gov/36338573/)]
7. Saadaldina SA, Eldwakhly E, Alnazzawi AA, et al. Awareness and practice of oral health measures in Medina, Saudi Arabia: an observational study. Int J Environ Res Public Health 2020 Dec 6;17(23):1-10. [doi: [10.3390/ijerph17239112](https://doi.org/10.3390/ijerph17239112)] [Medline: [33291281](https://pubmed.ncbi.nlm.nih.gov/33291281/)]
8. Oral health. World Health Organization. 2023. URL: https://www.who.int/health-topics/oral-health#tab=tab_1 [accessed 2025-01-20]
9. Duangthip D, Chu CH. Challenges in oral hygiene and oral health policy. Front Oral Health 2020 Oct 7;1:575428. [doi: [10.3389/froh.2020.575428](https://doi.org/10.3389/froh.2020.575428)] [Medline: [35047981](https://pubmed.ncbi.nlm.nih.gov/35047981/)]
10. Anil S, Anand PS. Early childhood caries: prevalence, risk factors, and prevention. Front Pediatr 2017 Jul 18;5:157. [doi: [10.3389/fped.2017.00157](https://doi.org/10.3389/fped.2017.00157)] [Medline: [28770188](https://pubmed.ncbi.nlm.nih.gov/28770188/)]
11. Hossain MS, Tasnim S, Chowdhury MA, Chowdhury FIF, Hossain D, Rahman MM. Under - five children's acute respiratory infection dropped significantly in Bangladesh: an evidence from Bangladesh demographic and health survey, 1996–2018. Acta Paediatr 2022 Oct;111(10):1981-1994. [doi: [10.1111/apa.16447](https://doi.org/10.1111/apa.16447)] [Medline: [35678484](https://pubmed.ncbi.nlm.nih.gov/35678484/)]

12. Islam GMR, Rahman MM, Hasan MI, Tadesse AW, Hamadani JD, Hamer DH. Hair, serum and urine chromium levels in children with cognitive defects: a systematic review and meta-analysis of case control studies. *Chemosphere* 2022 Mar;291(Pt 2):133017. [doi: [10.1016/j.chemosphere.2021.133017](https://doi.org/10.1016/j.chemosphere.2021.133017)] [Medline: [34813844](https://pubmed.ncbi.nlm.nih.gov/34813844/)]
13. Sony SA, Haseen F, Islam SS, Chowdhury SF. Knowledge and practice of oral health and hygiene and oral health status among school going adolescents in a rural area of Sylhet District. *Community Based Med J* 2022 Jan 10;10(1):30-36. [doi: [10.3329/cbmj.v10i1.58642](https://doi.org/10.3329/cbmj.v10i1.58642)]
14. Mohammadi TM, Hajizamani A, Bozorgmehr E. Improving oral health status of preschool children using motivational interviewing method. *Dent Res J (Isfahan)* 2015;12(5):476-481. [doi: [10.4103/1735-3327.166231](https://doi.org/10.4103/1735-3327.166231)] [Medline: [26604963](https://pubmed.ncbi.nlm.nih.gov/26604963/)]
15. Holm AK. Caries in the preschool child: international trends. *J Dent* 1990 Dec;18(6):291-295. [doi: [10.1016/0300-5712\(90\)90125-x](https://doi.org/10.1016/0300-5712(90)90125-x)] [Medline: [2074302](https://pubmed.ncbi.nlm.nih.gov/2074302/)]
16. Al-Batayneh OB, Al-Khateeb HO, Ibrahim WM, Khader YS. Parental knowledge and acceptance of different treatment options for primary teeth provided by dental practitioners. *Front Public Health* 2019 Nov 7;7:322. [doi: [10.3389/fpubh.2019.00322](https://doi.org/10.3389/fpubh.2019.00322)] [Medline: [31788466](https://pubmed.ncbi.nlm.nih.gov/31788466/)]
17. Chand S, Chand S, Dhanker K, Chaudhary A. Impact of mothers' oral hygiene knowledge and practice on oral hygiene status of their 12-year-old children: a cross-sectional study. *J Indian Assoc Public Health Dent* 2014;12(4):323-329. [doi: [10.4103/2319-5932.147681](https://doi.org/10.4103/2319-5932.147681)]
18. Khodadadi E, Niknahad A, Sistani MMN, Motallebnejad M. Parents' oral health literacy and its impact on their children's dental health status. *Electron Physician* 2016 Dec 25;8(12):3421-3425. [doi: [10.19082/3421](https://doi.org/10.19082/3421)] [Medline: [28163858](https://pubmed.ncbi.nlm.nih.gov/28163858/)]
19. Al-Zahrani AM, Al-Mushayt AS, Otaibi MF, Wyne AH. Knowledge and attitude of Saudi mothers towards their preschool children's oral health. *Pak J Med Sci* 2014 Jul;30(4):720-724. [doi: [10.12669/pjms.304.5069](https://doi.org/10.12669/pjms.304.5069)] [Medline: [25097504](https://pubmed.ncbi.nlm.nih.gov/25097504/)]
20. Mohandass B, Chaudhary H, Pal GK, Kaur S. Knowledge and practice of rural mothers on oral hygiene for children. *Indian J Continuing Nurs Education* 2021;22(1):39-43. [doi: [10.4103/IJCN.IJCN_7_20](https://doi.org/10.4103/IJCN.IJCN_7_20)]
21. Ali Leghari M, Tanwir F, Ali H. Association of dental caries and parents knowledge of oral health, a cross-sectional survey of schools of Karachi, Pakistan. *J Pakistan Dent Assoc* 2018 May 15;23(1) [FREE Full text]
22. Bennadi D, Reddy CVK, Sunitha S, Kshetrimayum N. Oral health status of 3-6 year old children and their mother's oral health related knowledge, attitude and practices in Mysore City, India. *Asian J Med Sci* 2014 Sep 15;6(2):66-71. [doi: [10.3126/ajms.v6i2.11097](https://doi.org/10.3126/ajms.v6i2.11097)]
23. Bakar N, Mamat Z. Parental knowledge and practices on preschool children oral healthcare in Nibong Tebal Penang, Malaysia. *JOJ Nurs Health Care* 2018 Apr;7(4). [doi: [10.19080/JOJNHC.2018.07.555716](https://doi.org/10.19080/JOJNHC.2018.07.555716)]
24. Kabar AME, Elzahaf RA, Shakhathreh FM. The relationship between oral health knowledge mothers and dental caries in Tripoli, Libya. *Saudi J Oral Dent Res* 2019 Jul 22;4(7):463-467. [doi: [10.21276/sjodr.2019.4.7.7](https://doi.org/10.21276/sjodr.2019.4.7.7)]
25. Alzaidi SS, Alanazi IA, Abo Nawas OM, Mulla MA. Childhood oral health: maternal knowledge and practice in Tabuk, Saudi Arabia. *Egyptian J Hosp Med* 2018 Jan;70(9):1544-1551. [doi: [10.12816/0044681](https://doi.org/10.12816/0044681)]
26. Das SK, Tamannur T, Nesa A, et al. Exploring the knowledge and practices on road safety measures among motorbikers in Dhaka, Bangladesh: a cross-sectional study. *Inj Prev* 2023 Nov 28;ip-2023-045071. [doi: [10.1136/ip-2023-045071](https://doi.org/10.1136/ip-2023-045071)] [Medline: [38050086](https://pubmed.ncbi.nlm.nih.gov/38050086/)]
27. Nachar N. The Mann-Whitney U: a test for assessing whether two independent samples come from the same distribution. *Tutorials Quant Methods Psychol* 2008;4(1):13-20. [doi: [10.20982/tqmp.04.1.p013](https://doi.org/10.20982/tqmp.04.1.p013)]
28. Kim HY. Statistical notes for clinical researchers: nonparametric statistical methods: 2. Nonparametric methods for comparing three or more groups and repeated measures. *Restor Dent Endod* 2014 Nov;39(4):329-332. [doi: [10.5395/rde.2014.39.4.329](https://doi.org/10.5395/rde.2014.39.4.329)] [Medline: [25383354](https://pubmed.ncbi.nlm.nih.gov/25383354/)]
29. Rahman MM, Hamiduzzaman M, Akter MS, et al. Frailty indexed classification of Bangladeshi older adults' physio-psychosocial health and associated risk factors- a cross-sectional survey study. *BMC Geriatr* 2021 Jan 6;21(1):3. [doi: [10.1186/s12877-020-01970-5](https://doi.org/10.1186/s12877-020-01970-5)] [Medline: [33402094](https://pubmed.ncbi.nlm.nih.gov/33402094/)]
30. Manzoor F, Iqbal Z, Ahmed K, Khayyam U, Malhi P, Khalid M. Assessment of parental knowledge and attitude regarding oral health status of their children in District Mirpurkhas Sindh, Pakistan. *Pakistan J Med Health Sci* 2021 Apr;15(4):1352-1355 [FREE Full text]
31. Salama AA, Konsowa EM, Alkalash SH. Mothers' knowledge, attitude, and practice regarding their primary school children's oral hygiene. *Menoufia Med J* 2020 Mar 25;33(1):11-17. [doi: [10.4103/mmj.mmj_300_19](https://doi.org/10.4103/mmj.mmj_300_19)]
32. Tonetti MS, Bottenberg P, Conrads G, et al. Dental caries and periodontal diseases in the ageing population: call to action to protect and enhance oral health and well-being as an essential component of healthy ageing - consensus report of group 4 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. *J Clin Periodontol* 2017 Mar;44 Suppl 18:S135-S144. [doi: [10.1111/jcpe.12681](https://doi.org/10.1111/jcpe.12681)] [Medline: [28266112](https://pubmed.ncbi.nlm.nih.gov/28266112/)]
33. Ibrahim R, Helaly MO, Ahmed EMA. Assessment of brushing techniques in school children and its association with dental caries, Omdurman, 2019. *Int J Dent* 2021 Jan 21;2021:4383418. [doi: [10.1155/2021/4383418](https://doi.org/10.1155/2021/4383418)] [Medline: [33552159](https://pubmed.ncbi.nlm.nih.gov/33552159/)]
34. Amin M, Nyachhyon P, Elyasi M, Al-Nuaimi M. Impact of an oral health education workshop on parents' oral health knowledge, attitude, and perceived behavioral control among African immigrants. *J Oral Dis* 2014 Jun 23;2014:1-7. [doi: [10.1155/2014/986745](https://doi.org/10.1155/2014/986745)]

35. Sharma S, Mohanty V, Balappanavar AY, Chahar P, Rijhwani K. Role of digital media in promoting oral health: a systematic review. *Cureus* 2022 Sep 7;14(9):e28893. [doi: [10.7759/cureus.28893](https://doi.org/10.7759/cureus.28893)] [Medline: [36225421](https://pubmed.ncbi.nlm.nih.gov/36225421/)]
36. Goldberg E, Eberhard J, Bauman A, Smith BJ. Mass media campaigns for the promotion of oral health: a scoping review. *BMC Oral Health* 2022 May 14;22(1):182. [doi: [10.1186/s12903-022-02212-3](https://doi.org/10.1186/s12903-022-02212-3)] [Medline: [35568896](https://pubmed.ncbi.nlm.nih.gov/35568896/)]
37. Suresh BS, Ravishankar TL, Chaitra TR, Mohapatra AK, Gupta V. Mother's knowledge about pre-school child's oral health. *J Indian Soc Pedod Prev Dent* 2010;28(4):282-287. [doi: [10.4103/0970-4388.76159](https://doi.org/10.4103/0970-4388.76159)] [Medline: [21273717](https://pubmed.ncbi.nlm.nih.gov/21273717/)]
38. Alshammari FS, Alshammari RA, Alshammari MH, et al. Parental awareness and knowledge toward their children's oral health in the city of Dammam, Saudi Arabia. *Int J Clin Pediatr Dent* 2021;14(1):100-103. [doi: [10.5005/jp-journals-10005-1894](https://doi.org/10.5005/jp-journals-10005-1894)] [Medline: [34326593](https://pubmed.ncbi.nlm.nih.gov/34326593/)]
39. Jumaa FA, Turki SG, Hattab KM. Mothers' knowledge toward oral health of children under 5 years old. *Pakistan J Med Health Sci* 2022;16(6):437-442. [doi: [10.53350/pjmhs22166437](https://doi.org/10.53350/pjmhs22166437)]
40. Nepal P, Mahomed O. Influence of parents' oral health knowledge and attitudes on oral health practices of children (5-12 years) in a rural school in KwaZulu-Natal, South Africa. *J Int Soc Prev Community Dent* 2020 Sep 28;10(5):605-612. [doi: [10.4103/jispcd.JISPCD_273_20](https://doi.org/10.4103/jispcd.JISPCD_273_20)] [Medline: [33282770](https://pubmed.ncbi.nlm.nih.gov/33282770/)]
41. Sehrawat P, Shivlingesh KK, Gupta B, Anand R, Sharma A, Chaudhry M. Oral health knowledge, awareness and associated practices of pre-school children's mothers in Greater Noida, India. *Niger Postgrad Med J* 2016;23(3):152-157. [doi: [10.4103/1117-1936.190344](https://doi.org/10.4103/1117-1936.190344)] [Medline: [27623728](https://pubmed.ncbi.nlm.nih.gov/27623728/)]
42. Đorđević A. Parents' knowledge about the effects of oral hygiene, proper nutrition and fluoride prophylaxis on oral health in early childhood. *Balkan J Dent Med* 2018;22(3):26-31. [doi: [10.2478/bjdm-2018-0005](https://doi.org/10.2478/bjdm-2018-0005)]
43. Gurunathan D, Moses J, Arunachalam SK. Knowledge, attitude, and practice of mothers regarding oral hygiene of primary school children in Chennai, Tamil Nadu, India. *Int J Clin Pediatr Dent* 2018;11(4):338-343. [doi: [10.5005/jp-journals-10005-1535](https://doi.org/10.5005/jp-journals-10005-1535)] [Medline: [30397379](https://pubmed.ncbi.nlm.nih.gov/30397379/)]
44. Das H, Janakiram C, Ramanarayanan V, et al. Effectiveness of an oral health curriculum in reducing dental caries increment and improving oral hygiene behaviour among schoolchildren of Ernakulam district in Kerala, India: study protocol for a cluster randomised trial. *BMJ Open* 2023 Feb 20;13(2):e069877. [doi: [10.1136/bmjopen-2022-069877](https://doi.org/10.1136/bmjopen-2022-069877)] [Medline: [36806129](https://pubmed.ncbi.nlm.nih.gov/36806129/)]
45. Chawłowska E, Karasiewicz M, Lipiak A, et al. Exploring the relationships between children's oral health and parents' oral health knowledge, literacy, behaviours and adherence to recommendations: a cross-sectional survey. *Int J Environ Res Public Health* 2022 Sep 8;19(18):11288. [doi: [10.3390/ijerph191811288](https://doi.org/10.3390/ijerph191811288)] [Medline: [36141563](https://pubmed.ncbi.nlm.nih.gov/36141563/)]
46. Rahman MM. Knowledge and practices towards oral hygiene of children aged 5-9 years old: a cross-sectional dataset among mothers visited tertiary level hospitals. *figshare*. 2024 Aug 31. URL: <https://doi.org/10.6084/m9.figshare.26886547.v1> [accessed 2024-01-13]

Abbreviations

ECC: early childhood caries

NIPSOM: National Institute of Preventive and Social Medicine

STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

Edited by E Meinert, T Leung; submitted 10.04.24; peer-reviewed by B Nwankwo, MH Islam; revised version received 13.09.24; accepted 27.11.24; published 03.02.25.

Please cite as:

Tamannur T, Das SK, Nesa A, Nahar F, Nowshin N, Binty TH, Shakil SA, Kundu SK, Siddik MAB, Rafsun SM, Habiba U, Farhana Z, Sultana H, Kamil AA, Rahman MM

Mothers' Knowledge of and Practices Toward Oral Hygiene of Children Aged 5-9 Years in Bangladesh: Cross-Sectional Study
JMIRx Med 2025;6:e59379

URL: <https://xmed.jmir.org/2025/1/e59379>

doi: [10.2196/59379](https://doi.org/10.2196/59379)

© Tahazid Tamannur, Sadhan Kumar Das, Arifatun Nesa, Foijun Nahar, Nadia Nowshin, Tasnim Haque Binty, Shafiul Azam Shakil, Shuvojit Kumar Kundu, Md Abu Bakkar Siddik, Shafkat Mahmud Rafsun, Umme Habiba, Zaki Farhana, Hafiza Sultana, Anton Abdulbasah Kamil, Mohammad Meshbahur Rahman. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 3.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis

Bernard Friedenson, PhD

Department of Biochemistry and Medical Genetics, Cancer Center, University of Illinois Chicago, 900 s Ashland, Chicago, IL, United States

Corresponding Author:

Bernard Friedenson, PhD

Department of Biochemistry and Medical Genetics, Cancer Center, University of Illinois Chicago, 900 s Ashland, Chicago, IL, United States

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2023.07.03.23292185v1>

Companion article: <https://med.jmirx.org/2025/1/e70039>

Companion article: <https://med.jmirx.org/2025/1/e70041>

Companion article: <https://med.jmirx.org/2025/1/e69307>

Abstract

Background: The causes of breast cancer are poorly understood. A potential risk factor is Epstein-Barr virus (EBV), a lifelong infection nearly everyone acquires. EBV-transformed human mammary cells accelerate breast cancer when transplanted into immunosuppressed mice, but the virus can disappear as malignant cells reproduce. If this model applies to human breast cancers, then they should have genome damage characteristic of EBV infection.

Objective: This study tests the hypothesis that EBV infection predisposes one to breast cancer by causing permanent genome damage that compromises cancer safeguards.

Methods: Publicly available genome data from approximately 2100 breast cancers and 25 ovarian cancers were compared to cancers with proven associations to EBV, including 70 nasopharyngeal cancers, 90 Burkitt lymphomas, 88 diffuse large B-cell lymphomas, and 34 gastric cancers. Calculation algorithms to make these comparisons were developed.

Results: Chromosome breakpoints in breast and ovarian cancer clustered around breakpoints in EBV-associated cancers. Breakpoint distributions in breast and EBV-associated cancers on some chromosomes were not confidently distinguished ($P > .05$), but differed from controls unrelated to EBV infection. Viral breakpoint clusters occurred in high-risk, sporadic, and other breast cancer subgroups. Breakpoint clusters disrupted gene functions essential for cancer protection, which remain compromised even if EBV infection disappears. As CRISPR (clustered regularly interspaced short palindromic repeats)-like reminders of past infection during evolution, EBV genome fragments were found regularly interspaced between Piwi-interacting RNA (piRNA) genes on chromosome 6. Both breast and EBV-associated cancers had inactivated genes that guard piRNA defenses and the major histocompatibility complex (MHC) locus. Breast and EBV-associated cancer breakpoints and other variations converged around the highly polymorphic MHC. Not everyone develops cancer because MHC differences produce differing responses to EBV infection. Chromosome shattering and mutation hot spots in breast cancers preferentially occurred at incorporated viral sequences. On chromosome 17, breast cancer breakpoints that clustered around those in EBV-mediated cancers were linked to estrogen effects. Other breast cancer breaks affected sites where EBV inhibits JAK-STAT and SWI-SNF signaling pathways. A characteristic EBV-cancer gene deletion that shifts metabolism to favor tumors was also found in breast cancers. These changes push breast cancer into metastasis and then favor survival of metastatic cells.

Conclusions: EBV infection predisposes one to breast cancer and metastasis, even if the virus disappears. Identifying this pathogenic viral damage may improve screening, treatment, and prevention. Immunizing children against EBV may protect against breast, ovarian, other cancers, and potentially even chronic unexplained diseases.

(*JMIRx Med* 2025;6:e50712) doi:[10.2196/50712](https://doi.org/10.2196/50712)

KEYWORDS

breast cancer; cancer; oncology; ovarian; virus; viral; Epstein-Barr; herpes; bioinformatics; chromosome; gene; genetic; chromosomal; DNA; genomic; BRCA; metastasis; biology

Introduction

In the United States, over 40,000 women die from breast cancer each year [1,2]. The causes of the disease are not well understood, making prevention and treatment empirical and hazardous. At the time of breast cancer diagnosis, its causes are difficult to isolate from multiple risk factors. A human cancer virus is one such risk factor. A tumor virus does not cause cancer by itself [3] but can make cancer more likely by inhibiting tumor suppressors [4] or activating oncogenes. Viral damage then increases cancer risks via mutations and chromosome breaks. Epstein-Barr virus (EBV), also called human herpesvirus 4, infects at least 90% of humans as a lifelong infection, often acquired at an early age [5], but the virus remains latent and asymptomatic in most people. EBV may be a risk factor for breast cancer. Active infection is significantly more prevalent in breast cancer tissues than in normal and benign controls [6], increasing risk by 4.75- to 6.29-fold [7]. EBV transformed human mammary epithelial cells in culture so that xenografts in immunosuppressed mice accelerated breast cancer. Once malignant transformation occurred, EBV was no longer required [8], but the cells remain malignant.

There has been no way to test the idea that EBV causes breast cancer and can then disappear. However, cancers in other tissues have proven relationships to EBV infection, so these known EBV-associated cancers can be compared to breast cancers at the genome level. Cancers with unambiguous EBV associations include nasopharyngeal cancer (NPC), EBV-positive diffuse large B-cell lymphoma (DLBCL), endemic Burkitt lymphoma (BL) [9], and gastric cancer (GC). Some genomic similarities between these EBV-associated cancers and breast cancer can be derived from the literature. In NPC, 100% of malignant cells are EBV positive [10]. Over 64% of NPCs are deficient in a pathway that depends on the breast cancer susceptibility genes *BRCA1* and *BRCA2* [11], which accurately repair DNA crosslinks and breaks via the homologous recombination pathway. This sprawling, interconnected pathway includes Fanconi anemia (FA) gene products and is often designated as the FA-BRCA pathway. In 126 patients with NPC, *BRCA1* and *BRCA2* were the most frequently mutated genes (55.5% and 33.3%, respectively) [12]. NPC mutations interfere with innate immunity and constitutively activate an inflammatory response. Overexpressed nuclear factor- κ B (NF- κ B) is a hallmark of NPC, occurring in 90% of NPCs [11]. Similarly, almost all stage-3 breast cancers overexpress NF- κ B [13].

In NPC and the other known EBV-associated cancers, EBV inhibits the FA-BRCA pathway by various methods, including using viral microRNAs to downregulate *BRCA1* [14], hijacking other pathway components [15,16], and destabilizing SMC5/6-mediated chromatin interactions [17,18]. In GC, EBV infection and FA-BRCA pathway status are mutually exclusive [19], implying that EBV infection is approximately equivalent to disabling the FA-BRCA pathway. In DLBCL, the best prognostic marker is FA-BRCA pathway status [20]. In DLBCL

and endemic BL, EBV variant infection accompanies *MYC* translocations. These translocations drive the disease and make a characteristic replacement of normal *MYC* control elements with highly active immunoglobulin regulatory sequences [21,22]. *MYC* amplification is frequent in breast cancers that have inactive *BRCA1* [23].

NPCs, DLBCLs, BLs, GCs, and breast cancers all have deficits in correctly repairing double-strand breaks and crosslinks. The compromised FA-BRCA pathway can produce chromosomes with too many centromeres. During cell division, mitotic spindles pull chromatids with multiple centromeres in too many directions, generating chromosome breaks to destabilize the human genome [24,25]. In breast cancer, these variations mark breakpoints at translocations and oncogene amplifications [26].

If EBV contributes to breast cancer, gene deficits in breast cancers and EBV-associated cancers should produce comparable changes in the human genome that do not depend on whether EBV infection persists. The aim of this study was to test for these virus-induced genome changes using bioinformatic calculations and analyses. The results could implicate EBV and its variants in disabling a variety of molecular and cellular safeguards that protect against breast cancer and its metastasis. Whether or not cancer develops in response to EBV infection depends on major histocompatibility complex (MHC) gene polymorphisms [27,28], so not everyone infected with EBV will develop cancer. In susceptible people, genome damage is permanent and does not require large numbers of viral particles, active infection, or continuing virus presence. Childhood immunization against selected EBV gene products may do much to prevent breast, ovarian, and other cancers.

Methods

Datasets Used in the Analysis

Overview

The initial data for analysis came from literature searches for studies on breast and EBV-associated cancers with large numbers of participants, unrestricted access to genome information, and complete whole-genome analysis. The first criterion for including breast cancer data was published intrachain or interchain chromosome breakpoints from high-quality, peer-reviewed publications produced by world-class laboratories. The second criterion was the availability of sufficient DNA sequence data to specify the location of these chromosome breakpoints. The third criterion was that genome sequencing had been done on samples taken before treatment began. These publicly available DNA sequence data were chosen to encompass diverse genetics, subtypes, stages, grades, morphologies, and outcomes. Initially, breast cancers were separated only broadly into those with a likely hereditary component versus those without this component. The cancers had to include typical morphologies such as ductal carcinomas, lobular carcinomas, medullary carcinomas, and

invasive carcinomas (ie, “no special type”). The included breast cancers were all primary stage-2 or stage-3 cancers. Although surgery usually removes these primary tumors, cells with only a few additional late mutations are responsible for seeding local recurrences or metastases, so primary and metastatic tumors are not very different [29]. Although the selected cancers are not a random sample representing all breast cancers [30], they are likely to have chromosome instability originating from diverse typical causes.

Specifically, the breast cancer data used came from 560 breast cancer genome sequences, familial cancer data from 78 patients, methylation data from 1538 breast cancers versus 244 controls, 243 triple-negative breast cancers, and 2658 human cancers [31-35]. Data also included 74 breast cancers from high-risk women who were typed as having *BRCA1*- or *BRCA2*-associated mutations or cancers diagnosed before the age of 40 years [36,37]. Another study of familial breast cancers contributed 65 familial breast cancers [33]. Gene breakpoints for many interchromosomal and intrachromosomal translocations and breakpoints were obtained from the COSMIC (Catalog of Somatic Mutations in Cancer) website, as curated from original publications or original articles and their supplemental information [31-33]. [Multimedia Appendix 1](#) provides a glossary of the terms used in this paper.

Breakpoints in Breast Cancers From High-Risk Women

Hereditary cancers were taken as breast cancers from women with a typed high-risk *BRCA1* or *BRCA2* mutation diagnosed before the age of 70 years. Cancers from patients with onset before the age of 50 years were also included to add more data, since these women are at high risk for an inherited, cancer-associated mutation. These patient samples were chosen based on descriptions in published data defining the breast cancer cohorts [31,33].

Sporadic Breast Cancers

Sporadic breast cancers were taken as breast cancers diagnosed after the age of 70 years that did not have a known inherited mutation [31].

Breast Cancer Subgroups

Human epidermal growth factor receptor 2 (HER2)-positive and triple-negative breast cancer data used for subgroup analysis were from original publications [33] and the COSMIC website.

Exclusions

Male breast cancers were excluded.

Data Source for Ovarian Cancers

Data for breakpoints in ovarian cancers were downloaded from the COSMIC website. The cancers corresponded to “mixed adenocarcinomas” and were arbitrarily taken from those with the largest number of structural variants. These cancers all had the prefix “AOCS-” with further identification numbers and *BRCA* mutation status in parentheses as follows: 170-1-8 (negative), 120-3-6 (*BRCA2*), 142-3-5 (negative), 139-1-5 (negative), 086-3-2 (negative), 147-1-1 (*BRCA1* and *BRCA2*), 094 - 6-X (*BRCA1*), 094-1-1 (*BRCA1*), 088-3-8 (negative), 139-6-3 (*BRCA2*), 150-3-1 (negative), 116-1-3

(negative), 155-3-5 (*BRCA2*), 093-3-6 (negative), 034-3-8 (*BRCA1*), 091-3-0 (*BRCA1*), 139-19-0 (*BRCA2*), 170-3-5 (negative), 114-1-8 (negative), 064-3-3 (negative), 064-1-6 (negative), 106-1-1 (*BRCA1*), 152 - 1-X (*BRCA1*), and 134-1-5 (unknown).

Original Data Sources for Cancers With Known EBV Associations: NPCs, Lymphomas, and GCs

Overview

NPC chromosome breakpoint positions were retrieved from Bruce et al [11] for 70 primary tumors of the nasopharynx at stages 1-4C. The data came from whole-genome sequencing of “63 micro-dissected tumors, 5-patient derived xenografts, and two cell lines.” DLBCL breakpoints were collected from 88 patients with DLBCL (aged >60 y) [22]. The *MYC* breakpoints included class I and II *MYC* translocation locus breakpoints defined in BL, encompassing areas far upstream of *c-myc* [38-40]. Downstream breakpoints included an enhancer region approximately 565 kilobases long on the nearest telomere side of the *MYC* coding sequence [22]. Older data provided fusion sequences as Gencode Accession numbers [21]. These fusion sequences were downloaded as FASTA files and copied to BLAST (Basic Local Alignment Search Tool) for placement on the human GRCh38/hg38 reference sequence. GCs with inferred EBV infection status came from 34 (20.2%) out of 168 samples subjected to whole-genome sequencing [41].

Selection Bias

As much as possible, selection bias was avoided by blindly selecting samples, replicating samples using cohorts from different publications, using the largest possible groups of samples, and avoiding convenience sampling. Some experiments used a newer dataset from 780 breast cancers [22] for comparisons to confirm that selection bias was unlikely.

Recruitment

Data from genome sequence studies did not include specific recruitment procedures for patients with cancer. However, patients are typically recruited through hospitals and clinics with referrals from medical professionals. Patients provide informed consent to have their genomes sequenced and used for research and to integrate cancer genome sequence data into treatment decisions [42].

Methods Used to Determine That DNA Breakpoints From Breast and Ovarian Cancers Clustered Around Breakpoints in EBV-Associated Cancers

Calculation of Distances Between Breakpoints in Breast and Ovarian Cancers Versus EBV-Related Cancers

Before combining or comparing datasets, they were all converted to the same genome version, usually GRCh38. The break position in breast cancer nearest to a break in NPC was taken as the Microsoft Excel *XLOOKUP* value for the number of base pairs (bp) from the closest NPC breakpoint 5' to the breast cancer break or the NPC breakpoint 3' to the breast cancer break, whichever was closer ([Multimedia Appendix 2](#)). For comparing a given breast cancer breakpoint A2 to EBV-associated cancer breakpoints B2 to B72, the initial

algorithm to find the nearest 5' break position was written in Excel as follows: $=XLOOKUP(\$A2, \$B\$2:\$B\$72, \$B\$2:\$B\$72, 0, -1, 1)$. Changing -1 to $+1$ gave D2, the nearest 3' position. Distance from the breast cancer breakpoint was then calculated as $=MIN(ABS(C2-A2), ABS(D2-A2))$. The same formulas were then continuously updated by Excel to calculate all other breast cancer comparisons in column A. Differences in the amount of data available for NPC versus breast cancer breakpoints complicated the calculations near chromosome telomeres. Several methods of handling these end regions made no discernible difference in the outcomes. For a 5000-bp window, an overflow window of 5,000,000 was used to limit the number of bins to a maximum of 1000. Another method of calculating distances between chromosome breakpoints in different cancers used the minimum of the absolute values of distances between breast cancers and the array of breakpoints in GCs, BLs, or NPCs. This method gave results identical to *XLOOKUP* values but was more convenient to compare clusters of breast cancer breakpoints to those in lymphoid and epithelial EBV-associated cancers. Hundreds of millions of calculations were repeated at least twice. Most of the calculations in this section are presented in [Multimedia Appendix 2](#).

DNA Sequence Homology Analyses to Determine Breakpoints in Human Cancer Sequences That Resemble Viral Sequences

The NCBI BLASTn program (MegaBLAST) and database [43-45] were used to compare DNA sequence homologies around breakpoints in breast cancers to all available viral DNA sequences. *E* ("expect") values are related to *P* values and represent the probability that a given homology bit score occurs by chance. *E* values $<1 \times 10^{-10}$ were considered significant homology. In many cases, *E* values were "0" ($<1 \times 10^{-180}$) and always far below 1×10^{-10} . The virus DNA was retrieved from BLAST searches using "viruses (taxid:10239)," with human sequences, mouse sequences, and uncharacterized sample mixtures excluded. Different strains and isolates of the same virus were tested for human homology. Specifically, the HKHD40 and HKNPC60 variants were often considered together as "EBV."

Methods Used for Chromosome Comparisons of Breakpoints in Breast Cancers in High-Risk Women Versus Breakpoints in Sporadic Breast Cancer

The NCBI Genome Decoration page provided chromosome annotation software [46].

Identifying Genes Around the Most Frequent EBV-Binding Site Locations and Tethering Sites

EBV nuclear antigen 1 (EBNA1)-binding location genome coordinates [47,48] were used to tabulate genes within or near anchoring sites where EBV docks on human DNA. Breaks in breast cancers were compared to the gene positions around their EBNA1-binding sites. The Palindrome Site Finder from NovoPro and the EMBOSS palindrome program were used to identify palindromic DNA sequences.

Comparisons for Similarities Among Human Herpesviruses

EBV variants HKHD40 and HKNPC60 were compared to human herpesviruses in BLASTn by entering the terms "human gamma herpesvirus 4," "herpesviridae," and "herpesvirales." Values with ≥ 2000 bp in common were selected. The EBV reference sequence was also tested against the following proven cancer viruses: human herpesvirus 8 (also called Kaposi sarcoma virus), herpes simplex virus 1, and human cytomegalovirus.

Locating Piwi-Interacting RNA Sequences as Evidence of Past EBV Infection

Piwi-interacting RNA (piRNA) locations were retrieved from the piRNA bank [49,50]. To compare the positions of piRNAs in virus homology versus genome position graphs, the midpoints of piRNA sequences were assigned arbitrary homology values. Positions of differentially methylated regions near breast cancer breakpoints on chromosome 6 [51] were compared to breakpoint positions for 70 NPCs based on published data analyses [11].

Viral Sequences in Human Genomes as Hypermutation and Rearrangement Sites in Breast Cancers

A graph of viral sequences in humans against chromothripsis breaks in breast cancers was so complex that it resisted interpretation, so only the 5 viral sequences nearest the chromothripsis breaks were used. The viral sequences nearest high-confidence chromothripsis breaks were determined in 5 iterations as genome coordinates where *XLOOKUP* values gave the minimum distances. Distances between all virus homology start points were then compared to all chromothripsis breakpoints.

Methods of Data Analyses and Statistical Software

DNA flanking sequences at breakpoints were downloaded primarily from the GRCh38/hg38 version of the University of California, Santa Cruz Genome Browser as FASTA files and copied directly into BLAST. Results were checked against breakpoints in 101 triple-negative breast cancers from a population-based study [32]. The University of California, Santa Cruz Genome Browser's *Liftover* function interconverted different versions of genome coordinates into GRCh38/hg38 coordinates.

Statistics

Excel, SPSS (IBM Corp), StatsDirect, Visual Basic (Microsoft), and Python (Python Software Foundation) scripts were used for data analysis. Mann-Whitney *U* tests compared overall breakpoint distributions [52] and tested the hypothesis that breakpoint distributions were identical or at least roughly the same. The Mann-Whitney *U* test was chosen because the comparisons involved unequal numbers of breakpoints, and each observation was likely independent. *P* values $>.05$ were taken to indicate that identical distributions could not be excluded. Tests for normality included kurtosis and skewness values and evaluation by Shapiro-Francia and Shapiro-Wilk methods [53] ([Multimedia Appendix 2](#)). The Fisher exact test compared breakpoints in breast cancers to those in known viral cancers. The unpaired 2-tailed Student *t* test was used to compare the means of numbers of breast cancers with severe

versus nil lymphocyte infiltrates, assuming the data approximated normality and that there were no extreme outliers. Both of these tests require independence and random sampling. All these test results are only approximate because they depend on underlying assumptions.

Fragile Site Sequence Data

Positions of common fragile sites were retrieved from a database [54] and original publications [55].

Ethical Considerations

This study presents analyses of publicly available data without recruiting additional human or animal subjects. Because this study is a secondary analysis, it is exempt from institutional review board and ethics approval. The data are in the public domain and are available for independent research and analysis [56]. It is not necessary to obtain permission to reuse public data. The original informed consent allows secondary analysis without additional permission.

Results

Breakpoints in Breast Cancers From High-Risk Backgrounds Clustered Around Breakpoints in NPC, an EBV-Mediated Cancer

EBV-mediated cancers such as NPC have defects in DNA repair and inflammatory pathways, resembling hereditary breast and ovarian cancers. To further characterize this resemblance, breakpoints in 70 NPC genomes were compared to breakpoints in 139 breast cancer genomes from high-risk women (*BRCA1/BRCA2* mutation, familial concentration, or young age).

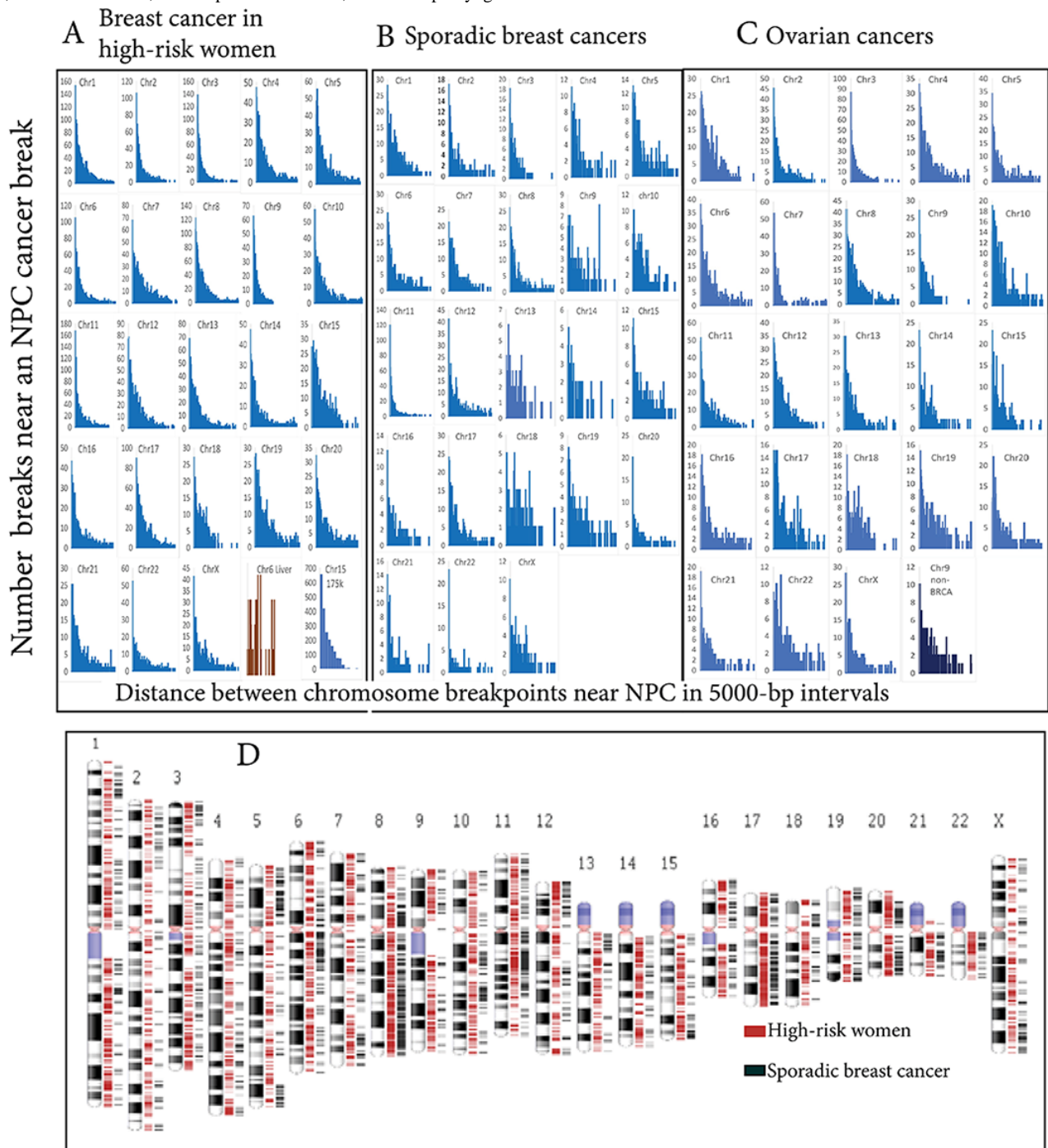
The distances from all breast cancer breakpoints to the nearest NPC breakpoints across the entire length of chromosome 1 produced results with so many points that they were difficult to interpret (Multimedia Appendix 3). Different laboratories collected these breakpoint data over many years. To allow for some variations, the data were grouped into 5000-bp increments

(2×10^{-5} relative error). As shown in Figure 1 and Multimedia Appendix 2, breast cancer breakpoints were most often clustered within 5000 bp of NPC breakpoints, but many breakpoints agreed much more closely. A total of 20 breast cancer breakpoints on chromosome 1 were within 500 bp of an NPC breakpoint, and several chromosomes had breast cancer and NPC breakpoints in essentially the same positions. As represented by Mann-Whitney *U* test results (Multimedia Appendix 2), breast cancer and NPC breakpoint distributions were statistically the same ($P > .05$) for chromosomes 6, 7, 10, 13, 14, 15, 22, and X, but different on chromosome 1 and other chromosomes ($P < .05$).

In contrast, liver cancer breakpoints at hepatitis B virus integration sites [57] differed from those in breast cancer or NPC (Figure 1). No breaks in 114 liver cancers on chromosome 1 were within 5000 bp of breaks in any NPC; only one break on chromosome 6 in 61 liver cancers fit this window. According to a meta-analysis, the chance that breakpoints on chromosomes 1, 2, 6, and 8 were not within 5000 bp in liver cancer versus NPC was 4.4 (95% CI 1.9 - 10). NPC and liver cancer did not have the same breakpoint distributions ($P < .001$).

The above results revealed that breast cancer breakpoints in high-risk women were clustered near those in the EBV-associated cancer, NPC, on every chromosome. The next step was to decide whether these similarities depended on mutations in the breast cancer susceptibility genes, *BRCA1* or *BRCA2*, by comparisons to sporadic breast cancers. The sporadic breast cancer group comprised 74 women, aged ≥ 70 years, with normal *BRCA* genes and no other known inherited, cancer-associated mutations [31]. Like breakpoints from high-risk women, many sporadic breast cancer breakpoints clustered around those in NPC (Figure 1). Breakpoints in these sporadic breast cancers clustered at chromosomal locations similar to breast cancers from high-risk women, although the frequencies and distributions sometimes differed significantly. The patients with sporadic breast cancer were older than the high-risk women, arguing against age as responsible for similarity to NPC breakpoints.

Figure 1. (A) Breakpoints in 139 breast cancers from high-risk women (*BRCA* mutation, familial concentration, or early onset) clustered around breakpoints in 70 NPCs. The data were grouped in 5000-bp increments to allow for methodological and laboratory differences. An unrelated set of hepatocellular data associated with hepatitis B insertions did not show a similar relationship to NPC. Breast cancer and NPC breakpoint distributions could not be confidently distinguished ($P>.05$) for chromosomes 6, 7, 10, 13, 14, 15, 22, and X (Multimedia Appendix 2). Many breakpoints were virtually the same on some chromosomes. The panel at the lower right shows how the selection of a larger bin size of 175,000 bp (the approximate length of EBV) affects the distributions of breakpoints. (B) Like the breast cancers from high-risk women, breakpoints in 74 sporadic breast cancers clustered around the breakpoints found in 70 NPCs. Breast cancer breakpoints within 5000 bp of an NPC breakpoint were the largest single category on most chromosomes. (C) Breakpoints in 25 mixed adenosquamous ovarian cancers also clustered around breakpoints in the 70 NPCs. The data show both *BRCA*-associated and nonassociated ovarian cancers. The panel in the lower right corner represents chromosome-9 data after removing all *BRCA*-associated ovarian cancers. The sporadic cancers show the same results as the complete set but with less data. (D) Many breakpoints in sporadic breast cancers clustered at chromosomal locations similar to those from high-risk women. Interchromosome translocation break positions in 74 mutation-associated, familial, or early-onset female breast cancers (red) versus 74 likely sporadic female breast cancers (black) are shown. bp: base pairs; Chr: chromosome; EBV: Epstein-Barr virus; NPC: nasopharyngeal cancer.



Viral Homologies Around Breakpoints in Mixed Adenosquamous Ovarian Carcinoma Also Clustered Around Breakpoints in EBV-Mediated Cancer

Ovarian cancer data enabled an additional test for EBV involvement in breast cancer because, like breast cancer, *BRCA1* or *BRCA2* mutations can also predispose patients to ovarian cancer [58]. Chromosome breakpoints in 25 mixed adenosquamous ovarian cancers were compared to breakpoints in NPCs. The results depicted in Figure 1 emulated breast cancer comparisons. Nearly half (12/25, 48%) the ovarian cancer cases had likely hereditary *BRCA* mutations. The remaining sporadic ovarian cancers gave the same results as the complete set but with less data. As in breast cancer, ovarian cancer breakpoint distributions clustered around NPC breakpoints, even without a hereditary *BRCA1* or *BRCA2* gene mutation driver.

Breaks in Lymphomas Associated With EBV Infection Also Matched Breast Cancer and NPC

EBV drives lymphomas as well as NPCs. Based on epidemiologic research results, FA-BRCA pathways protect against lymphomas [59,60]. If EBV is genuinely associated with breast cancer breakpoints, then breakpoint positions in EBV-mediated lymphomas should also resemble those of breast and ovarian cancers. Because *MYC* gene rearrangements are characteristic of EBV-associated lymphomas, the first test of this idea was to survey virus-like sequences surrounding the *MYC* gene locus on the human reference genome. Figure 2 shows that *MYC* resides in a literal forest of retrovirus sequences (eg, human immunodeficiency virus type 1 [HIV1], feline leukemia virus, porcine endogenous retrovirus, and human endogenous retrovirus [HERV]) interspersed with EBV-like sequences.

The concentration of virus sequences around *MYC* on chromosome 8 prompted the addition of the EBV-associated lymphoma DLBCL to breakpoint comparisons. As shown in Figure 2, the results revealed that hundreds of breast cancer and NPC breakpoints congregated around breakpoint positions in 88 DLBCLs [22]. This agreement was consistent with other similarities between breast cancers and these EBV-associated cancers, including deficits in FA-BRCA pathway-mediated DNA repair by homologous recombination [61] and NF- κ B activation [11,62-64].

EBV is also a proven driver of at least one subset of BLs, typically those with *MYC* translocations. BL subsets can have mutations that impair homologous recombination [65], so results in Figure 2 revealed many breast cancer breakpoint positions near corresponding BL breakpoints. An older dataset from BLs [21] had translocation breakpoints in the virus sequence-rich area near the *MYC* locus, agreeing with about 140 breast cancer breakpoints. Four different NPC breakpoints produced over 100 matches to BL translocation breakpoints, beginning at 8250 bp apart. An unpaired, 2-tailed *t* test did not support a statistically significant difference between BL and NPC breakpoints in this area ($P=.69$).

Further tests were conducted to determine whether the functions of genes near clustered breakpoints supported a relationship between breast cancers and EBV-related cancers (GC [41], BL, and NPC). As illustrated in Figure 3, breast cancer breakpoints on chromosomes 6, 8, 11, and 17 aggregated near positions where breakpoints occurred in EBV-associated cancers. Many aggregated breakpoints were in the same areas as genes that control inflammation, antiviral defenses, apoptosis, intermediate filaments, epigenetic and chromatin regulation, estrogen receptor activity, mitotic structures, and mitotic controls (Table S1 in Multimedia Appendix 4). Breast cancer breakpoints that clustered around EBV-associated cancer breakpoints were especially numerous on chromosome 17. One of these clusters marked in Figure 3 included the *HER2* amplicon and the topoisomerase 2a gene, with *BRCA1* and *SMARCE1* genes nearby. *SMARCE1* encodes a part of a chromatin regulation complex. Chromosome 17 breakpoints near *CNTROB* and *CTCI* genes connect EBV to centriole and telomere malfunctions during mitosis (Table S1 in Multimedia Appendix 4). Rearrangements near breakpoints may cause over- or underexpression of nearby genes (Table S2 in Multimedia Appendix 4). Many additional correlations were also likely revealed in Figure 3 but were not investigated further.

Results in this section show that breast cancer breakpoints clustered around breakpoints in additional EBV-associated cancers, where they affect critical functions needed to prevent breast cancer. Once these functions are compromised, cancer can occur without the continuing presence of EBV.

Figure 2. (A) Human DNA around the *MYC* locus on chromosome 8 was filled with virus-like sequences. *CASC11* is an RNA gene that several cancers overexpress. Breast cancer and lymphoma breakpoints were dispersed throughout the *MYC* region and beyond, but NPC breakpoints were less common. (B) On chromosome 8, hundreds of breakpoints in breast cancers and NPCs clustered around breakpoints in data from 88 patients with DLBCL who were likely EBV positive. This agreement highlights multiple similarities among these cancers. (C) EBV drives a subset of BLs, typically with *MYC* translocations and impaired homologous recombination. Based on *MYC* fusion sequences in BL, breast cancer breakpoints on chromosome 8 also clustered around BL breakpoints. BLs from an older dataset [21] had translocation breakpoints in the virus-rich area near the *MYC* locus, agreeing with ≥ 140 breast cancer breakpoints. *MYC* locus translocations had not been reported in NPCs, but NPC breakpoints still clustered around BL fusion breakpoints, although at greater distances. Four different NPC breakpoints produced over 100 matches to BL translocation breakpoints beginning at about 8250 bp apart. An unpaired, 2-tailed *t* test did not support a statistically significant difference between BL and NPC breakpoints in this area ($P=.69$). BL: Burkitt lymphoma; bp: base pairs; DLBCL: diffuse large B-cell lymphoma; EBV: Epstein-Barr virus; FeLV: feline leukemia virus; HERV: human endogenous retrovirus; HERVK: human endogenous retrovirus K; HIV1: human immunodeficiency virus type 1; HPV18: human papillomavirus 18; HRV: human retrovirus; NPC: nasopharyngeal cancer; PERV: porcine endogenous retrovirus; Stealth: stealth virus 1.

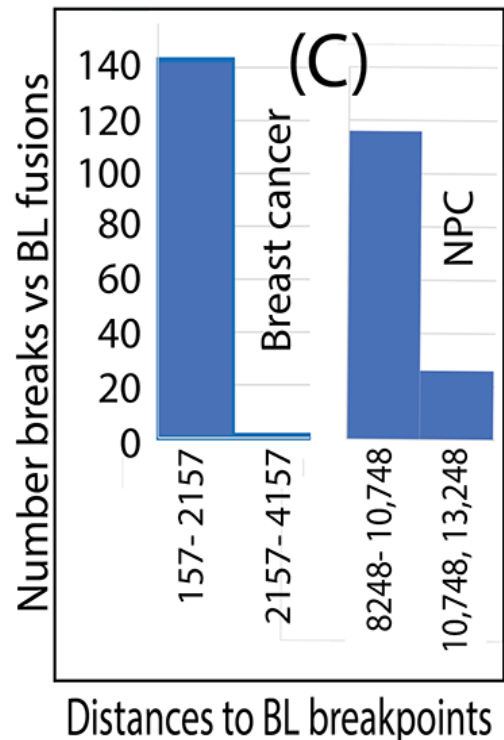
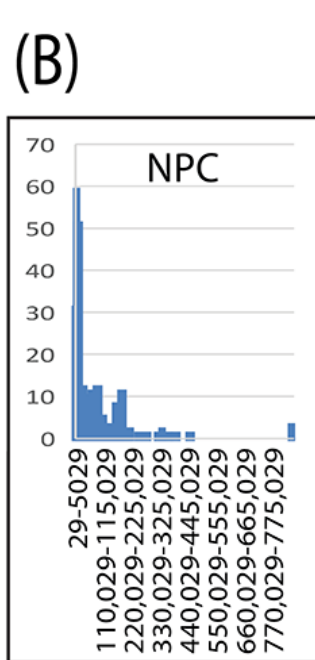
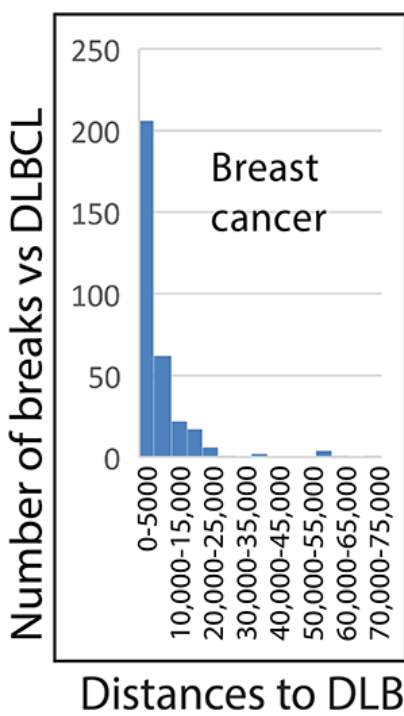
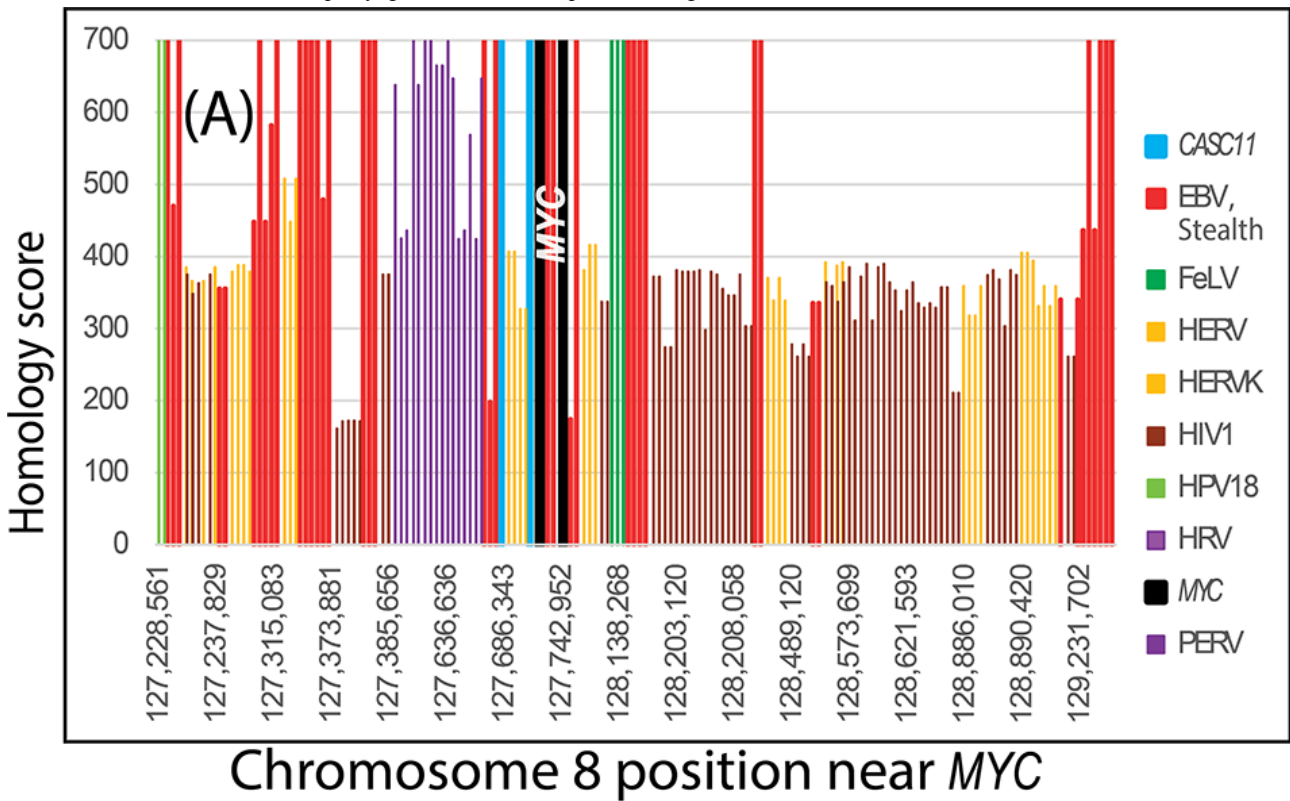
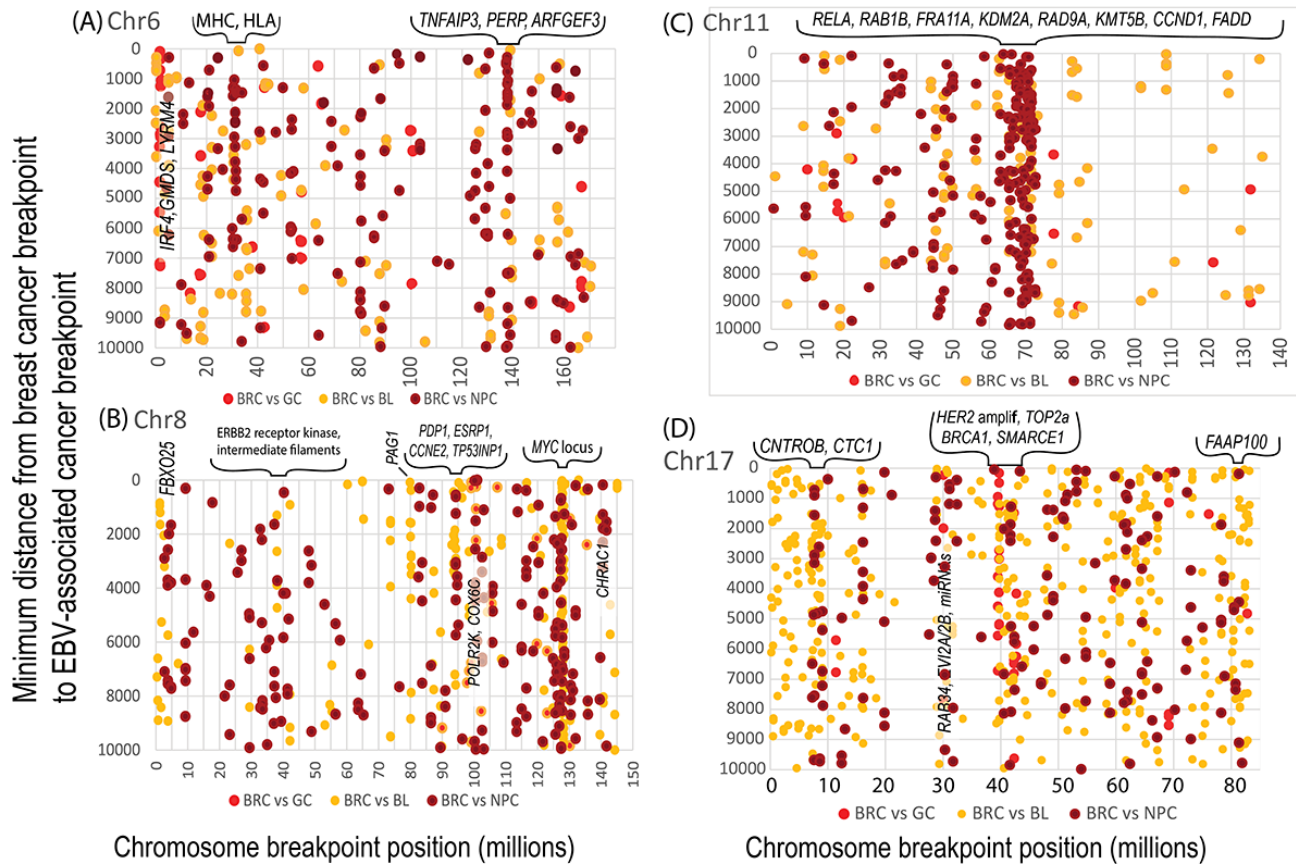


Figure 3. Breakpoints in breast cancers clustered around breakpoints in EBV-positive cancers in 3 different tissues. The EBV-positive cancers comprised 34 GCs, 90 BLs, and 70 NPCs. The clustering of breast cancer breakpoints and EBV-related cancer breakpoints was pronounced on chromosomes (A) 6, (B) 8, (C) 11, and (D) 17. Selected genes around some of the clustered breaks are indicated. Functions of the genes can have profound effects on the human genome and are summarized in Table S1 in [Multimedia Appendix 4](#). BL: Burkitt lymphoma; BRC: breast cancer; Chr: chromosome; EBV: Epstein-Barr virus; GC: gastric cancer; HLA: human leukocyte antigen; MHC: major histocompatibility complex; NPC: nasopharyngeal cancer.



Genes at the Most Frequent EBV-Tethering Sites Clustered Around Breast Cancer Breakpoints

In preceding sections, breast and ovarian cancer breakpoints were found to distribute most frequently near characteristic sets of breakpoints associated with EBV-related cancers. The virus first attaches its EBNA1 protein to human DNA in the nucleus. Then, circular EBV episomes dock to this attached EBNA1 anchor. To test whether the initial EBNA1 attachment sites were related to breast cancer chromosome breakpoints, breast cancer breakpoints were compared to genes near EBV-docking sites. EBV-positive BL cells providing the data had up to 1569 EBV-docking sites on all chromosomes identified by 4C-chromatin capture experiments [47]. As shown in [Figure](#)

[4A](#), the largest numbers of breast cancer breakpoints on most chromosomes clustered around the genes [47] nearest to genes at EBV-docking sites. In support of these comparisons, graphical estimation of virus-tethering sites on chromosome 2 from chromatin capture data for these EBV-positive cells also agreed with breast cancer breakpoints ([Figure 4A](#)). In an unrelated study [48], EBV-docking sites on chromosome 11 near known EBV anchor sites at the *FAM-D* and *FAM-B* genes were found near groups of breast cancer breakpoints, but imperfect palindrome sequences [66] were more distant ([Figure 4B](#)). This finding independently supports the idea that EBV-docking sites are near breast cancer breakpoints. Results in this section raise the possibility that EBV directly contributes to breast cancer chromosome breakpoints and fragmentation.

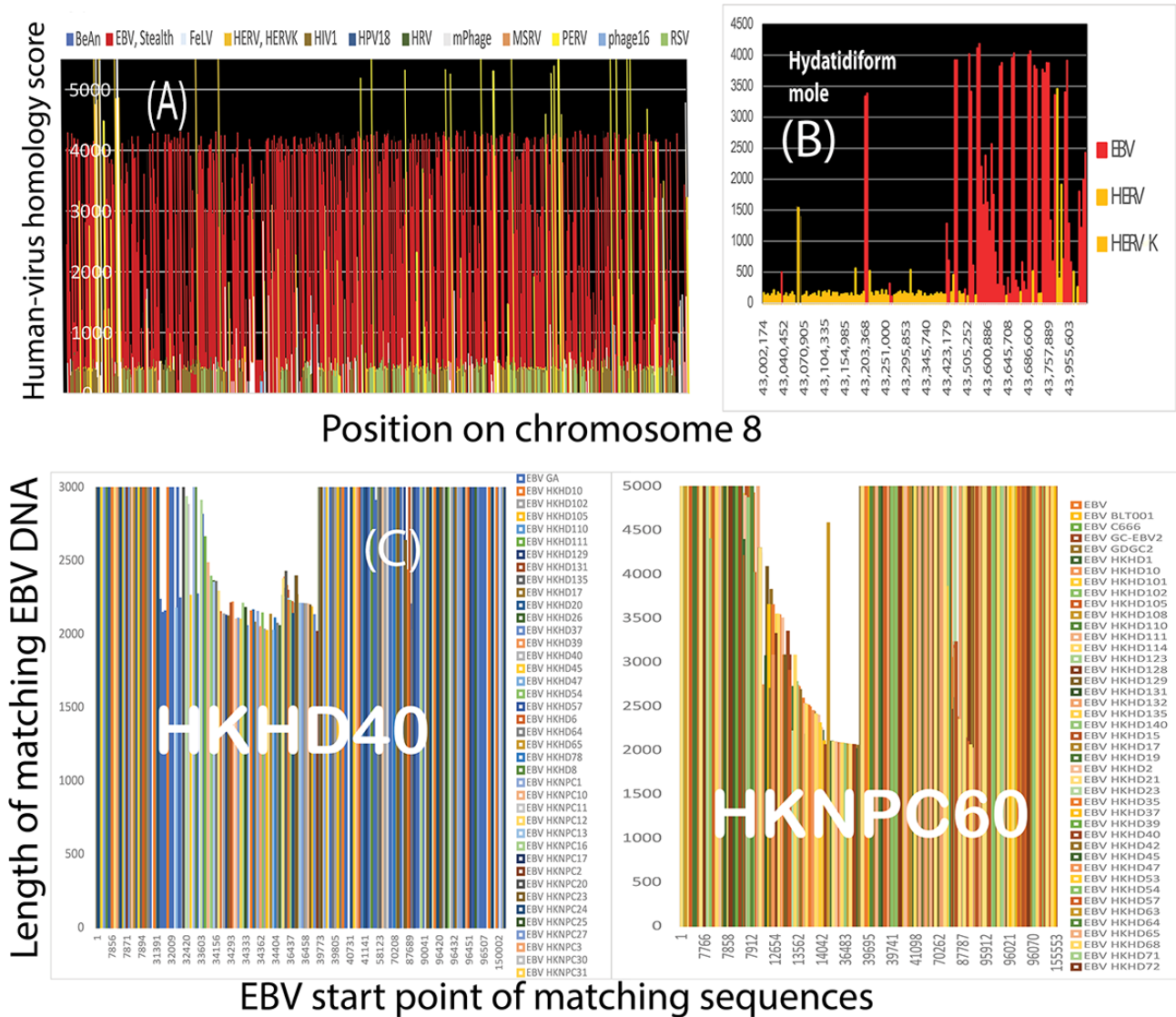
Figure 4. Relationships of EBV-docking sites to breast cancer breakpoints. (A) Breast cancer breakpoints clustered around the top 10% most frequently found genes near EBV-tethering sites in BL cells. Some of the best information on EBV-docking sites comes from 4C-chromatin capture experiments in EBV-positive BL cells [47]. The largest number of breast cancer breakpoints on most chromosomes clustered around the genes nearest EBV-tethering sites. BL cells providing the data had up to 1569 EBV-docking sites distributed over all chromosomes [47]. EBV-docking sites on chromosome 11 near the *LUZP2* and *FAT3* genes in BL cells were millions of bp from the 18-bp imperfect palindrome interval. Graphical estimation of virus-tethering sites on chromosome 2 (green) from these EBV-positive cells also agreed with breast cancer breakpoints. (B) Independent evidence relating breast cancer chromosome breakpoints to EBV-docking sites. Maximum homology to human DNA for all viruses (y-axis) is plotted around known EBV genome anchor sites on chromosome 11 near the *FAM55D* and *FAM55B* gene coordinates. A posited imperfect palindrome sequence [66] as an EBV-docking site was more distant from the *FAM55* genes. BL: Burkitt lymphoma; bp: base pairs; Chr: chromosome; chrom: chromatin; EBV: Epstein-Barr virus; HERV: human endogenous retrovirus; HERVK: human endogenous retrovirus K; HIV1: human immunodeficiency virus type 1; HTLV1: human T-cell lymphotropic virus type 1; MSRV: multiple sclerosis retrovirus; RSV: respiratory syncytial virus.

Breakpoints Occurred Near Human Sequences That Resemble Viruses in All Breast Cancers Tested

To further test whether EBV itself has some role in breaking chromosomes or altering their structures, human chromosomes were compared to all known viruses. As shown in Figure 5A, the results showed that nearly every breast cancer likely had undergone breakages near EBV-like sequences. Chromosome

8 alone had 59,566 significant (>200) viral homology scores. Based on data from 128 patients with breast cancers and 43,491 unique breakpoints, breakpoints in 123 (96.1%) out of 128 breast cancers were within 10,000 bp of a virus sequence. In 106 patients, the virus was an EBV tumor variant (HKHD40 or HKNPC60) with 3086 matching human sequences. According to the Fisher exact test, chromosome 8 breakpoints and EBV variant sequence matches were not independent ($P < .001$).

Figure 5. (A) All viral homologies on the entire lengths of chromosome 8 (a total of 145,138,636 bp) are shown in 200k-bp increments. Maximum homology scores over 4000 for human DNA versus herpes viral DNA were abundant. The 4000 score corresponds to 97% human-virus identity over nearly 2500 bp, with *E* (“expect”) values (essentially *P* values) effectively equal to 0. The EBV tumor variants, HKNPC60 and HKHD40, were nearly identical to human breast cancer DNA at many positions throughout chromosome 8. (B) It is unlikely that homologies to EBV sequences occurred because the human reference genome was contaminated with EBV episomes. Homozygous hydatidiform mole cells that had lost the paternal chromosomes after fertilization still had strong homology to EBV sequences, such as HKHD40 and HKNPC60 variants. (C) EBV variants HKHD40 and HKNPC60 are typical of hundreds of other EBV variants. Hundreds of human gamma herpesvirus 4 variants are almost identical to HKHD40 and HKNPC60 over at least 2000 bp. The matching sets of viruses included many high-risk herpesvirus isolates from NPCs [67]. BeAn: BeAn 58058 virus; bp: base pairs; EBV: Epstein-Barr virus; FeLV: feline leukemia virus; HERV: human endogenous retrovirus; HERVK: human endogenous retrovirus K; HIV1: human immunodeficiency virus type 1; HPV18: human papillomavirus 18; HRV: human retrovirus; mPhage: mycolicibacterium phage J1; MSRV: multiple sclerosis retrovirus; NPC: nasopharyngeal cancer; PERV: porcine endogenous retrovirus; RSV: respiratory syncytial virus; Stealth: stealth virus 1.



Many areas on other chromosomes also had 97% human-virus identity over nearly 2500 bp. It is implausible that this much similarity comes from EBV DNA being carried over into the human reference genome. Viral homology occurred with only a small, select portion of viral DNA [68]. Viral homologies were determined for a human genome in a homozygous

karyotype, haploid cell line (46,XX) hydatidiform mole derived only from the paternal chromosomes in an X-bearing sperm cell after fertilization [69]. Results still showed extensive homology between the mole and EBV variants HKHD40 and HKNPC60 (Figure 5B).

HKHD40 and HKNPC60 variant sequences kept appearing in comparisons to human sequences, so these variants were tested against other herpesviruses to determine whether they were unusual. Hundreds of human gamma herpesvirus 4 variants were almost identical to HKHD40 and HKNPC60 over at least 2000 bp (Figure 5C). The matching sets of viruses included many high-risk herpesvirus isolates from NPCs [67]. Based on this information, HKHD40 and HKNPC60 strongly resembled other herpesvirus isolates, including many that confer high risks for NPC [10]. These results show that humans have interacted extensively with EBV; the results are not due to EBV impurities in the human reference genome, and the human genome has had close relationships with oncogenic EBV forms.

Evidence of Past EBV Infection

The evidence thus far supports a central hypothesis that EBV disables tumor suppressor mechanisms in breast cancer and can then disappear. This absence of viral particles is a significant experimental obstacle to testing this hypothesis. Unlike retroviruses, EBV and its variants do not have integrase enzymes, so EBV has no conventional way to insert itself into the human genome. EBV rarely integrates, with only one or two copies in BL cell lines [70].

BLAST analysis found about 65,000 areas of strong homology ($E < 1 \times 10^{-10}$) between the human reference genome and EBV. Because 65,000 is far more than realistic EBV integration events, it suggested the possibility that some EBV sequences were fragments created by a human version of the bacterial CRISPR (clustered regularly interspaced short palindromic repeats) system. As shown previously in Figure 3, breast cancers have breakpoints that cluster around breakpoints in EBV-associated cancers and involve MHC genes.

MHC genes are encoded on chromosome 6p21.3 in a region that becomes a candidate for such a human CRISPR version. Variants of human leukocyte antigens (HLAs) in the MHC are strong risk factors for NPC infections [71] because HLAs are required to break down and display fragments of some antigens to the immune system. A total of 13 breast cancers listed on the COSMIC website had a deletion near this HLA region. About 23% of breast cancers had mutations directly affecting HLA class I or II genes. Many more breast cancers had indirect connections because they had damage to multiple genes that interact with HLAs or were otherwise essential for immunity. The MHC region also holds *NFKB1L1*, a negative regulator of the NPC overexpressed gene hallmark, NF- κ B. The 139 breast cancers from high-risk women had 284 breakpoints at chromosome 6p21.3. Breakpoints in the 70 NPC cancers also clustered there, with 40 breakpoints within the 27,865,296-34,017,013 segment on chromosome 6. Variability in the inactivation of MHC genes reflects the extreme diversity of this region.

In general, the bacterial CRISPR/Cas system loosely resembles the human piRNA system, so the distribution of piRNAs was graphed. As shown in Figure 6A, hundreds of piRNA sequences cluster near the MHC region (at ~29.7 - 33.3 megabases). The piRNA system is known to inactivate virus-derived transposons (related to HERVs) by methylating or cleaving them. The distribution of piRNA fragments was then compared to the distribution of viral DNA fragments in the MHC region of chromosome 6. Figure 6B-F reveals striking similarities in how remnants of exogenous and endogenous viruses distribute relative to piRNAs. Remnants of both virus types were homologous to the same human sequence, and both types were interspaced between piRNA sequences, sometimes right next to each other. Most of these sandwiches were at a regular interval or a multiple of a regular interval.

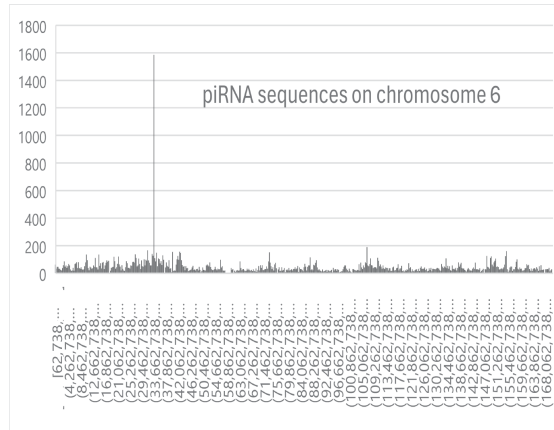
This interspaced arrangement looked so much like CRISPR that it raised the question of whether piRNA defense mechanisms have inactivated some EBV variants in addition to their canonical role with endogenous viruses. Long stretches of endogenous transposon-like DNA sequences routinely matched exogenous viruses. As shown in Figure 6C and E, the same human DNA interval had homology both to endogenous transposons (HERV) and exogenous viral sequences (EBV variants, stealth virus 1, chikungunya virus, BeAn 58058 virus, human papillomavirus [HPV] 16, HIV1, and HERV). This result shows that the piRNA system can store the same piece of DNA to protect DNA against these different viruses.

Chromosome 6p21.3 also contains an EBV infection marker [72]. The marker was examined in 1538 breast cancers using existing methylation data [34]. As indicated in Figure 7, promoter methylation differed significantly from normal controls in the segment shown (30,523,984 - 33,216,811 on chromosome 6). Hypermethylation occurred on *STK19*, a MHC class III gene for RNA surveillance [73,74]. Hypermethylation also occurred on a gene for preventing tumors (*TNFB*) [75] and a gene for responding to antigen-antibody complexes (*C2*). Polymorphisms in *HLA-DMB* antigen and *SAPCD1*, another class III MHC gene [76], at chromosome 6p21.3 had links to Kaposi sarcoma [77]. Human herpesvirus 8 (Kaposi sarcoma virus) is a Kaposi sarcoma driver and is closely related to EBV.

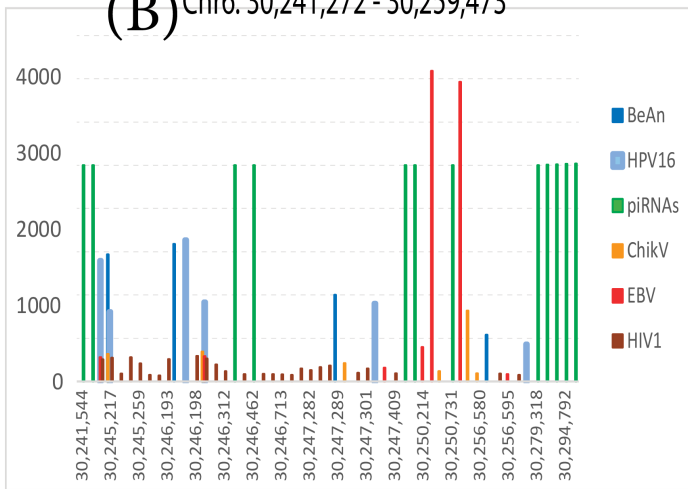
These results reveal that EBV has been attacking human DNA during evolution. There is a piRNA defense mechanism for human DNA near critical immune system genes, but both EBV-associated cancers and breast cancers inactivate some of the genes that guard piRNA defenses. The histocompatibility antigen gene region of chromosome 6 can be extensively fragmented in EBV-associated and breast cancers. MHC genes have the largest number of polymorphic forms in the human genome. This variation creates differences in viral susceptibility and inactivation. Even though most people are infected, not everyone will get an EBV-related disease or cancer.

Figure 6. The human genome organizes piRNA sequences into clusters near the MHC region of chromosome 6 (6p21.3 at ~29.7-33.3 megabases), with hundreds of piRNAs nearby. (A) The levels of various piRNAs varied by more than 1000-fold, but the most abundant piRNAs were the only ones present in every cell. These abundant sequences drive the inactivation of foreign DNA. Rare piRNAs do not function in every cell but can potentially adapt to new genome invaders. (B-F) Arbitrarily selected areas of the chromosome region where piRNAs are most abundant. piRNAs were assigned sufficient homology scores to mark their positions relative to positions with homology to viruses. (C and E) Remnants of both exogenous and endogenous virus types were homologous to the same human sequence, and both types were sandwiched between piRNA sequences, sometimes right next to each other. Most sandwiches were at a regular interval or a multiple of a regular interval. The same human DNA interval has homology to endogenous transposons (HERV) and exogenous viral sequences (ChikV, HIV1, Stealth, BeAn, and HPV16). The piRNA system can store the same piece of DNA to protect DNA against these different viruses. BeAn: BeAn 58058 virus; ChikV: chikungunya virus; Chr: chromosome; EBV: Epstein-Barr virus; HERV: human endogenous retrovirus; HERVK: human endogenous retrovirus K; HIV1: human immunodeficiency virus type 1; HPV16: human papillomavirus 16; MHC: major histocompatibility complex; PERV: porcine endogenous retrovirus; piRNA: Piwi-interacting RNA; Stealth: stealth virus 1.

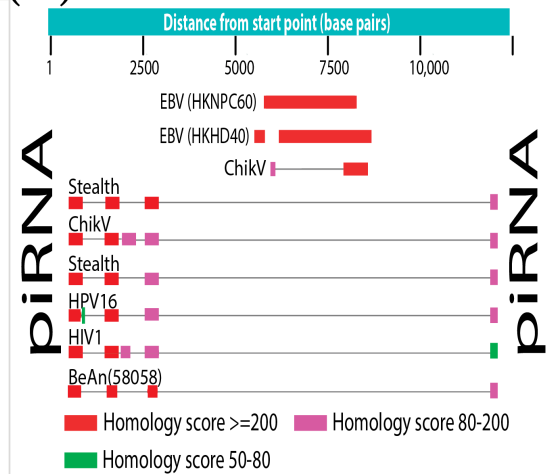
(A)



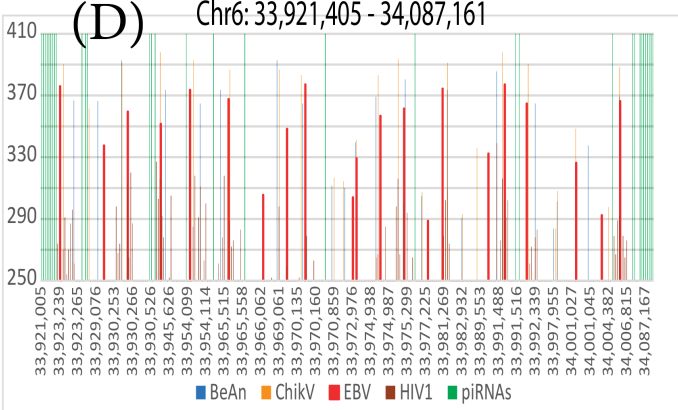
(B) Chr6: 30,241,272 - 30,259,473



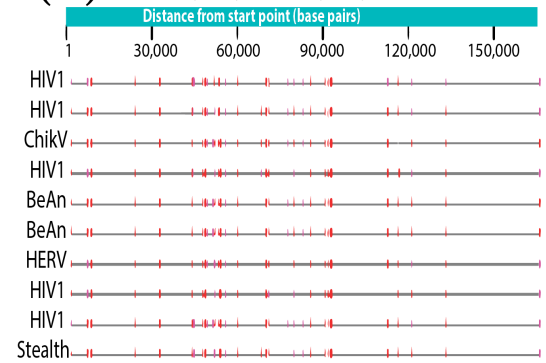
(C) Chr6: 30,241,272 - 30,259,473



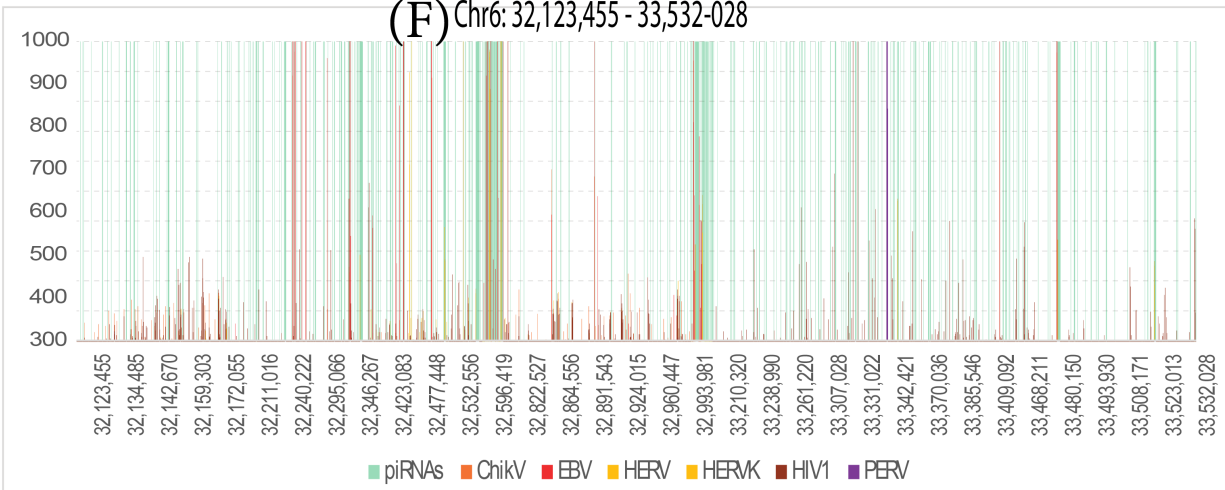
(D) Chr6: 33,921,405 - 34,087,161



(E) Chr6: 33,921,405 - 34,087,161

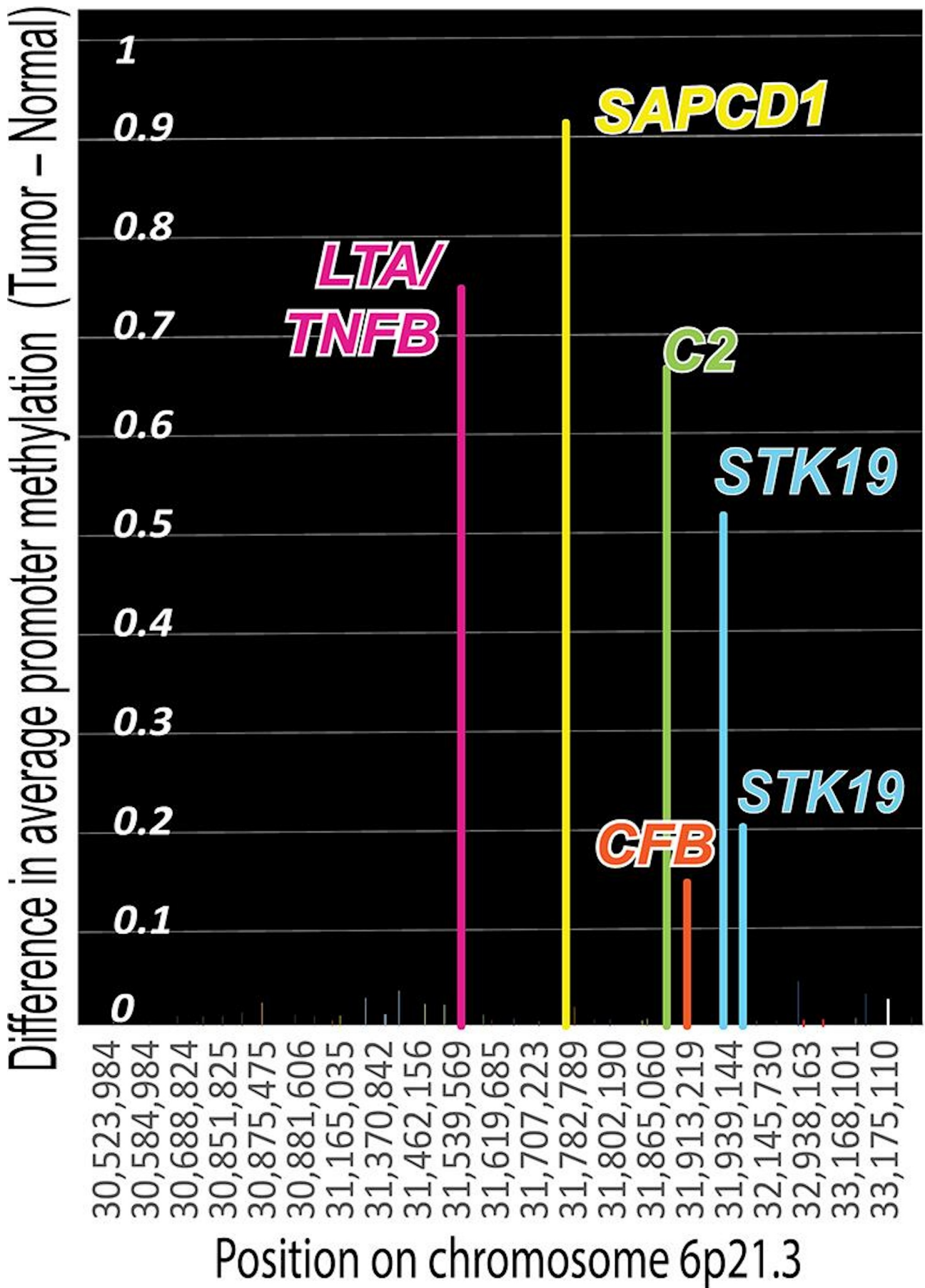


(F) Chr6: 32,123,455 - 33,532,028



Maximum homology score

Figure 7. Chromosome 6p21.3 contains an EBV infection signature [72]. Using existing methylation data [34], the marker was examined in 1538 breast cancers. Promoter methylation in this marker region differed significantly from normal controls. Hypermethylation occurs on *STK19*, an MHC class III region gene [73] for RNA surveillance [74]. Hypermethylation also inhibited *LTA/TNFB*, a gene for preventing tumors [75], and *C2*, which encodes antigen-antibody complex responses. Polymorphisms in *HLA-DMB* antigen and *SAPCD1*, another class III MHC gene [76], at chromosome 6p21.3 have links to Kaposi sarcoma [77]. HHV8 is a Kaposi sarcoma virus closely related to EBV. EBV: Epstein-Barr virus; HHV8: human herpesvirus 8; HLA: human leukocyte antigen; MHC: major histocompatibility complex.



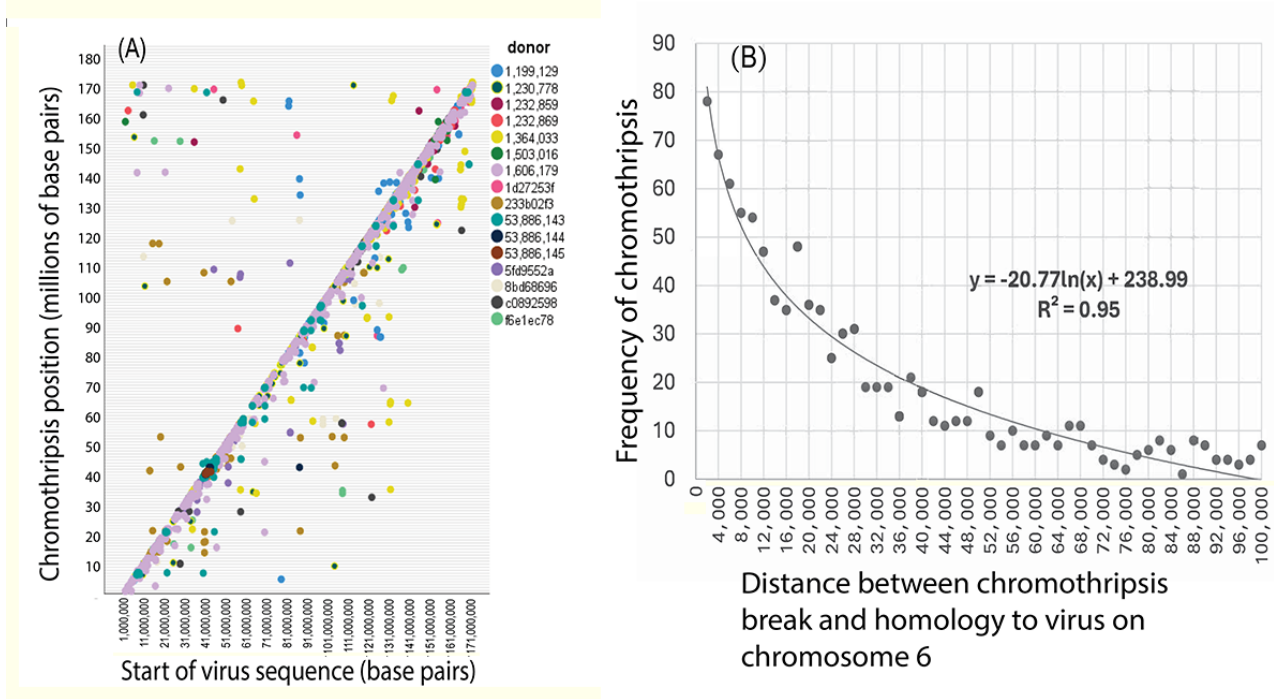
Viral Sequences in Human Genomes as Hypermutation and Rearrangement Sites in Breast Cancers

The next question was whether EBV or other virus-like sequences in the human genome cause multiple rearrangements and clustered hypermutations (chromothripsis). As shown in [Figure 8A](#), many positions on chromosome 6 where chromothripsis occurs [35] congregated around virus sequence start positions. A total of 1090 genome coordinates described chromothripsis fragments with copy number ≥ 3 . These coordinates were unlikely to be random since they did not follow a normal distribution ($P < .001$). By simple linear regression analysis ($R^2 = 0.93$), many viral sequence coordinates strongly

correlated with chromothripsis positions. [Figure 8B](#) shows that as you move further away from a chromothripsis breakpoint, the frequency of breast cancer homology (score > 500) to viruses decreases. This result implicates viral sequences as preferred sites where breast cancer chromosomes begin to fall apart. The equation shown mathematically describes the relationship between chromothripsis frequency and distance from viral sequences, and the constant in the equation suggests a baseline level of breakpoints.

These results suggest that homologous virus sequences at multiple positions could confuse DNA repairs already compromised by EBV in breast cancer and contribute to chromothripsis and clustered rearrangements.

Figure 8. Repetitive copies of virus sequences may confuse compromised DNA repairs and contribute to hypermutation clusters and rearrangements. (A) High-confidence positions where chromosome 6 shatters in 16 breast cancer genomes [35] were plotted against start points of viral sequence homologies. EBV or other viruses then cause groups of rearrangements and hypermutation clusters (chromothripsis). A total of 1090 genome coordinates described chromothripsis fragments with copy number ≥ 3 . These coordinates were unlikely to be completely random since they did not follow a normal distribution ($P < .001$). Genome coordinates on chromosome 6 matching virus sequences were strongly correlated by simple linear regression analysis ($R^2 = 0.93$). (B) As you move further from chromothripsis breakpoints, the frequency of breast cancer homology to viruses decreases, according to the equation shown. The constant in the equation suggests a baseline level of breaks.



EBV and Metastasis

The last question was whether EBV contributes to breast cancer metastasis. According to Yates et al [29], relapsed and metastatic breast cancer tumors keep their tumor-driver gene mutations and continue acquiring new ones. Late mutations in JAK-STAT and SWI-SNF signaling pathways drive established breast cancers into metastasis.

NPC often loses type-1 interferon genes (*IFNA1*, *IFNA2*, *IFNA8*, and *IFNE*) and nearby *MTAP* (32%-34% [11]) by homozygous deletions at chromosome 9p21.3. Interferons initiate canonical JAK-STAT signaling by binding to cell surface receptors that then activate internal Janus kinases (JAKs). The activated JAKs phosphorylate cytoplasmic STAT (signal transducer and

activator of transcription) proteins, which travel to the cell nucleus to activate interferon-responsive genes. The percentages of breast cancers on the COSMIC website with mutations in a “JAK” or “STAT” isoform or transcript variant were calculated: 7.8% had a JAK mutation and 36.7% had a STAT mutation. Deletions of interferon genes in NPC also facilitate viral replication and block interferon from activating JAK-STAT signaling. Breast cancers ([Multimedia Appendix 2](#)) have 65 breakpoints strictly within this interferon-*MTAP* region (21,579,478 - 20,503,534 on chromosome 9), not counting longer fragments that include the interval. As shown in [Figure 9](#), breast cancer breakpoints align well with EBV-associated cancer breakpoints near the large cluster of interferon genes on chromosome 9.

Figure 9. Damage to JAK-STAT and SWI-SNF signals pushes breast cancer into metastasis [29]. EBV interferes with these signaling pathways to facilitate viral replication. (A) Breakpoints in breast cancers on chromosome 9 facilitated viral replication and blocked sources of JAK-STAT signaling, including a large cluster of interferon genes on chromosome 9. Breast cancers can disable SWI-SNF by targeting *ARID* genes. (B) *ARIDIA* was encoded on chromosome 1 near a hot spot where multiple breast cancer breakpoints approximately aligned with breakage points in EBV-associated cancers. Another site at about 150,000,000 bp had a histone-rich region nearby. SWI-SNF affects histones, which also profoundly affects metastasis [78]. The GRCh38 genome version does not include centromere sequences due to technical limitations. *ANXA1*: Annexin A1; BL: Burkitt lymphoma; bp: base pairs; BRC: breast cancer; Chr: chromosome; EBV: Epstein-Barr virus; GC: gastric cancer; NPC: nasopharyngeal cancer; SWI-SNF: switch/sucrose non-fermentable.

Mutations in EBV-associated cancers show that Yates metastasis driver gene damage accompanies EBV infection. SWI-SNF (switch/sucrose non-fermentable) is a complex that repositions nucleosomes and supports genome stability [79]. SWI-SNF addresses obstacles to replication sensed by the FA-BRCA pathway [79,80]. Referring back to Figure 3, clustered breast cancer breakpoints on chromosome 17 around EBV breakpoints affect the SWI-SNF component *SMARCE1*. In addition, breast cancers can disable SWI-SNF by targeting *ARID* genes [29]. *ARIDIA* is a COSMIC top-20 most frequently mutated gene in breast cancer. Like breast cancer, NPC has multiple recurrent aberrations in *ARIDIA* genes. As shown in Figure 9, *ARIDIA* lies near a hot spot where multiple breast cancer breakpoints approximately aligned with breakage points in EBV-associated cancers. The loss of *ARIDIA* activates Annexin A1, which aligned closely with a region targeted by EBV-associated cancers on chromosome 9. A chromosome-1 site at about 150 million bp had a nearby histone-rich gene region. Histones are chromatin structures that SWI-SNF dynamically remodels to regulate access to genetic information. Histones can profoundly affect metastasis [78]. Figure 9 also reveals many additional alignments between breakpoints in breast and EBV-associated cancers that were not investigated further.

NPC often inactivates SWI-SNF components *BAP1* and *PBRM1* within a frequently damaged 3p21.3 gene cluster [11] at 52,400,000 - 53,000,000 on chromosome 3. Analyses of breast cancers found 18 breakpoints within this short interval. DLBCL, another EBV-linked cancer, also had recurrent alterations in components of SWI-SNF complexes [81].

The Warburg effect (oxidative glycolysis) [68] favors metastasis. The Warburg effect occurs in NPC because pyruvate dehydrogenase (*PDHB*) genes on chromosome 3p are deleted or rearranged in almost all cases. Similar changes to chromosome 3p were found in breast cancers, which also undergo the Warburg effect [68]. This Warburg metabolic switch favors metastasis because it mitigates oxidative stress on cancer cells. Large amounts of lactate accumulate in the absence of *PDHB* to acidify the tumor microenvironment and interfere with the destruction of metastatic cells [82].

This section's results show that EBV may push breast cancer into metastasis by interfering with JAK-STAT and SWI-SNF signaling pathways to facilitate viral replication while making the microenvironment more favorable to tumor growth.

Alternative Explanations for Breast Cancer Breakpoints That Do Not Involve EBV Variants

Subgroups

To determine whether breakpoint similarities in viral and breast cancers depended on specific subgroups, relationships to NPC were compared in triple-negative and HER2-positive breast cancers (20 and 22 patients, respectively). Triple-negative breast cancers are likely to be *BRCA1* mutation positive [83], while HER2 amplification is uncommon in *BRCA1* and *BRCA2* mutation carriers [84]. Although subgroup differences are noticeable, results still show that both subgroups had breakpoints on all chromosomes related to NPC (Figure S1A in Multimedia Appendix 5).

Tumor-Infiltrating Lymphocytes

Tumor-infiltrating lymphocytes (TILs) are biomarkers for predicting breast cancer prognosis [85,86]. To test whether TILs cause chromosome breaks, breakpoint numbers in 16 breast cancers with severe lymphocyte infiltration were compared to 17 breast cancers with nil lymphocyte infiltration. The 2-tailed Student *t* test could not reject the null hypothesis that the numbers of breakpoints were statistically identical ($P=.70$; Figure S1B in Multimedia Appendix 5). This result does not rule out differences in prognosis due to differences in lymphocyte infiltration.

Retroviruses

Retrovirus contributions to structural variations were estimated using data from cancer in 38 different tissues [87]. Retrotransposons make relatively modest contributions to breast cancer compared to, say, esophageal or oral (gums) cancer (Multimedia Appendix 5). EBV can transactivate endogenous retroviruses [11,87,88]. DNA near some breast cancer breakpoints resembles porcine endogenous retrovirus, HERV, and HIV1 (eg, Figure 5). The human genome also contains DNA matching the retrovirus mouse mammary tumor virus [89,90] at 23 sites that give BLAST homology scores >200. HPV variants are DNA viruses that are also implicated in breast cancer. HPVs were not assessed further, but they occasionally matched DNA near breast cancer breakpoints.

Common Fragile Sites

Common fragile sites are site-specific breaks seen on metaphase chromosomes after inhibiting DNA synthesis via DNA polymerase inhibitors. Some common fragile sites [54] aligned with breast cancer breaks on chromosome 1, but breakpoints on most other chromosomes were incompatible. Chromosomes 8, 9, 11 - 15, 17-19, 21, and 22 do not have common fragile sites but still have many breast cancer breaks [91]. However, the human genome has over 13 million palindromes that are ≤40 bp [92]. The generation of rare fragile sites by palindromes or their attraction to EBV cannot be excluded.

Imperfect Palindrome Repeats

An alternative explanation for EBV-related carcinogenesis involves the docking of EBNA1 virus-tethering protein at imperfect palindromes [93] tandemly repeated on chromosome 11. The docked EBNA1 binds EBV circular episomes, and chromosome 11 breaks initiate malignancy. To test this explanation, existing literature data were first compared to the specific human EBNA1-binding site [48,66,94]. The results (Table S2 in Multimedia Appendix 4) are incompatible with a single host sequence binding EBNA1.

BLAST analysis showed that matches to the imperfect palindrome were likely due to pure chance with *E* values between 16 and 964 for 4352 matches, from 12 to 18 bp. Chromosome 11 had only 197 of these 4352 matches, and none were near the palindromic region. The prototype DNA palindrome (Table S2 in Multimedia Appendix 4, line 2) produced 7074 matches with *E* values ranging from 0.25 to 964. Further BLAST analyses of the slightly different docking sequence in EBNA1-DNA crystals (Table S3 in Multimedia Appendix 4, line 1) against other genome assemblies [95]

revealed matches on chromosomes 2, 19, 4, and 12. Various isolates of HIVs had 52 matching sequences.

In 94 BL samples from patients who were EBV positive, breakpoints concentrated within chromosomes 2, 8, 13, 14, and 22 (Figure S1D in [Multimedia Appendix 5](#)). Chromosome 14 contained 610 breakpoints (*IgVH* regions), and chromosome 2 (*IgVK* regions) contained 522 breakpoints. EBV hijacks activation-induced cytidine deaminase, a mutagenic enzyme that generates antibody gene variants in response to myriad antigens. In the 94 EBV-positive BL cases, the palindromic locus was nearly 100 million bp away from the principal breakpoint coordinates (Figure S1E in [Multimedia Appendix 5](#)). Only 19 (20%) of the 94 patients who were EBV positive [96] had breakpoints anywhere on chromosome 11. The palindromic locus was also not involved in diverse cancers from 8227 patients [97] ([Multimedia Appendix 5](#)).

The results in this section show that alternative explanations that invoke subgroups, TILs, retroviruses, or a specific palindromic repeat locus are incompatible with the associations between EBV-associated and breast cancers .

Discussion

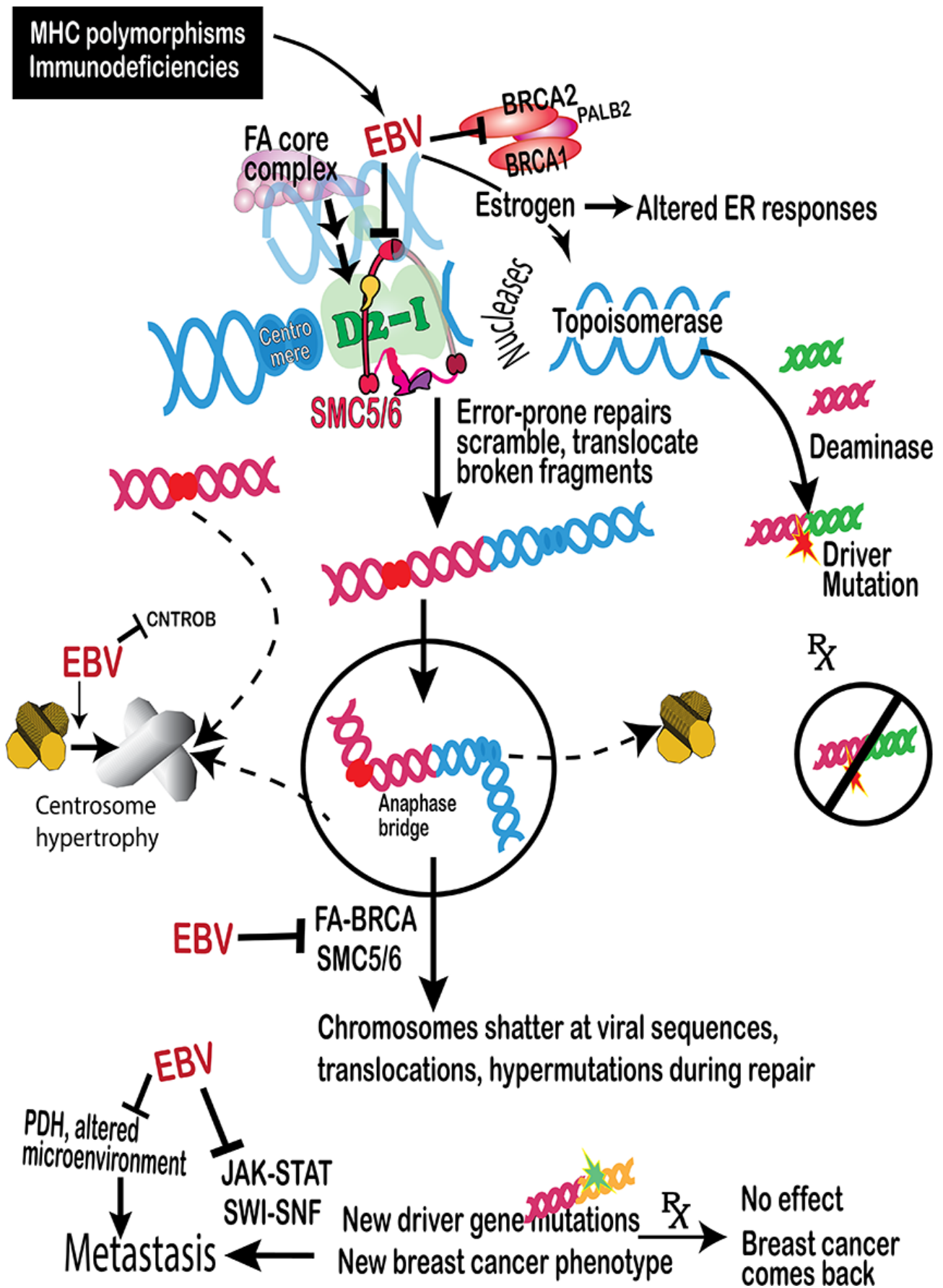
Principal Findings

This study finds that EBV contributes to breast cancer by disabling safeguards against tumors. Cancer then occurs because the safeguards remain disabled even if the virus is cleared. Multiple independent analyses identified residual genetic and epigenetic damage in cancer genomes and formed the basis of the model in [Figure 10](#). Breakpoints in breast cancers in high-risk women, sporadic breast cancers, and even ovarian cancers cluster around breakpoints in known EBV-related cancers, including NPC, BL, DLBCL, and GC. Some genes clustered near breakpoints in these diverse EBV-associated cancers are critical to preventing breast cancers. Some breast cancer breakpoints are near genes at EBV-docking sites. Varying numbers of DNA breaks occur within the highly polymorphic forms of MHC region genes on chromosome 6. This damage adds to susceptible polymorphisms and immunodeficiencies to

help explain why not everyone develops EBV-related cancers. Near the MHC region on chromosome 6, piRNA sequences are regularly interspaced between viral DNA sequences. The sandwiched arrangements are presumptive evidence of past infection and probably represent a DNA defense mechanism. These defenses fail when chromosome 6 breaks apart near start points of the large number of repetitive viral sequences in the human genome. The viral sequences confuse repairs already damaged by EBV, and bursts of mutation occur where scrambled fragments ligate. EBV disables the most reliable restoration of broken chromosomes back to their native forms, so repairs form structures with multiple centromeres. These structures undergo additional rounds of fragmentation during cell division. The process continually forms new cancer driver mutations and allows cancer to come back after successful therapy ([Figure 10](#)). An EBV methylation signature on chromosome 6 was far more abundant in 1538 breast cancers than in normal controls. Finally, EBV facilitates its own replication by damaging JAK-STAT and SWI-SNF signaling pathways, which pushes breast cancer into metastasis, while virus-associated changes on chromosome 3p interfere with the destruction of metastatic cells. Models [8,98] of EBV-infected human mammary cell cultures transplanted into immunosuppressed mice and EBV loss from NPC cells are consistent with these results.

The study herein has current and future clinical implications in addressing cancers and chronic diseases. An early childhood vaccine against EBV may reduce the incidence of breast cancer on a global scale. If this vaccine even approaches the effectiveness of the HPV vaccine for cervical cancer, then the reduction of breast cancer incidence would be substantial. In breast cancer cases where active infection can be demonstrated, immunotherapy or antivirals can be considered. The results also heighten concern about hidden dangers from viral infections. EBV infection leaves behind persistent genome abnormalities (“long EBV”) linked to breast cancer. Not everyone develops an EBV-related cancer even though almost everyone is infected, suggesting risk assessment should include MHC polymorphisms. MHC genes have abundant connections to both EBV infection [99] and breast cancer [100-102].

Figure 10. Model proposed to explain the results. EBV causes serious disease in only some people due to MHC variants and other damage to the immune system. Viral nucleases are one source of chromosome breaks. EBV causes inappropriate expression of estrogen and transcription targets of occupied estrogen receptors. Transcription induced by artificially high estrogen levels then induces topoisomerase-mediated DNA breaks. EBV-mediated deregulation of estrogen production, topoisomerase activity, and deaminase activation then collaborate to cause chromosome breaks and drive translocations [68]. EBV-associated cancers share additional genome deficits with breast cancers, which interfere with restoring the genome from DNA crosslinks and DNA double-strand breaks. If crosslinks and DNA breaks persist during cell division, they also cause chromosome rearrangements and cancer. The cancer safeguards targeted by EBV extend to the *BRCA* pathway, FA proteins, an SMC5/6 scaffold, JAK-STAT signaling, and the SWI-SNF chromatin remodeling complex. EBV: Epstein-Barr virus; ER: estrogen receptor; FA: Fanconi anemia; MHC: major histocompatibility complex; PDH: pyruvate dehydrogenase; SWI-SNF: switch/sucrose non-fermentable.



The strategy of using bioinformatics to identify markers of “long EBV” may well work for other cancers, multiple sclerosis [103], and other chronic diseases that are currently unexplained.

Testing for persistent viral damage in genomes from biopsies is a new method for screening for breast cancer risk. The results may inform further prevention and treatment decisions. Cancer

drug therapy has focused on finding and destroying cancer-driver gene products. The drugs are initially effective, sometimes for long periods, but then stop working. The cycles represented in [Figure 10](#) are an occult, underlying process that can now be evaluated. Cancer treatment generates new clones that do not exist in the original population [104]. The underlying genome damage and EBV scars continually produce new cancer-driver mutations. Some antigens targeted by successful therapy for hematologic malignancies [105], such as DLBCL, may also be effective for breast cancers. The idea that breast cancers and hematologic malignancies can have similar breakpoints and translocation fusions suggests that there may be many more susceptible targets and that there are options to overcome resistance or tolerance [106]. The findings may further stimulate research into other EBV-associated diseases and cancers, leading to better and broader understanding.

Estrogen has been thought to generate the initial chromosome breakpoints leading to translocations in human breast cancer. However, young boys with BL do not produce estrogen from ovaries, yet [Figure 3](#) shows that their malignant B-cells have many breakpoints [68,107] that approximately match breast cancer breakpoints. Normally, aromatase catalyzes the rate-limiting step in estrogen production [108], and aromatase acting on androgens is the primary source of most estrogens in breast tissue [109]. EBV-infected cells lose control of aromatase activity [108]. An EBV-mediated increase in aromatase activity explains why locations of breakpoints ([Multimedia Appendix 5](#)) are relatively independent of estrogen receptor status in breast cancer [68] and resemble locations in lymphoid cells ([Figures 1-4 and 9](#)). Transcription in response to artificially high estrogen levels created by EBV then induces topoisomerase-mediated DNA breaks. Double-strand break repair genes remove topoisomerase from these complexes, but damage to this process leaves pathological enzyme complexes still bound at a DNA breakpoint [110-112]. As shown in [Figure 3](#), topoisomerase itself may be damaged. In either case, EBV-mediated deregulation of estrogen production, topoisomerase activity, and deaminases then collaborate to cause chromosome breaks and drive breast cancer.

Breast cancer chromosome breakpoints cluster around genes near EBV-binding sites ([Figure 4](#)), further suggesting that EBV participates in causing the breaks. The breaks lead to pathogenic chromosome rearrangements because EBV-induced damage forces restoration into error-prone methods by suppressing FA-BRCA pathway intermediates [14,15]. Repairs using the FA-BRCA pathway [113] need chromatin access, which requires the SMC5/6 cohesin complex [114,115]. In one scenario shown in [Figure 10](#), SMC5/6 interacts with a crucial pathway intermediate, the FANCD2-FANCI heterodimer (“D2-I”) [17,116]. EBV variants deplete SMC5/6, preventing FA-BRCA-mediated DNA repairs and leading to chromosomes with too many centromeres. When mitosis pulls apart multicentromere chromosome structures, the forces shatter the chromosome and induce mutation storms [35]. EBV thus threatens a sprawling, interconnected repair system, including the BRCA pathway, FA proteins, an SMC5/6 scaffold, JAK-STAT signaling, and the SWI-SNF chromatin remodeling complex ([Figure 10](#)).

Of course, other environmental, genetic, or lifestyle factors also participate in breast cancer development, but EBV infection exacerbates their effects. Genome deficits in EBV-associated cancers and breast cancers interfere with restoring chromosomes from damage due to natural processes and exogenous mutagens. Some of this damage requires repair pathways that are subject to EBV interference.

Evidence underlying the model in [Figure 10](#) has independent support from the literature. For example, viral load is a marker for the extent of cell-free DNA fragmentation [117]. EBV-mediated transformation routinely generates abnormal karyotypes [118]. The binding of EBNA1 sequence variants increases NPC risk and drives EBV lytic gene expression [119,120], which requires EBV-encoded nucleases [121-123]. Other herpesviruses related to EBV share the ability to fragment DNA and subvert DNA repair pathways [124-126]. EBV facilitates its own replication by interfering with signaling pathways that prevent metastasis [29,127-130]. Independent literature supports EBV participation in metastasis and the results shown in [Figure 9](#). NPC has the highest metastatic rate among all head and neck cancers, and the levels of circulating EBV markers are highly predictive [10]. Finding EBV in lymph nodes of patients with NPC or primary cancer at an unknown site helps detect metastasis [131]. NPC patients with ≥ 500 copies of EBV per mL plasma had significantly higher rates of liver metastasis than patients with lower EBV levels [132]. EBV-infected B-cells and breast cancer cells both have amplified centrosomes ([Figure 10](#)), the mitosis-organizing centers that exert structural control over cell division. The EBV protein thymidine kinase takes up residence in the centrosome [133], and another EBV protein, BNRF1, initiates centrosome amplification in infected B-cells [134]. Overduplication of centrosomes confuses chromatid attachments to spindle fibers during mitosis. Chromosomes do not distribute properly into daughter cells, creating mistakes when the genome replicates [134,135]. Neither centrosome amplification nor chromosome fragmentation (chromothripsis) requires large numbers of viral particles or active infection.

Further bioinformatic tests may still add significant additional information. EBV activation brings massive changes to host chromatin methylation and structure [47,51,136]. Breast cancers have hundreds of these changes [34]. Results here further implicate epigenetic effects, so EBV effects on breast cancer epigenetics should be explored in more detail. EBV is implicated in cancers in multiple additional organs, and the methods developed here may help clarify its potential contributions. Predictions based on virus-human interaction structural biology may also be helpful. The ultimate direct test will be whether childhood recipients of an anti-EBV vaccine have reduced breast cancer incidence. If it even approaches the reduction of cervical cancer achieved by the HPV vaccine (up to 94%), a childhood EBV vaccine could effectively prevent many cases of breast cancer.

Limitations

EBV itself creates a limitation because the virus can disappear after causing pathogenic genome damage that allows breast cancer to develop. This transitory virus presence forces the use

of bioinformatics to look for persistent genome damage EBV leaves behind. EBV disappearance questions whether a group of cancers with EBV connections also contains “sporadic” cancers typed as EBV negative. The EBV-negative forms may have merely lost the criteria used to identify EBV infection, but EBV-related genome damage may still remain. Another limitation is that compared to breast cancers, known EBV-linked cancers such as GC, BL, and NPC are less common, so genome sequence data are also less common.

Conclusions

In summary, early childhood immunizations against inactivated EBV or selected EBV gene products may significantly reduce the incidence of breast, ovarian, and other cancers, and potentially unexplained chronic diseases. EBV variants lead to

DNA breaks, mitotic abnormalities, and the loss of safeguards that protect against breast cancer and its metastasis. Breast cancer breakpoints cluster around breakpoints in EBV cancers, disrupting genes essential to prevent viral infection and breast cancers. A CRISPR-like region on chromosome 6 sequesters some of the thousands of pieces of EBV sequences in the human genome. The same area of chromosome 6 undergoes variable damage in breast cancer, contributing to the reason not everyone with EBV infection develops cancer. In susceptible people, EBV infection leaves behind pathogenic cancer-associated genome abnormalities (“long EBV”). Clinical implications include improvements in evaluating the chances that cancer will return, increased use of immunotherapy for patients with breast cancer that have active infection, and greater urgency in developing an effective EBV vaccine.

Data Availability

The primary dataset and calculations that were generated or analyzed during this study are included. Datasets not included are freely available from the original sources or the corresponding author on reasonable request.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Glossary and abbreviations.

[[DOCX File, 20 KB](#) - [xmed_v6i1e50712_app1.docx](#)]

Multimedia Appendix 2

Most of the calculations used in this work.

[[XLSX File, 7644 KB](#) - [xmed_v6i1e50712_app2.xlsx](#)]

Multimedia Appendix 3

The exact distances between nasopharyngeal cancer and breast cancer breakpoints on chromosome 1. These breaks gather around a few low valleys that periodically occur across the whole chromosome, but the data points are too numerous to display, making the results difficult to interpret.

[[PNG File, 213 KB](#) - [xmed_v6i1e50712_app3.png](#)]

Multimedia Appendix 4

Gene functions at breast cancer breakpoints that clustered around breakpoints in EBV-associated cancers (GC, BL, and NPC), and EBNA1-binding sequences reported in the human genome. BL: Burkitt lymphoma; EBNA1: Epstein-Barr virus nuclear antigen 1; EBV: Epstein-Barr virus; NPC: nasopharyngeal cancer.

[[DOCX File, 31 KB](#) - [xmed_v6i1e50712_app4.docx](#)]

Multimedia Appendix 5

Alternative explanations.

[[PNG File, 374 KB](#) - [xmed_v6i1e50712_app5.png](#)]

References

1. DeSantis CE, Ma J, Gaudet MM, et al. Breast cancer statistics, 2019. *CA Cancer J Clin* 2019 Nov;69(6):438-451. [doi: [10.3322/caac.21583](#)] [Medline: [31577379](#)]
2. QuickStats: age-adjusted death rates* for female breast cancer,† by state — National Vital Statistics System, United States, 2019§. *MMWR Morb Mortal Wkly Rep* 2021 Oct 1;70(39):1391. [doi: [10.15585/mmwr.mm7039a6](#)] [Medline: [34591833](#)]
3. Sarid R, Gao SJ. Viruses and human cancer: from detection to causality. *Cancer Lett* 2011 Jun 28;305(2):218-227. [doi: [10.1016/j.canlet.2010.09.011](#)] [Medline: [20971551](#)]

4. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002 May;2(5):342-350. [doi: [10.1038/mrc798](https://doi.org/10.1038/mrc798)] [Medline: [12044010](https://pubmed.ncbi.nlm.nih.gov/12044010/)]
5. Balfour HHJ, Sifakis F, Sliman JA, Knight JA, Schmeling DO, Thomas W. Age-specific prevalence of Epstein-Barr virus infection among individuals aged 6–19 years in the United States and factors affecting its acquisition. *J Infect Dis* 2013 Oct 15;208(8):1286-1293. [doi: [10.1093/infdis/jit321](https://doi.org/10.1093/infdis/jit321)] [Medline: [23868878](https://pubmed.ncbi.nlm.nih.gov/23868878/)]
6. Huo Q, Zhang N, Yang Q. Epstein-Barr virus infection and sporadic breast cancer risk: a meta-analysis. *PLoS One* 2012;7(2):e31656. [doi: [10.1371/journal.pone.0031656](https://doi.org/10.1371/journal.pone.0031656)] [Medline: [22363698](https://pubmed.ncbi.nlm.nih.gov/22363698/)]
7. Fina F, Romain S, Ouafik L, et al. Frequency and genome load of Epstein-Barr virus in 509 breast cancers from different geographical areas. *Br J Cancer* 2001 Mar 23;84(6):783-790. [doi: [10.1054/bjoc.2000.1672](https://doi.org/10.1054/bjoc.2000.1672)] [Medline: [11259092](https://pubmed.ncbi.nlm.nih.gov/11259092/)]
8. Hu H, Luo ML, Desmedt C, et al. Epstein-Barr virus infection of mammary epithelial cells promotes malignant transformation. *EBioMedicine* 2016 Jul;9:148-160. [doi: [10.1016/j.ebiom.2016.05.025](https://doi.org/10.1016/j.ebiom.2016.05.025)] [Medline: [27333046](https://pubmed.ncbi.nlm.nih.gov/27333046/)]
9. God JM, Haque A. Burkitt lymphoma: pathogenesis and immune evasion. *J Oncol* 2010;2010:516047. [doi: [10.1155/2010/516047](https://doi.org/10.1155/2010/516047)] [Medline: [20953370](https://pubmed.ncbi.nlm.nih.gov/20953370/)]
10. Xu M, Yao Y, Chen H, et al. Genome sequencing analysis identifies Epstein-Barr virus subtypes associated with high risk of nasopharyngeal carcinoma. *Nat Genet* 2019 Jul;51(7):1131-1136. [doi: [10.1038/s41588-019-0436-5](https://doi.org/10.1038/s41588-019-0436-5)] [Medline: [31209392](https://pubmed.ncbi.nlm.nih.gov/31209392/)]
11. Bruce JP, To KF, Lui VWY, et al. Whole-genome profiling of nasopharyngeal carcinoma reveals viral-host co-operation in inflammatory NF- κ B activation and immune escape. *Nat Commun* 2021 Jul 7;12(1):4193. [doi: [10.1038/s41467-021-24348-6](https://doi.org/10.1038/s41467-021-24348-6)] [Medline: [34234122](https://pubmed.ncbi.nlm.nih.gov/34234122/)]
12. Fountzilas G, Psyrris A, Giannoulidou E, et al. Prevalent somatic BRCA1 mutations shape clinically relevant genomic patterns of nasopharyngeal carcinoma in Southeast Europe. *Int J Cancer* 2018 Jan 1;142(1):66-80. [doi: [10.1002/ijc.31023](https://doi.org/10.1002/ijc.31023)] [Medline: [28857155](https://pubmed.ncbi.nlm.nih.gov/28857155/)]
13. Devanaboyina M, Kaur J, Whiteley E, et al. NF- κ B signaling in tumor pathways focusing on breast and ovarian cancer. *Oncol Rev* 2022 Oct 3;16:10568. [doi: [10.3389/or.2022.10568](https://doi.org/10.3389/or.2022.10568)] [Medline: [36531159](https://pubmed.ncbi.nlm.nih.gov/36531159/)]
14. Lung RWM, Tong JHM, Ip LM, et al. EBV-encoded miRNAs can sensitize nasopharyngeal carcinoma to chemotherapeutic drugs by targeting BRCA1. *J Cell Mol Med* 2020 Nov;24(22):13523-13535. [doi: [10.1111/jcmm.16007](https://doi.org/10.1111/jcmm.16007)] [Medline: [33074587](https://pubmed.ncbi.nlm.nih.gov/33074587/)]
15. Hau PM, Tsao SW. Epstein-Barr virus hijacks DNA damage response transducers to orchestrate its life cycle. *Viruses* 2017 Nov 16;9(11):341. [doi: [10.3390/v9110341](https://doi.org/10.3390/v9110341)] [Medline: [29144413](https://pubmed.ncbi.nlm.nih.gov/29144413/)]
16. Dheekollu J, Wiedmer A, Ayyanathan K, Deakye JS, Messick TE, Lieberman PM. Cell-cycle-dependent EBNA1-DNA crosslinking promotes replication termination at oriP and viral episome maintenance. *Cell* 2021 Feb 4;184(3):643-654.e13. [doi: [10.1016/j.cell.2020.12.022](https://doi.org/10.1016/j.cell.2020.12.022)] [Medline: [33482082](https://pubmed.ncbi.nlm.nih.gov/33482082/)]
17. Rossi F, Helbling-Leclerc A, Kawasumi R, et al. SMC5/6 acts jointly with Fanconi anemia factors to support DNA repair and genome stability. *EMBO Rep* 2020 Feb 5;21(2):e48222. [doi: [10.15252/embr.201948222](https://doi.org/10.15252/embr.201948222)] [Medline: [31867888](https://pubmed.ncbi.nlm.nih.gov/31867888/)]
18. Yiu SPT, Guo R, Zerbe C, Weekes MP, Gewurz BE. Epstein-Barr virus BNRF1 destabilizes SMC5/6 cohesin complexes to evade its restriction of replication compartments. *Cell Rep* 2022 Mar 8;38(10):110411. [doi: [10.1016/j.celrep.2022.110411](https://doi.org/10.1016/j.celrep.2022.110411)] [Medline: [35263599](https://pubmed.ncbi.nlm.nih.gov/35263599/)]
19. Fan Y, Ying H, Wu X, et al. The mutational pattern of homologous recombination (HR)-associated genes and its relevance to the immunotherapeutic response in gastric cancer. *Cancer Biol Med* 2020 Nov 15;17(4):1002-1013. [doi: [10.20892/j.issn.2095-3941.2020.0089](https://doi.org/10.20892/j.issn.2095-3941.2020.0089)] [Medline: [33299649](https://pubmed.ncbi.nlm.nih.gov/33299649/)]
20. Parvin S, Labrada AR, Santiago GE, et al. Novel role of LMO2 in DNA repair control in diffuse large B cell lymphoma. *Blood* 2016 Dec 2;128(22):776-776. [doi: [10.1182/blood.V128.22.776.776](https://doi.org/10.1182/blood.V128.22.776.776)]
21. Busch K, Keller T, Fuchs U, et al. Identification of two distinct MYC breakpoint clusters and their association with various IGH breakpoint regions in the t(8;14) translocations in sporadic Burkitt-lymphoma. *Leukemia* 2007 Aug;21(8):1739-1751. [doi: [10.1038/sj.leu.2404753](https://doi.org/10.1038/sj.leu.2404753)] [Medline: [17541401](https://pubmed.ncbi.nlm.nih.gov/17541401/)]
22. Chong LC, Ben-Neriah S, Slack GW, et al. High-resolution architecture and partner genes of MYC rearrangements in lymphoma with DLBCL morphology. *Blood Adv* 2018 Oct 23;2(20):2755-2765. [doi: [10.1182/bloodadvances.2018023572](https://doi.org/10.1182/bloodadvances.2018023572)] [Medline: [30348671](https://pubmed.ncbi.nlm.nih.gov/30348671/)]
23. Xu J, Chen Y, Olopade OI. MYC and breast cancer. *Genes Cancer* 2010 Jun;1(6):629-640. [doi: [10.1177/1947601910378691](https://doi.org/10.1177/1947601910378691)] [Medline: [21779462](https://pubmed.ncbi.nlm.nih.gov/21779462/)]
24. Umbreit NT, Zhang CZ, Lynch LD, et al. Mechanisms generating cancer genome complexity from a single cell division error. *Science* 2020 Apr 17;368(6488):eaba0712. [doi: [10.1126/science.aba0712](https://doi.org/10.1126/science.aba0712)] [Medline: [32299917](https://pubmed.ncbi.nlm.nih.gov/32299917/)]
25. McClintock B. The stability of broken ends of chromosomes in *Zea mays*. *Genetics* 1941 Mar;26(2):234-282. [doi: [10.1093/genetics/26.2.234](https://doi.org/10.1093/genetics/26.2.234)] [Medline: [17247004](https://pubmed.ncbi.nlm.nih.gov/17247004/)]
26. Lee JJK, Jung YL, Cheong TC, et al. ER α -associated translocations underlie oncogene amplifications in breast cancer. *Nature New Biol* 2023 Jun;618(7967):1024-1032. [doi: [10.1038/s41586-023-06057-w](https://doi.org/10.1038/s41586-023-06057-w)] [Medline: [37198482](https://pubmed.ncbi.nlm.nih.gov/37198482/)]
27. McAulay KA, Higgins CD, Macsween KF, et al. HLA class I polymorphisms are associated with development of infectious mononucleosis upon primary EBV infection. *J Clin Invest* 2007 Oct;117(10):3042-3048. [doi: [10.1172/JCI32377](https://doi.org/10.1172/JCI32377)] [Medline: [17909631](https://pubmed.ncbi.nlm.nih.gov/17909631/)]
28. Aboulghras S, Khalid A, Makeen HA, et al. Polymorphism of HLA and susceptibility of breast cancer. *Front Biosci (Landmark Ed)* 2024 Feb 5;29(2):55. [doi: [10.31083/j.fbl2902055](https://doi.org/10.31083/j.fbl2902055)] [Medline: [38420797](https://pubmed.ncbi.nlm.nih.gov/38420797/)]

29. Yates LR, Knappskog S, Wedge D, et al. Genomic evolution of breast cancer metastasis and relapse. *Cancer Cell* 2017 Aug 14;32(2):169-184.e7. [doi: [10.1016/j.ccell.2017.07.005](https://doi.org/10.1016/j.ccell.2017.07.005)] [Medline: [28810143](https://pubmed.ncbi.nlm.nih.gov/28810143/)]
30. Friedenson B. Dewey defeats Truman and cancer statistics. *J Natl Cancer Inst* 2009 Aug 19;101(16):1157. [doi: [10.1093/jnci/djp203](https://doi.org/10.1093/jnci/djp203)] [Medline: [19561316](https://pubmed.ncbi.nlm.nih.gov/19561316/)]
31. Nik-Zainal S, Davies H, Staaf J, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature New Biol* 2016 Jun 2;534(7605):47-54. [doi: [10.1038/nature17676](https://doi.org/10.1038/nature17676)] [Medline: [27135926](https://pubmed.ncbi.nlm.nih.gov/27135926/)]
32. Staaf J, Glodzik D, Bosch A, et al. Whole-genome sequencing of triple-negative breast cancers in a population-based clinical study. *Nat Med* 2019 Oct;25(10):1526-1533. [doi: [10.1038/s41591-019-0582-4](https://doi.org/10.1038/s41591-019-0582-4)] [Medline: [31570822](https://pubmed.ncbi.nlm.nih.gov/31570822/)]
33. Nones K, Johnson J, Newell F, et al. Whole-genome sequencing reveals clinically relevant insights into the aetiology of familial breast cancers. *Ann Oncol* 2019 Jul 1;30(7):1071-1079. [doi: [10.1093/annonc/mdz132](https://doi.org/10.1093/annonc/mdz132)] [Medline: [31090900](https://pubmed.ncbi.nlm.nih.gov/31090900/)]
34. Batra RN, Lifshitz A, Vidakovic AT, et al. DNA methylation landscapes of 1538 breast cancers reveal a replication-linked clock, epigenomic instability and cis-regulation. *Nat Commun* 2021 Sep 13;12(1):5406. [doi: [10.1038/s41467-021-25661-w](https://doi.org/10.1038/s41467-021-25661-w)] [Medline: [34518533](https://pubmed.ncbi.nlm.nih.gov/34518533/)]
35. Cortés-Ciriano I, Lee JJK, Xi R, et al. Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing. *Nat Genet* 2020 Mar;52(3):331-341. [doi: [10.1038/s41588-019-0576-7](https://doi.org/10.1038/s41588-019-0576-7)] [Medline: [32025003](https://pubmed.ncbi.nlm.nih.gov/32025003/)]
36. Petrucelli N, Daly MB, Pal T. BRCA1- and BRCA2-associated hereditary breast and ovarian cancer. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, editors. *GeneReviews: University of Washington, Seattle; 1993*. URL: <https://www.ncbi.nlm.nih.gov/books/NBK1247/> [accessed 2025-01-09]
37. Lalloo F, Varley J, Moran A, et al. BRCA1, BRCA2 and TP53 mutations in very early-onset breast cancer with associated risks to relatives. *Eur J Cancer* 2006 May;42(8):1143-1150. [doi: [10.1016/j.ejca.2005.11.032](https://doi.org/10.1016/j.ejca.2005.11.032)] [Medline: [16644204](https://pubmed.ncbi.nlm.nih.gov/16644204/)]
38. Joos S, Falk MH, Lichter P, et al. Variable breakpoints in Burkitt lymphoma cells with chromosomal t(8;14) translocation separate c-myc and the IgH locus up to several hundred kb. *Hum Mol Genet* 1992 Nov;1(8):625-632. [doi: [10.1093/hmg/1.8.625](https://doi.org/10.1093/hmg/1.8.625)] [Medline: [1301171](https://pubmed.ncbi.nlm.nih.gov/1301171/)]
39. Joos S, Haluska FG, Falk MH, et al. Mapping chromosomal breakpoints of Burkitt's t(8;14) translocations far upstream of c-myc. *Cancer Res* 1992 Dec 1;52(23):6547-6552. [Medline: [1330296](https://pubmed.ncbi.nlm.nih.gov/1330296/)]
40. López C, Kleinheinz K, Aukema SM, et al. Genomic and transcriptomic changes complement each other in the pathogenesis of sporadic Burkitt lymphoma. *Nat Commun* 2019 Mar 29;10(1):1459. [doi: [10.1038/s41467-019-08578-3](https://doi.org/10.1038/s41467-019-08578-3)] [Medline: [30926794](https://pubmed.ncbi.nlm.nih.gov/30926794/)]
41. Xing R, Zhou Y, Yu J, et al. Whole-genome sequencing reveals novel tandem-duplication hotspots and a prognostic mutational signature in gastric cancer. *Nat Commun* 2019 May 2;10(1):2037. [doi: [10.1038/s41467-019-09644-6](https://doi.org/10.1038/s41467-019-09644-6)] [Medline: [31048690](https://pubmed.ncbi.nlm.nih.gov/31048690/)]
42. Nangalia J, Campbell PJ. Genome sequencing during a patient's journey through cancer. *N Engl J Med* 2019 Nov 28;381(22):2145-2156. [doi: [10.1056/NEJMr1910138](https://doi.org/10.1056/NEJMr1910138)] [Medline: [31774959](https://pubmed.ncbi.nlm.nih.gov/31774959/)]
43. Mount DW. Using the Basic Local Alignment Search Tool (BLAST). *CSH Protoc* 2007 Jul 1;2007(7):pdb.top17. [doi: [10.1101/pdb.top17](https://doi.org/10.1101/pdb.top17)] [Medline: [21357135](https://pubmed.ncbi.nlm.nih.gov/21357135/)]
44. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990 Oct 5;215(3):403-410. [doi: [10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)] [Medline: [2231712](https://pubmed.ncbi.nlm.nih.gov/2231712/)]
45. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. *J Comput Biol* 2000;7(1-2):203-214. [doi: [10.1089/10665270050081478](https://doi.org/10.1089/10665270050081478)] [Medline: [10890397](https://pubmed.ncbi.nlm.nih.gov/10890397/)]
46. Rangwala SH, Kuznetsov A, Ananiev V, et al. Accessing NCBI data using the NCBI Sequence Viewer and Genome Data Viewer (GDV). *Genome Res* 2021 Jan;31(1):159-169. [doi: [10.1101/gr.266932.120](https://doi.org/10.1101/gr.266932.120)] [Medline: [33239395](https://pubmed.ncbi.nlm.nih.gov/33239395/)]
47. Kim KD, Tanizawa H, de Leo A, et al. Epigenetic specifications of host chromosome docking sites for latent Epstein-Barr virus. *Nat Commun* 2020 Feb 13;11(1):877. [doi: [10.1038/s41467-019-14152-8](https://doi.org/10.1038/s41467-019-14152-8)] [Medline: [32054837](https://pubmed.ncbi.nlm.nih.gov/32054837/)]
48. Lu F, Wikramasinghe P, Norseen J, et al. Genome-wide analysis of host-chromosome binding sites for Epstein-Barr virus nuclear antigen 1 (EBNA1). *Virology* 2010 Oct 7;7:262. [doi: [10.1186/1743-422X-7-262](https://doi.org/10.1186/1743-422X-7-262)] [Medline: [20929547](https://pubmed.ncbi.nlm.nih.gov/20929547/)]
49. Sai Lakshmi S, Agrawal S. piRNABank: a web resource on classified and clustered Piwi-interacting RNAs. *Nucleic Acids Res* 2008 Jan;36(Database issue):D173-D177. [doi: [10.1093/nar/gkm696](https://doi.org/10.1093/nar/gkm696)] [Medline: [17881367](https://pubmed.ncbi.nlm.nih.gov/17881367/)]
50. Wang J, Zhang P, Lu Y, et al. piRBase: a comprehensive database of piRNA sequences. *Nucleic Acids Res* 2019 Jan 8;47(D1):D175-D180. [doi: [10.1093/nar/gky1043](https://doi.org/10.1093/nar/gky1043)] [Medline: [30371818](https://pubmed.ncbi.nlm.nih.gov/30371818/)]
51. Tang MH, Varadan V, Kamalakaran S, Zhang MQ, Dimitrova N, Hicks J. Major chromosomal breakpoint intervals in breast cancer co-localize with differentially methylated regions. *Front Oncol* 2012 Dec 27;2:197. [doi: [10.3389/fonc.2012.00197](https://doi.org/10.3389/fonc.2012.00197)] [Medline: [23293768](https://pubmed.ncbi.nlm.nih.gov/23293768/)]
52. Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. *Ann Math Statist* 1947 Mar;18(1):50-60. [doi: [10.1214/aoms/1177730491](https://doi.org/10.1214/aoms/1177730491)]
53. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika* 1965 Dec;52(3/4):591-611. [doi: [10.2307/2333709](https://doi.org/10.2307/2333709)]
54. Kumar R, Nagpal G, Kumar V, Usmani SS, Agrawal P, Raghava GPS. HumCFS: a database of fragile sites in human chromosomes. *BMC Genomics* 2019 Apr 18;19(Suppl 9):985. [doi: [10.1186/s12864-018-5330-5](https://doi.org/10.1186/s12864-018-5330-5)] [Medline: [30999860](https://pubmed.ncbi.nlm.nih.gov/30999860/)]

55. Maccaroni K, Balzano E, Mirimao F, Giunta S, Pelliccia F. Impaired replication timing promotes tissue-specific expression of common fragile sites. *Genes (Basel)* 2020 Mar 19;11(3):326. [doi: [10.3390/genes11030326](https://doi.org/10.3390/genes11030326)] [Medline: [32204553](https://pubmed.ncbi.nlm.nih.gov/32204553/)]
56. European Commission: Directorate-General for Research and Innovation. Improving access to and reuse of research results, publications and data for scientific purposes – study to evaluate the effects of the EU copyright framework on research and the effects of potential interventions and to identify and present relevant provisions for research in EU data and digital legislation, with a focus on rights and obligations. : Publications Office of the European Union; 2024 URL: <https://data.europa.eu/doi/10.2777/633395> [accessed 2025-01-09]
57. Zheng B, Liu XL, Fan R, et al. The landscape of cell-free HBV integrations and mutations in cirrhosis and hepatocellular carcinoma patients. *Clin Cancer Res* 2021 Jul 1;27(13):3772-3783. [doi: [10.1158/1078-0432.CCR-21-0002](https://doi.org/10.1158/1078-0432.CCR-21-0002)] [Medline: [33947693](https://pubmed.ncbi.nlm.nih.gov/33947693/)]
58. Foster KA, Harrington P, Kerr J, et al. Somatic and germline mutations of the BRCA2 gene in sporadic ovarian cancer. *Cancer Res* 1996 Aug 15;56(16):3622-3625. [Medline: [8705994](https://pubmed.ncbi.nlm.nih.gov/8705994/)]
59. Friedenson B. The BRCA1/2 pathway prevents hematologic cancers in addition to breast and ovarian cancers. *BMC Cancer* 2007 Aug 6;7:152. [doi: [10.1186/1471-2407-7-152](https://doi.org/10.1186/1471-2407-7-152)] [Medline: [17683622](https://pubmed.ncbi.nlm.nih.gov/17683622/)]
60. Friedenson B. Comment on 'The incidence of leukaemia in women with BRCA1 and BRCA2 mutations: an International Prospective Cohort Study'. *Br J Cancer* 2016 Aug 23;115(5):e2. [doi: [10.1038/bjc.2016.192](https://doi.org/10.1038/bjc.2016.192)] [Medline: [27459694](https://pubmed.ncbi.nlm.nih.gov/27459694/)]
61. Parvin S, Ramirez-Labrada A, Aumann S, et al. LMO2 confers synthetic lethality to PARP inhibition in DLBCL. *Cancer Cell* 2019 Sep 16;36(3):237-249.e6. [doi: [10.1016/j.ccell.2019.07.007](https://doi.org/10.1016/j.ccell.2019.07.007)] [Medline: [31447348](https://pubmed.ncbi.nlm.nih.gov/31447348/)]
62. Compagno M, Lim WK, Grunn A, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. *Nature* 2009 Jun 4;459(7247):717-721. [doi: [10.1038/nature07968](https://doi.org/10.1038/nature07968)] [Medline: [19412164](https://pubmed.ncbi.nlm.nih.gov/19412164/)]
63. Ceribelli M, Kelly PN, Shaffer AL, et al. Blockade of oncogenic IκB kinase activity in diffuse large B-cell lymphoma by bromodomain and extraterminal domain protein inhibitors. *Proc Natl Acad Sci U S A* 2014 Aug 5;111(31):11365-11370. [doi: [10.1073/pnas.1411701111](https://doi.org/10.1073/pnas.1411701111)] [Medline: [25049379](https://pubmed.ncbi.nlm.nih.gov/25049379/)]
64. Montes-Moreno S, Odqvist L, Diaz-Perez JA, et al. EBV-positive diffuse large B-cell lymphoma of the elderly is an aggressive post-germinal center B-cell neoplasm characterized by prominent nuclear factor-kB activation. *Mod Pathol* 2012 Jul;25(7):968-982. [doi: [10.1038/modpathol.2012.52](https://doi.org/10.1038/modpathol.2012.52)] [Medline: [22538516](https://pubmed.ncbi.nlm.nih.gov/22538516/)]
65. Gröbner SN, Worst BC, Weischenfeldt J, et al. The landscape of genomic alterations across childhood cancers. *Nature* 2018 Mar 15;555(7696):321-327. [doi: [10.1038/nature25480](https://doi.org/10.1038/nature25480)] [Medline: [29489754](https://pubmed.ncbi.nlm.nih.gov/29489754/)]
66. Li JSZ, Abbasi A, Kim DH, Lippman SM, Alexandrov LB, Cleveland DW. Chromosomal fragile site breakage by EBV-encoded EBNA1 at clustered repeats. *Nature* 2023 Apr;616(7957):504-509. [doi: [10.1038/s41586-023-05923-x](https://doi.org/10.1038/s41586-023-05923-x)] [Medline: [37046091](https://pubmed.ncbi.nlm.nih.gov/37046091/)]
67. Hui KF, Chan TF, Yang W, et al. High risk Epstein - Barr virus variants characterized by distinct polymorphisms in the EBV locus are strongly associated with nasopharyngeal carcinoma. *Int J Cancer* 2019 Jun 15;144(12):3031-3042. [doi: [10.1002/ijc.32049](https://doi.org/10.1002/ijc.32049)] [Medline: [30536939](https://pubmed.ncbi.nlm.nih.gov/30536939/)]
68. Friedenson B. Evidence of lesions from Epstein-Barr virus infection in human breast cancer genomes. medRxiv. Preprint posted online on Jun 26, 2024. [doi: [10.1101/2024.06.24.24309410](https://doi.org/10.1101/2024.06.24.24309410)]
69. Nurk S, Koren S, Rhie A, et al. The complete sequence of a human genome. *Science* 2022 Apr;376(6588):44-53. [doi: [10.1126/science.abj6987](https://doi.org/10.1126/science.abj6987)] [Medline: [35357919](https://pubmed.ncbi.nlm.nih.gov/35357919/)]
70. Luo WJ, Takakuwa T, Ham MF, et al. Epstein-Barr virus is integrated between REL and BCL-11A in American Burkitt lymphoma cell line (NAB-2). *Lab Invest* 2004 Sep;84(9):1193-1199. [doi: [10.1038/labinvest.3700152](https://doi.org/10.1038/labinvest.3700152)] [Medline: [15241441](https://pubmed.ncbi.nlm.nih.gov/15241441/)]
71. Tsao SW, Tsang CM, Lo KW. Epstein-Barr virus infection and nasopharyngeal carcinoma. *Philos Trans R Soc Lond B Biol Sci* 2017 Oct 19;372(1732):20160270. [doi: [10.1098/rstb.2016.0270](https://doi.org/10.1098/rstb.2016.0270)] [Medline: [28893937](https://pubmed.ncbi.nlm.nih.gov/28893937/)]
72. Scott RS. Epstein-Barr virus: a master epigenetic manipulator. *Curr Opin Virol* 2017 Oct;26:74-80. [doi: [10.1016/j.coviro.2017.07.017](https://doi.org/10.1016/j.coviro.2017.07.017)] [Medline: [28780440](https://pubmed.ncbi.nlm.nih.gov/28780440/)]
73. Schott G, Garcia-Blanco MA. MHC class III RNA binding proteins and immunity. *RNA Biol* 2021 May;18(5):640-646. [doi: [10.1080/15476286.2020.1860388](https://doi.org/10.1080/15476286.2020.1860388)] [Medline: [33280511](https://pubmed.ncbi.nlm.nih.gov/33280511/)]
74. Zhou D, Lai M, Luo A, Yu CY. An RNA metabolism and surveillance quartet in the major histocompatibility complex. *Cells* 2019 Aug 30;8(9):1008. [doi: [10.3390/cells8091008](https://doi.org/10.3390/cells8091008)] [Medline: [31480283](https://pubmed.ncbi.nlm.nih.gov/31480283/)]
75. Fernandes MT, Dejardin E, dos Santos NR. Context-dependent roles for lymphotoxin-β receptor signaling in cancer development. *Biochim Biophys Acta* 2016 Apr;1865(2):204-219. [doi: [10.1016/j.bbcan.2016.02.005](https://doi.org/10.1016/j.bbcan.2016.02.005)] [Medline: [26923876](https://pubmed.ncbi.nlm.nih.gov/26923876/)]
76. Shiina T, Blancher A, Inoko H, Kulski JK. Comparative genomics of the human, macaque and mouse major histocompatibility complex. *Immunology* 2017 Feb;150(2):127-138. [doi: [10.1111/imm.12624](https://doi.org/10.1111/imm.12624)] [Medline: [27395034](https://pubmed.ncbi.nlm.nih.gov/27395034/)]
77. Aissani B, Boehme AK, Wiener HW, Shrestha S, Jacobson LP, Kaslow RA. SNP screening of central MHC-identified HLA-DMB as a candidate susceptibility gene for HIV-related Kaposi's sarcoma. *Genes Immun* 2014 Sep;15(6):424-429. [doi: [10.1038/gene.2014.42](https://doi.org/10.1038/gene.2014.42)] [Medline: [25008864](https://pubmed.ncbi.nlm.nih.gov/25008864/)]
78. Zhuang J, Huo Q, Yang F, Xie N. Perspectives on the role of histone modification in breast cancer progression and the advanced technological tools to study epigenetic determinants of metastasis. *Front Genet* 2020 Oct 29;11:603552. [doi: [10.3389/fgene.2020.603552](https://doi.org/10.3389/fgene.2020.603552)] [Medline: [33193750](https://pubmed.ncbi.nlm.nih.gov/33193750/)]

79. Bayona-Feliu A, Barroso S, Muñoz S, Aguilera A. The SWI/SNF chromatin remodeling complex helps resolve R-loop-mediated transcription–replication conflicts. *Nat Genet* 2021 Jul;53(7):1050-1063. [doi: [10.1038/s41588-021-00867-2](https://doi.org/10.1038/s41588-021-00867-2)] [Medline: [33986538](https://pubmed.ncbi.nlm.nih.gov/33986538/)]
80. Harrod A, Lane KA, Downs JA. The role of the SWI/SNF chromatin remodelling complex in the response to DNA double strand breaks. *DNA Repair (Amst)* 2020 Sep;93:102919. [doi: [10.1016/j.dnarep.2020.102919](https://doi.org/10.1016/j.dnarep.2020.102919)] [Medline: [33087260](https://pubmed.ncbi.nlm.nih.gov/33087260/)]
81. Andrades A, Peinado P, Alvarez-Perez JC, et al. SWI/SNF complexes in hematological malignancies: biological implications and therapeutic opportunities. *Mol Cancer* 2023 Feb 21;22(1):39. [doi: [10.1186/s12943-023-01736-8](https://doi.org/10.1186/s12943-023-01736-8)] [Medline: [36810086](https://pubmed.ncbi.nlm.nih.gov/36810086/)]
82. Lu J. The Warburg metabolism fuels tumor metastasis. *Cancer Metastasis Rev* 2019 Jun;38(1-2):157-164. [doi: [10.1007/s10555-019-09794-5](https://doi.org/10.1007/s10555-019-09794-5)] [Medline: [30997670](https://pubmed.ncbi.nlm.nih.gov/30997670/)]
83. Chen H, Wu J, Zhang Z, et al. Association between BRCA status and triple-negative breast cancer: a meta-analysis. *Front Pharmacol* 2018 Aug 21;9:909. [doi: [10.3389/fphar.2018.00909](https://doi.org/10.3389/fphar.2018.00909)] [Medline: [30186165](https://pubmed.ncbi.nlm.nih.gov/30186165/)]
84. Evans DG, Lalloo F, Howell S, Verhoef S, Woodward ER, Howell A. Low prevalence of HER2 positivity amongst BRCA1 and BRCA2 mutation carriers and in primary BRCA screens. *Breast Cancer Res Treat* 2016 Feb;155(3):597-601. [doi: [10.1007/s10549-016-3697-z](https://doi.org/10.1007/s10549-016-3697-z)] [Medline: [26888723](https://pubmed.ncbi.nlm.nih.gov/26888723/)]
85. Locy H, Verhulst S, Cools W, et al. Assessing tumor-infiltrating lymphocytes in breast cancer: a proposal for combining immunohistochemistry and gene expression analysis to refine scoring. *Front Immunol* 2022 Feb 11;13:794175. [doi: [10.3389/fimmu.2022.794175](https://doi.org/10.3389/fimmu.2022.794175)] [Medline: [35222378](https://pubmed.ncbi.nlm.nih.gov/35222378/)]
86. Takada K, Kashiwagi S, Asano Y, et al. Prediction of distant metastatic recurrence by tumor-infiltrating lymphocytes in hormone receptor-positive breast cancer. *BMC Womens Health* 2021 May 29;21(1):225. [doi: [10.1186/s12905-021-01373-7](https://doi.org/10.1186/s12905-021-01373-7)] [Medline: [34051785](https://pubmed.ncbi.nlm.nih.gov/34051785/)]
87. Meier UC, Cipian RC, Karimi A, Ramasamy R, Middeldorp JM. Cumulative roles for Epstein-Barr virus, human endogenous retroviruses, and human herpes virus-6 in driving an inflammatory cascade underlying MS pathogenesis. *Front Immunol* 2021 Nov 1;12:757302. [doi: [10.3389/fimmu.2021.757302](https://doi.org/10.3389/fimmu.2021.757302)] [Medline: [34790199](https://pubmed.ncbi.nlm.nih.gov/34790199/)]
88. Mameli G, Poddighe L, Mei A, et al. Expression and activation by Epstein Barr virus of human endogenous retroviruses-W in blood cells and astrocytes: inference for multiple sclerosis. *PLoS One* 2012;7(9):e44991. [doi: [10.1371/journal.pone.0044991](https://doi.org/10.1371/journal.pone.0044991)] [Medline: [23028727](https://pubmed.ncbi.nlm.nih.gov/23028727/)]
89. Lawson JS, Glenn WK. Evidence for a causal role by mouse mammary tumour-like virus in human breast cancer. *NPJ Breast Cancer* 2019 Nov 7;5:40. [doi: [10.1038/s41523-019-0136-4](https://doi.org/10.1038/s41523-019-0136-4)] [Medline: [31728407](https://pubmed.ncbi.nlm.nih.gov/31728407/)]
90. Dudley JP, Golovkina TV, Ross SR. Lessons learned from mouse mammary tumor virus in animal models. *ILAR J* 2016;57(1):12-23. [doi: [10.1093/ilar/ilv044](https://doi.org/10.1093/ilar/ilv044)] [Medline: [27034391](https://pubmed.ncbi.nlm.nih.gov/27034391/)]
91. Li Y, Roberts ND, Wala JA, et al. Patterns of somatic structural variation in human cancer genomes. *Nature* 2020 Feb;578(7793):112-121. [doi: [10.1038/s41586-019-1913-9](https://doi.org/10.1038/s41586-019-1913-9)] [Medline: [32025012](https://pubmed.ncbi.nlm.nih.gov/32025012/)]
92. Ganapathiraju MK, Subramanian S, Chaparala S, Karunakaran KB. A reference catalog of DNA palindromes in the human genome and their variations in 1000 Genomes. *Hum Genome Var* 2020 Nov 20;7(1):40. [doi: [10.1038/s41439-020-00127-5](https://doi.org/10.1038/s41439-020-00127-5)] [Medline: [33298903](https://pubmed.ncbi.nlm.nih.gov/33298903/)]
93. Rawlins DR, Milman G, Hayward SD, Hayward GS. Sequence-specific DNA binding of the Epstein-Barr virus nuclear antigen (EBNA-1) to clustered sites in the plasmid maintenance region. *Cell* 1985 Oct;42(3):859-868. [doi: [10.1016/0092-8674\(85\)90282-x](https://doi.org/10.1016/0092-8674(85)90282-x)] [Medline: [2996781](https://pubmed.ncbi.nlm.nih.gov/2996781/)]
94. Bochkarev A, Barwell JA, Pfuetzner RA, Bochkareva E, Frappier L, Edwards AM. Crystal structure of the DNA-binding domain of the Epstein-Barr virus origin-binding protein, EBNA1, bound to DNA. *Cell* 1996 Mar 8;84(5):791-800. [doi: [10.1016/s0092-8674\(00\)81056-9](https://doi.org/10.1016/s0092-8674(00)81056-9)] [Medline: [8625416](https://pubmed.ncbi.nlm.nih.gov/8625416/)]
95. Altemose N, Logsdon GA, Bzikadze AV, et al. Complete genomic and epigenetic maps of human centromeres. *Science* 2022 Apr;376(6588):eabl4178. [doi: [10.1126/science.abl4178](https://doi.org/10.1126/science.abl4178)] [Medline: [35357911](https://pubmed.ncbi.nlm.nih.gov/35357911/)]
96. Grande BM, Gerhard DS, Jiang A, et al. Genome-wide discovery of somatic coding and noncoding mutations in pediatric endemic and sporadic Burkitt lymphoma. *Blood* 2019 Mar 21;133(12):1313-1324. [doi: [10.1182/blood-2018-09-871418](https://doi.org/10.1182/blood-2018-09-871418)] [Medline: [30617194](https://pubmed.ncbi.nlm.nih.gov/30617194/)]
97. Kim TM, Xi R, Luquette LJ, Park RW, Johnson MD, Park PJ. Functional genomic analysis of chromosomal aberrations in a compendium of 8000 cancer genomes. *Genome Res* 2013 Feb;23(2):217-227. [doi: [10.1101/gr.140301.112](https://doi.org/10.1101/gr.140301.112)] [Medline: [23132910](https://pubmed.ncbi.nlm.nih.gov/23132910/)]
98. Dittmer DP, Hilscher CJ, Gulley ML, Yang EV, Chen M, Glaser R. Multiple pathways for Epstein-Barr virus episome loss from nasopharyngeal carcinoma. *Int J Cancer* 2008 Nov 1;123(9):2105-2112. [doi: [10.1002/ijc.23685](https://doi.org/10.1002/ijc.23685)] [Medline: [18688856](https://pubmed.ncbi.nlm.nih.gov/18688856/)]
99. Li Q, Cohen JL. Epstein-Barr virus and the human leukocyte antigen complex. *Curr Clin Microbiol Rep* 2019 Sep;6(3):175-181. [doi: [10.1007/s40588-019-00120-9](https://doi.org/10.1007/s40588-019-00120-9)] [Medline: [33094090](https://pubmed.ncbi.nlm.nih.gov/33094090/)]
100. Lei PJ, Pereira ER, Andersson P, et al. Cancer cell plasticity and MHC-II-mediated immune tolerance promote breast cancer metastasis to lymph nodes. *J Exp Med* 2023 Sep 4;220(9):e20221847. [doi: [10.1084/jem.20221847](https://doi.org/10.1084/jem.20221847)] [Medline: [37341991](https://pubmed.ncbi.nlm.nih.gov/37341991/)]
101. Park IA, Hwang SH, Song IH, et al. Expression of the MHC class II in triple-negative breast cancer is associated with tumor-infiltrating lymphocytes and interferon signaling. *PLoS One* 2017 Aug 17;12(8):e0182786. [doi: [10.1371/journal.pone.0182786](https://doi.org/10.1371/journal.pone.0182786)] [Medline: [28817603](https://pubmed.ncbi.nlm.nih.gov/28817603/)]

102. Song IH, Kim YA, Heo SH, et al. The association of estrogen receptor activity, interferon signaling, and MHC class I expression in breast cancer. *Cancer Res Treat* 2022 Oct;54(4):1111-1120. [doi: [10.4143/crt.2021.1017](https://doi.org/10.4143/crt.2021.1017)] [Medline: [34942685](https://pubmed.ncbi.nlm.nih.gov/34942685/)]
103. Bjornevik K, Cortese M, Healy BC, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science* 2022 Jan 21;375(6578):296-301. [doi: [10.1126/science.abj8222](https://doi.org/10.1126/science.abj8222)] [Medline: [35025605](https://pubmed.ncbi.nlm.nih.gov/35025605/)]
104. Aissa AF, Islam ABMMK, Ariss MM, et al. Single-cell transcriptional changes associated with drug tolerance and response to combination therapies in cancer. *Nat Commun* 2021 Mar 12;12(1):1628. [doi: [10.1038/s41467-021-21884-z](https://doi.org/10.1038/s41467-021-21884-z)] [Medline: [33712615](https://pubmed.ncbi.nlm.nih.gov/33712615/)]
105. Cao Y, Efetov SK, He M, et al. Updated clinical perspectives and challenges of chimeric antigen receptor-T cell therapy in colorectal cancer and invasive breast cancer. *Arch Immunol Ther Exp (Warsz)* 2023 Aug 11;71(1):19. [doi: [10.1007/s00005-023-00684-x](https://doi.org/10.1007/s00005-023-00684-x)] [Medline: [37566162](https://pubmed.ncbi.nlm.nih.gov/37566162/)]
106. Upton R, Banuelos A, Feng D, et al. Combining CD47 blockade with trastuzumab eliminates HER2-positive breast cancer cells and overcomes trastuzumab tolerance. *Proc Natl Acad Sci U S A* 2021 Jul 20;118(29):e2026849118. [doi: [10.1073/pnas.2026849118](https://doi.org/10.1073/pnas.2026849118)] [Medline: [34257155](https://pubmed.ncbi.nlm.nih.gov/34257155/)]
107. Burkhardt B, Michgehl U, Rohde J, et al. Clinical relevance of molecular characteristics in Burkitt lymphoma differs according to age. *Nat Commun* 2022 Jul 6;13(1):3881. [doi: [10.1038/s41467-022-31355-8](https://doi.org/10.1038/s41467-022-31355-8)] [Medline: [35794096](https://pubmed.ncbi.nlm.nih.gov/35794096/)]
108. Dochi H, Kondo S, Murata T, et al. Estrogen induces the expression of EBV lytic protein ZEBRA, a marker of poor prognosis in nasopharyngeal carcinoma. *Cancer Sci* 2022 Aug;113(8):2862-2877. [doi: [10.1111/cas.15440](https://doi.org/10.1111/cas.15440)] [Medline: [35633182](https://pubmed.ncbi.nlm.nih.gov/35633182/)]
109. Zhu BT, Conney AH. Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis* 1998 Jan 1;19(1):1-27. [doi: [10.1093/carcin/19.1.1](https://doi.org/10.1093/carcin/19.1.1)] [Medline: [9472688](https://pubmed.ncbi.nlm.nih.gov/9472688/)]
110. Pommier Y, Nussenzweig A, Takeda S, Austin C. Human topoisomerases and their roles in genome stability and organization. *Nat Rev Mol Cell Biol* 2022 Jun;23(6):407-427. [doi: [10.1038/s41580-022-00452-3](https://doi.org/10.1038/s41580-022-00452-3)] [Medline: [35228717](https://pubmed.ncbi.nlm.nih.gov/35228717/)]
111. Sasanuma H, Tsuda M, Morimoto S, et al. BRCA1 ensures genome integrity by eliminating estrogen-induced pathological topoisomerase II-DNA complexes. *Proc Natl Acad Sci U S A* 2018 Nov 6;115(45):E10642-E10651. [doi: [10.1073/pnas.1803177115](https://doi.org/10.1073/pnas.1803177115)] [Medline: [30352856](https://pubmed.ncbi.nlm.nih.gov/30352856/)]
112. Manguso N, Kim M, Joshi N, et al. TDP2 is a regulator of estrogen-responsive oncogene expression. *NAR Cancer* 2024 Apr 8;6(2):zcae016. [doi: [10.1093/narcan/zcae016](https://doi.org/10.1093/narcan/zcae016)] [Medline: [38596431](https://pubmed.ncbi.nlm.nih.gov/38596431/)]
113. de Piccoli G, Cortes-Ledesma F, Ira G, et al. Smc5-Smc6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination. *Nat Cell Biol* 2006 Sep;8(9):1032-1034. [doi: [10.1038/ncb1466](https://doi.org/10.1038/ncb1466)] [Medline: [16892052](https://pubmed.ncbi.nlm.nih.gov/16892052/)]
114. Tapia-Alveal C, Lin SJ, O'Connell MJ. Functional interplay between cohesin and Smc5/6 complexes. *Chromosoma* 2014 Oct;123(5):437-445. [doi: [10.1007/s00412-014-0474-9](https://doi.org/10.1007/s00412-014-0474-9)] [Medline: [24981336](https://pubmed.ncbi.nlm.nih.gov/24981336/)]
115. Irwan ID, Cullen BR. The SMC5/6 complex: an emerging antiviral restriction factor that can silence episomal DNA. *PLoS Pathog* 2023 Mar 2;19(3):e1011180. [doi: [10.1371/journal.ppat.1011180](https://doi.org/10.1371/journal.ppat.1011180)] [Medline: [36862666](https://pubmed.ncbi.nlm.nih.gov/36862666/)]
116. Agashe S, Joseph CR, Reyes TAC, et al. Smc5/6 functions with Sgs1-Top3-Rmi1 to complete chromosome replication at natural pause sites. *Nat Commun* 2021 Apr 8;12(1):2111. [doi: [10.1038/s41467-021-22217-w](https://doi.org/10.1038/s41467-021-22217-w)] [Medline: [33833229](https://pubmed.ncbi.nlm.nih.gov/33833229/)]
117. Truszevska A, Wirkowska A, Gala K, et al. EBV load is associated with cfDNA fragmentation and renal damage in SLE patients. *Lupus* 2021 Jul;30(8):1214-1225. [doi: [10.1177/09612033211010339](https://doi.org/10.1177/09612033211010339)] [Medline: [33866897](https://pubmed.ncbi.nlm.nih.gov/33866897/)]
118. Volleth M, Zenker M, Joksic I, Liehr T. Long-term culture of EBV-induced human lymphoblastoid cell lines reveals chromosomal instability. *J Histochem Cytochem* 2020 Apr;68(4):239-251. [doi: [10.1369/002155420910113](https://doi.org/10.1369/002155420910113)] [Medline: [32108534](https://pubmed.ncbi.nlm.nih.gov/32108534/)]
119. Dheekollu J, Malecka K, Wiedmer A, et al. Carcinoma-risk variant of EBNA1 deregulates Epstein-Barr virus episomal latency. *Oncotarget* 2017 Jan 31;8(5):7248-7264. [doi: [10.18632/oncotarget.14540](https://doi.org/10.18632/oncotarget.14540)] [Medline: [28077791](https://pubmed.ncbi.nlm.nih.gov/28077791/)]
120. Buisson M, Géoui T, Flot D, et al. A bridge crosses the active-site canyon of the Epstein-Barr virus nuclease with DNase and RNase activities. *J Mol Biol* 2009 Aug 28;391(4):717-728. [doi: [10.1016/j.jmb.2009.06.034](https://doi.org/10.1016/j.jmb.2009.06.034)] [Medline: [19538972](https://pubmed.ncbi.nlm.nih.gov/19538972/)]
121. Wu CC, Liu MT, Chang YT, et al. Epstein-Barr virus DNase (BGLF5) induces genomic instability in human epithelial cells. *Nucleic Acids Res* 2010 Apr;38(6):1932-1949. [doi: [10.1093/nar/gkp1169](https://doi.org/10.1093/nar/gkp1169)] [Medline: [20034954](https://pubmed.ncbi.nlm.nih.gov/20034954/)]
122. Chiu SH, Wu MC, Wu CC, et al. Epstein-Barr virus BALF3 has nuclease activity and mediates mature virion production during the lytic cycle. *J Virol* 2014 May;88(9):4962-4975. [doi: [10.1128/JVI.00063-14](https://doi.org/10.1128/JVI.00063-14)] [Medline: [24554665](https://pubmed.ncbi.nlm.nih.gov/24554665/)]
123. Chiu SH, Wu CC, Fang CY, et al. Epstein-Barr virus BALF3 mediates genomic instability and progressive malignancy in nasopharyngeal carcinoma. *Oncotarget* 2014 Sep 30;5(18):8583-8601. [doi: [10.18632/oncotarget.2323](https://doi.org/10.18632/oncotarget.2323)] [Medline: [25261366](https://pubmed.ncbi.nlm.nih.gov/25261366/)]
124. O'Neill FJ, Miles CP. Chromosome changes in human cells induced by herpes simplex, types 1 and 2. *Nature* 1969 Aug 23;223(5208):851-852. [doi: [10.1038/223851a0](https://doi.org/10.1038/223851a0)] [Medline: [4307971](https://pubmed.ncbi.nlm.nih.gov/4307971/)]
125. Mertens ME, Knipe DM. Herpes simplex virus 1 manipulates host cell antiviral and proviral DNA damage responses. *mBio* 2021 Feb 9;12(1):e03552-20. [doi: [10.1128/mBio.03552-20](https://doi.org/10.1128/mBio.03552-20)] [Medline: [33563816](https://pubmed.ncbi.nlm.nih.gov/33563816/)]
126. Schumacher AJ, Mohni KN, Kan Y, Hendrickson EA, Stark JM, Weller SK. The HSV-1 exonuclease, UL12, stimulates recombination by a single strand annealing mechanism. *PLoS Pathog* 2012;8(8):e1002862. [doi: [10.1371/journal.ppat.1002862](https://doi.org/10.1371/journal.ppat.1002862)] [Medline: [22912580](https://pubmed.ncbi.nlm.nih.gov/22912580/)]
127. Ezeonwumelu IJ, Garcia-Vidal E, Ballana E. JAK-STAT pathway: a novel target to tackle viral infections. *Viruses* 2021 Nov 27;13(12):2379. [doi: [10.3390/v13122379](https://doi.org/10.3390/v13122379)] [Medline: [34960648](https://pubmed.ncbi.nlm.nih.gov/34960648/)]

128. Jangra S, Bharti A, Lui WY, et al. Suppression of JAK-STAT signaling by Epstein-Barr virus tegument protein BGLF2 through recruitment of SHP1 phosphatase and promotion of STAT2 degradation. *J Virol* 2021 Sep 27;95(20):e0102721. [doi: [10.1128/JVI.01027-21](https://doi.org/10.1128/JVI.01027-21)] [Medline: [34319780](https://pubmed.ncbi.nlm.nih.gov/34319780/)]
129. Wu DY, Krumm A, Schubach WH. Promoter-specific targeting of human SWI-SNF complex by Epstein-Barr virus nuclear protein 2. *J Virol* 2000 Oct;74(19):8893-8903. [doi: [10.1128/jvi.74.19.8893-8903.2000](https://doi.org/10.1128/jvi.74.19.8893-8903.2000)] [Medline: [10982332](https://pubmed.ncbi.nlm.nih.gov/10982332/)]
130. Su MT, Wang YT, Chen YJ, Lin SF, Tsai CH, Chen MR. The SWI/SNF chromatin regulator BRG1 modulates the transcriptional regulatory activity of the Epstein-Barr virus DNA polymerase processivity factor BMRF1. *J Virol* 2017 Apr 13;91(9):e02114-16. [doi: [10.1128/JVI.02114-16](https://doi.org/10.1128/JVI.02114-16)] [Medline: [28228591](https://pubmed.ncbi.nlm.nih.gov/28228591/)]
131. Nakao K, Yuge T, Mochiki M, Nibu KI, Sugawara M. Detection of Epstein-Barr virus in metastatic lymph nodes of patients with nasopharyngeal carcinoma and a primary unknown carcinoma. *Arch Otolaryngol Head Neck Surg* 2003 Mar;129(3):338-340. [doi: [10.1001/archotol.129.3.338](https://doi.org/10.1001/archotol.129.3.338)] [Medline: [12622545](https://pubmed.ncbi.nlm.nih.gov/12622545/)]
132. Tao D, Zhang N, Huang Q, et al. Association of Epstein-Barr virus infection with peripheral immune parameters and clinical outcome in advanced nasopharyngeal carcinoma. *Sci Rep* 2020 Dec 15;10(1):21976. [doi: [10.1038/s41598-020-78892-0](https://doi.org/10.1038/s41598-020-78892-0)] [Medline: [33319825](https://pubmed.ncbi.nlm.nih.gov/33319825/)]
133. Gill MB, Kutok JL, Fingerhuth JD. Epstein-Barr virus thymidine kinase is a centrosomal resident precisely localized to the periphery of centrioles. *J Virol* 2007 Jun 15;81(12):6523-6535. [doi: [10.1128/JVI.00147-07](https://doi.org/10.1128/JVI.00147-07)] [Medline: [17428875](https://pubmed.ncbi.nlm.nih.gov/17428875/)]
134. Shumilov A, Tsai MH, Schlosser YT, et al. Epstein-Barr virus particles induce centrosome amplification and chromosomal instability. *Nat Commun* 2017 Feb 10;8:14257. [doi: [10.1038/ncomms14257](https://doi.org/10.1038/ncomms14257)] [Medline: [28186092](https://pubmed.ncbi.nlm.nih.gov/28186092/)]
135. Lingle WL, Lutz WH, Ingle JN, Maihle NJ, Salisbury JL. Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. *Proc Natl Acad Sci U S A* 1998 Mar 17;95(6):2950-2955. [doi: [10.1073/pnas.95.6.2950](https://doi.org/10.1073/pnas.95.6.2950)] [Medline: [9501196](https://pubmed.ncbi.nlm.nih.gov/9501196/)]
136. Buschle A, Mrozek-Gorska P, Cernilogar FM, et al. Epstein-Barr virus inactivates the transcriptome and disrupts the chromatin architecture of its host cell in the first phase of lytic reactivation. *Nucleic Acids Res* 2021 Apr 6;49(6):3217-3241. [doi: [10.1093/nar/gkab099](https://doi.org/10.1093/nar/gkab099)] [Medline: [33675667](https://pubmed.ncbi.nlm.nih.gov/33675667/)]

Abbreviations

- BL:** Burkitt lymphoma
- BLAST:** Basic Local Alignment Search Tool
- bp:** base pairs
- COSMIC:** Catalog of Somatic Mutations in Cancer
- CRISPR:** clustered regularly interspaced short palindromic repeats
- DLBCL:** diffuse large B-cell lymphoma
- EBNA1:** Epstein-Barr virus nuclear antigen 1
- EBV:** Epstein-Barr virus
- FA:** Fanconi anemia
- GC:** gastric cancer
- HER2:** human epidermal growth factor receptor 2
- HERV:** human endogenous retrovirus
- HIV1:** human immunodeficiency virus type 1
- HLA:** human leukocyte antigen
- HPV:** human papillomavirus
- JAK:** Janus kinase
- MHC:** major histocompatibility complex
- NF- κ B:** nuclear factor- κ B
- NPC:** nasopharyngeal cancer
- piRNA:** Piwi-interacting RNA
- SWI-SNF :** switch/sucrose non-fermentable
- TIL:** tumor-infiltrating lymphocyte

Edited by A Schwartz; submitted 10.07.23; peer-reviewed by Anonymous, Anonymous; revised version received 19.11.24; accepted 20.11.24; published 29.01.25.

Please cite as:

Friedenson B

Identifying Safeguards Disabled by Epstein-Barr Virus Infections in Genomes From Patients With Breast Cancer: Chromosomal Bioinformatics Analysis

JMIRx Med 2025;6:e50712

URL: <https://xmed.jmir.org/2025/1/e50712>

doi: [10.2196/50712](https://doi.org/10.2196/50712)

© Bernard Friedenson. Originally published in JMIRx Med (<https://med.jmirx.org>), 29.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: A Qualitative Study

Ajit Kerketta*, MHA; Raghavendra A N*, PhD

CHRIST (Deemed to be University), Hosur Road, Bhavani Nagar, Bengaluru, India

* all authors contributed equally

Corresponding Author:

Ajit Kerketta, MHA

CHRIST (Deemed to be University), Hosur Road, Bhavani Nagar, Bengaluru, India

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2023.04.12.23288461v1>

Companion article: <https://med.jmirx.org/2025/1/e70808>

Companion article: <https://med.jmirx.org/2025/1/e70059>

Abstract

Background: Rural health care delivery remains a global challenge and India is no exception, particularly in regions with Indigenous populations such as the state of Jharkhand. The Community Health Centres in Jharkhand, India, are staffed by Indigenous workers who play a crucial role in bridging the health care gap. However, their motivation and retention in these challenging areas are often influenced by a complex mix of sociocultural and environmental factors. One such significant but understudied influencing factor is alimentation, or nutrition, in rural settings. Previous studies have identified several motivators, including community ties, cultural alignment, job satisfaction, and financial incentives. However, the role of alimentation in their motivation and retention in rural areas has not been sufficiently explored.

Objective: This study aims to explore how the strong bond with locally produced food products impacts the retention of Indigenous community health workers (CHWs) in Jharkhand, India, and shed light on a crucial aspect of rural health care workforce sustainability.

Methods: This study adopted a phenomenological research design to explore the lived experiences and perspectives of Indigenous CHWs in Jharkhand. A purposive sampling method was used to select CHWs who had worked in rural areas for at least five years. Data were collected through semistructured interviews, focusing on the participants' experiences of rural alimentation and how it influences their motivation and retention for rural health care. The interviews were audio recorded, transcribed, and analyzed using thematic analysis to identify common themes and patterns in their experiences related to nutrition and retention.

Results: The study revealed that rural alimentation plays a significant role in both the motivation and retention of CHWs in Jharkhand. CHWs who experienced consistent access to local food reported higher job satisfaction, better physical well-being, and a stronger commitment to their roles. It has also been perceived that consuming nutrient-dense food products decreases the risk of chronic illness among rural populations. Additionally, community support systems related to alimentation were found to be crucial in maintaining motivation, with many CHWs emphasizing the importance of local food availability and cultural ties. The findings suggest that improving access to organic nutrition can positively influence the retention of CHWs in rural areas.

Conclusions: Indigenous communities have unique food habits and preferences deeply rooted in agriculture and arboriculture. Their traditional eating practices are integral to their rich cultural heritage, with significant social, symbolic, and spiritual importance. This study highlights the critical role of rural alimentation in motivating and retaining CHWs in rural Community Health Centres in Jharkhand. Therefore, addressing organic versus conventional food in rural health care policies plays a vital role in improving the retention rates of CHWs. By recognizing the interconnectedness of nutrition and workforce sustainability, health care systems can better support Indigenous CHWs and continue delivering health care services.

(JMIRx Med 2025;6:e48346) doi:[10.2196/48346](https://doi.org/10.2196/48346)

KEYWORDS

rural alimentation; community health workers; motivation; retention; rural health; rural nutrition; workforce

Introduction

Rural Health and Alimentation

Community health workers (CHWs) play a vital role in providing primary health care services to rural populations in low- and middle-income countries [1,2]. However, retaining them in rural areas is challenging, largely due to low motivation. One potential factor influencing their motivation and retention is access to a diverse and nutritious diet or rural alimentation [3]. Although the term “alimentation” has existed in the English language since the late 16th century, it is rarely used. In Latin-based languages like French, “alimentation” conveys a holistic view of how humans produce, procure, prepare, share, consume, and digest their food, encompassing human, technological, sociocultural, and environmental aspects [4].

Significance of Alimentation in Rural Health Systems

The term “rural alimentation” in this study refers to the food that Indigenous people produce, acquire, prepare, share, consume, and digest; it is intimately linked to their sociocultural and environmental surroundings. Indigenous CHWs are also among those who are devoted to these tastes and preferences and will find it difficult to give up their native cuisine. Rural food products that are fresh, pure, unadulterated, nutrient dense, and low in pesticides appeal to CHWs [5]. This experience has lured CHWs to continue serving in rural health centers in Jharkhand, India. However, the lack of local and traditional food in metropolitan cities has negatively impacted their motivation, causing many health workers to select rural health care jobs [6]. Previous studies indicate that the desire for nutritious food in the urban setting significantly affects their motivation, job satisfaction, and retention rates. For example, a study in Ethiopia showed that providing nutritious food to CHWs increased job satisfaction and reduced attrition rates [7]. Similarly, a study conducted in Malawi demonstrated that CHWs accessing local food products were less likely to leave their jobs [8]. Despite the potential impact of rural alimentation on CHWs’ motivation and retention, there is limited research on this topic in Jharkhand. Therefore, the study seeks to explore the question: “How does access to diverse and organic food in rural Jharkhand influence the motivation and retention of Indigenous community health workers?”

The study also aims to explore how Indigenous CHWs in Jharkhand perceive the impact of rural alimentation on their motivation and retention. Investigating the connection between rural alimentation and the motivation and retention of Indigenous CHWs will provide valuable insights into the factors that influence their engagement and commitment. We also intend to share the findings with policy makers and health care stakeholders, invoking the implementation of policies that support the well-being of CHWs, promote local food, and attract adequate CHWs to rural Jharkhand.

Study Background

Jharkhand is an eastern Indian state with a population of 39 million spread across 79,714 square kilometers (2019 census). Out of the total population, 24.05% reside in urban areas, while 75.95% live in rural areas [9]. Agriculture and agroforestry products are the primary sources of livelihood. However, the younger generation is increasingly migrating to metropolitan cities in search of better, more sustainable living opportunities. Most state regions are characterized by hills, rugged terrain, lakes, and rivers, presenting significant challenges. While some areas have plains and level topography nestled within natural surroundings, socioeconomic difficulties and a lack of infrastructure make it challenging for CHWs to stay in these locations. Consequently, Jharkhand faces a severe shortage of health workforce [10,11]. Approximately 80% of health care workers are stationed in metropolitan cities catering to the 24.05% of the population residing in urban areas, while 20% health care workers serve the 75.95% population living in rural areas [11-13]. Additionally, the population’s strong beliefs in spirit worship and reliance on local quacks and tantric practices for their ill health further contribute to the short supply of CHWs. This study aims to provide evidence-based insights into the factors that promote the retention of CHWs in rural areas.

Methods

Study Design

We used a qualitative case research design to help understand the perspectives, emotions, and behaviors of Indigenous CHWs and uncover their in-depth experiences [14]. This approach focuses on understanding the subjective meaning that drove CHWs to work in rural Jharkhand [15,16]. This study selected participants with 5 years of service records in the respective Community Health Centers (CHCs).

Ethical Considerations

The study is not a clinical trial and, therefore, does not require registration to establish safety and efficacy standards. Nevertheless, ethical approval was obtained from the Institutional Review Board of CHRIST (Deemed to be University), Bangalore, India (CU: RCEC/00371/11/22). Written informed consent was obtained from each participant before conducting the interview. To further ensure the privacy of the participants, all names were changed to pseudonyms during the transcriptions of the text. However, the interviewers (AK and RNA) know the actual names of the interview participants. Each participant received a fixed remuneration of US \$5.75 after completion of the interview as an acknowledgment of their time and contribution.

Setting and Sample

The corresponding author randomly selected and visited 3 CHCs in the eastern districts of Jharkhand to pilot the survey. This visit played a key role in shaping the development of the research objective: to explore the impact of rural alimentation (local and traditional food systems) on the motivation and

retention of Indigenous CHWs in rural India, as well as to establish a suitable research framework. CHWs from these randomly selected CHCs participated by completing a self-validated questionnaire with open-ended questions. In the main study, 30 CHWs were selected; of these, 10, 12, and 8 CHWs from the respective CHCs met the study criteria. They had served more than five years, expressed willingness to continue residing in rural areas, and were government employees. The study adopted a purposive sampling technique, which helped obtain rich, detailed, and relevant data that influenced the motivation and retention of CHWs in Jharkhand [17]. The male and female respondents were selected irrespective of their rural and urban backgrounds.

Process of Data Generation

A total of 14 participants (4 male individuals and 10 female individuals) ultimately consented to participate in the interviews. However, 16 individuals declined, with some initially agreeing but later withdrawing due to hesitation from the novelty of such an interview process and discomfort with having their comments audio recorded. The participants were aged 30-60 years and expressed their desire to participate in individual, face-to-face or telephone interviews within 10 months. A follow-up interview was done after 4 and 6 months. Within 4 months, 8 interviews were conducted at the CHCs and 6 interviews were conducted in the home district of the reviewer [18,19]. [Multimedia Appendix 1](#) shows the interview guidelines and questionnaire.

The round-1 interview was precise and relevant to the objectives mentioned above (in the *Setting and Sample* section) and, hence, did not require a reinterview of any participants. Interviews were conducted both face-to-face and remotely in Hindi, a language in which the authors are fluent and experienced in conducting qualitative case research. While consent was sought to audio record the interviews, many participants expressed unwillingness; as a result, the researchers took detailed notes instead.

The data were collected through individual, semistructured qualitative case research, with in-depth interviews conducted according to the established protocol matrix [20]. Questions regarding all main areas were posed, albeit in varying order. The interviews in the 4-month follow-up ranged between 6 and 37 minutes (average of 10 min), and interviews in the 6-month follow-up ranged between 5 and 13 minutes (average of 7 min).

Research Team and Reflexibility

AK (research scholar in human resource management, male, aged 40 y) and RAN (PhD in human resource management, male, aged 48 y) solely conducted the interviews. After the interviews, the corresponding author listened to the audio recordings, with several breaks between every audio recording, and transcribed them.

Analysis

We employed the general data analysis methodologies indicated below in the context of thematic analysis and read the texts

multiple times to familiarize and better understand them [21]. Descriptive codes were then applied to data segments [22] relevant to the research question: how do the local food habits influence motivation and continuation of work, and do these factors impact decisions to remain in rural areas? This question was aligned with the objective of the study [23]. The coded data were grouped into themes using QDA Miner Lite software (Provalis Research), demonstrating the relationships between them and identifying themes using inductive methods. The themes were assessed and modified depending on their relevance to the data and the research topic, and they were blended as appropriate. After the themes were developed, they were further defined and given titles that accurately expressed their meanings [24]. Then, the researcher drafted the report. The thematic analysis involves a recursive process of moving back and forth between the data and the emerging themes. It is an iterative and reflexive process, requiring the researcher to consider their biases and assumptions throughout the analysis.

- In-depth investigation: This method provided an in-depth understanding of the study's objectives and phenomena [25]. It enabled the researchers to collect data from multiple sources and examine them comprehensively.
- Contextual analysis: The qualitative case research design allowed the researchers to focus on the social, cultural, economic, and political factors influencing the phenomenon [26].
- Interpretive analysis: This approach involved identifying themes and interpreting them in the context of the research objectives [21,27].
- Flexible design: The qualitative case research design is adaptable, allowing the researchers to evolve the design as data are collected and analyzed [28]. To explore complex and context-specific issues in real-life settings, the interview provided comprehensive insights into the CHWs' experiences, opinions, and perspectives regarding rural alimentation and its impact on their motivation and retention.

Results

Study Participants

We contacted 4 CHCs; however, the medical officer at one center declined to grant permission, citing concerns that the study might inadvertently violate government protocols. A total of 64 CHWs were contacted across the remaining 3 CHCs, who were directly appointed by the government and were under the age of 60 years. [Table 1](#) shows that the majority of health workers were female, accounting for 52% (13/25), 61% (11/18), and 57% (12/21) across the 3 CHCs. Among the 14 participants, 71% (n=10) were female and 29% (n=4) were male. This sex disparity could be a potential area for further research, exploring why fewer male CHWs tend to remain in rural locations.

Table . Participants characteristics from CHCs^a A, B, and C. This table combines the demographic and workplace preferences of health care workers across the 3 centers (A, B, and C).

Characteristics	Center A (n=25), n (%)	Center B (n=18), n (%)	Center (n=21), n (%)
Sex			
Male	12 (48)	7 (39)	9 (43)
Female	13 (52)	11 (61)	12 (57)
Age group (years)			
≤30	3 (12)	5 (28)	4 (19)
≥30	22 (88)	13 (72)	17 (81)
Residence			
Rural origin	25 (100)	18 (100)	21 (100)
Urban origin	0 (0)	0 (0)	0 (0)
Preferred workplace			
Rural area	8 (32)	12 (67)	15 (71)
Male	2 (25) ^b	4 (33) ^b	4 (27) ^b
Female	6 (75) ^b	9 (67) ^b	11 (73) ^b
Urban area	17 (68)	6 (33)	6 (29)
Male	3 (18) ^c	1 (17) ^c	2 (33) ^c
Female	14 (82) ^c	5 (83) ^c	4 (67) ^c

^aCHC: Community Health Centre.

^bPercentages are based the number of workers who preferred a rural workplace as the denominator.

^cPercentages are based the number of workers who preferred an urban workplace as the denominator.

Data were analyzed by constructing a thematic analysis, identifying patterns and themes as guided by the research questions and objectives [24,29]. Emerging themes were verified through member checking to ensure accuracy and validity. This study offers a comprehensive understanding and valid representations [30] of the perspectives and experiences of CHWs staying in rural Jharkhand. The focus is on a specific area within the CHCs, which is predominantly tribal dominated. The analysis identified themes that offered insights into the barriers and facilitators affecting CHWs' access to and consumption of diverse and nutritious food, as well as how their food habits intersect with their roles as health promoters and caregivers.

The study explored three major themes, presented as main themes and their corresponding minor themes, as illustrated below. These themes reflect the perspectives, experiences, and perceptions of the Indigenous CHWs regarding their reasons for remaining in rural Jharkhand.

1. The impact of rural alimentation on Indigenous CHWs' motivation
2. Retention trends among Indigenous CHWs
3. Correlations between nutritional support and job satisfaction

Impact of Rural Alimentation on Indigenous CHWs' Motivation

Health and Nutrition

Local food, often known as "field to plate," plays a vital role in connecting Indigenous CHWs to rural health centers. Free from preservatives, pesticides, additives, and flavorings, this food comes straight from the field, offering freshness and abundance, which enhances both its quality and appeal.

Whenever people call me to see patients or visit their house, they offer me fresh produce from their farm and sometimes even "desi" (country) chicken for free. Where can you get such nutritious and healthy food in cities? [Nurse BY, 4-month interview]

Community Engagement

A unique characteristic of Indigenous communities is their emphasis on communitarian living, characterized by strong bonds of sharing and caring for one another [31,32]. Farming serves as both a livelihood and a means of fostering community engagement and identity. Their connection to the land, local markets, and cultural festivals centered around regional cuisine strengthens their sense of belonging and deepens social ties within the community.

I visit the villages whenever I have time. During these visits, many people gather to sit and discuss the health and well-being of the community, and we motivate the children. On holidays and Sundays, I often take

the village youth to the rivers for fishing. [Doctor BA, 4-month interview]

Work-Life Balance

The concern among these CHWs is their inability to manage their domestic chores, as distance limits regular visits to the family and family affairs. The opportunity to serve in their home town facilitates work-life balance and positively impacts their physical and mental health, reducing stress, increasing job satisfaction, and enhancing productivity [33].

Cultural Connection

Food habits often represent a deep cultural bond and sense of belonging [34]. It makes them feel a strong connection to their heritage and traditions through the food they grew up with, making it more appealing to remain in their hometown.

We gather together and prepare meals for every celebration in common for all young and old. [Accredited social health activist PK, 4-month interview]

Retention Trends Among Indigenous CHWs

Recognition

In rural areas, doctors often receive deep respect and appreciation from the rural community. This sense of being valued and recognized enforced emotional fulfillment, encouraging CHWs to continue serving in these regions.

I feel like a celebrity, as wherever I go—whether it's the market, the community, or my workplace—people honour and respect me immensely. [Doctor DM, 4-month interview]

Career Intentions

The state government implemented various strategies to encourage medical students to serve in rural areas, including career growth incentives such as district quotas for entrance into Bachelor of Medicine, Bachelor of Surgery programs; specialized training programs (eg, barefoot doctor training) for rural service; a 3-month community medicine internship in rural settings; government-sponsored quotas for postgraduate, diploma, and degree course selections; as well as the introduction of the Diplomate of National Board program with training conducted in district hospitals [35]. As a result, professional development opportunities, a supportive work environment, community integration, and work-life balance were factors that encouraged CHWs to choose rural areas [36].

Once I complete the rural posting then there is an opportunity for further professional growth and other career intentions. [Doctor SM, 4-month interview]

Promote Local Food and Lifestyle

Access to local food and a lifestyle that aligns with their cultural values and traditions contribute to higher retention rates [37]. The availability of fresh, familiar foods and a slower pace of life compared to urban centers created a more appealing working environment for Indigenous CHWs.

When I eat food outside of my region, I face digestion problems. It may be because I am not used to spices and tastemakers. Our tribal food is simple and organic resulting in better health outcomes. Therefore, I prefer to be in rural areas. [Nurse PK, 6-month interview]

Role of Cultural Beliefs and Practices

The study of sociocultural and economic factors that affected food consumption patterns in Arab countries demonstrates that the cultural beliefs and practices related to food significantly shaped dietary habits and food choices among rural communities [38]. However, in this study, CHWs reported that the ancient practices have a great impact and were driven by a need for local cuisine [39].

Correlations Between Nutritional Support and Job Satisfaction

Better Health and Productivity

Access to nutritional support ensures that health care workers in rural areas stay physically fit and energized, which enhances their job performance [40]. Knowing that their health and well-being will be supported through nutritious, locally sourced food can make rural postings more attractive.

I have observed that rural people generally don't suffer from chronic diseases, but rather face issues like accidents, sunburn, sunstroke, or water-borne diseases. We are fortunate to have access to nutritious and healthy food. [Nurse SH, 4-month interview]

Incentives of Fresh, Organic, and Local Food

Rural areas offer access to fresh, organic, and culturally significant local food. The availability of healthy, farm-to-table meals can serve as a strong motivator for health care workers, making rural postings more appealing due to the unique lifestyle benefits they offer.

They don't pay me that time for the treatment I provide when I visit or am called to see patients. They often can't afford to pay, but they give me fresh vegetables, pulses, or fruits that they harvest on the spot. Where else, in urban areas, can you find such genuine incentives and fresh produce? [Nurse RJ, 6-month interview]

Low Cost of Living

In rural areas, access to fresh, local food can be more affordable than in urban settings. The prospect of spending less on quality food while still enjoying a nutritious diet can make rural postings more financially appealing.

I go to a market with 1000 INR [US \$15] and buy groceries for the next two to three weeks. Everything is so cheap and fresh in the village markets. Do you think the same in the cities? [Lab technician AG, 4-month interview]

Ethnicity

The findings demonstrated that ethnicity substantially impacted the food habits of a person owing to traditions, social norms,

migration, and acculturation, which is evident within and outside India [41]. When one travels outside of their home country or region, this becomes quite apparent.

Discussion

Principal Findings

The study findings underscore the positive impact that rural alimentation plays in enhancing the contentment of CHWs and highlight the complex interplay between the rural work environment and the factors that drive their motivation [42]. The results indicate that CHWs with access to nutritious food experienced higher motivation and retention rates [43]. The objective of the study also aligns with previous research showing that psychological factors related to adopting a healthy diet can significantly boost life satisfaction and job motivation. In this study, CHWs expressed satisfaction and a sense of contentment with the availability and quality of food in rural areas. This is similar to the study conducted in Tanzania, which showed that access to nutritious food made CHWs more likely to remain in their positions for extended periods [44].

Role of Nutrition in Enhancing Job Satisfaction

Previous studies have determined that a healthy diet helps protect against many chronic diseases, reducing the risk of developing such conditions [45,46]. The availability of locally sourced, nourishing food enhances rural health care workers' motivation and urges providers and administrators to promote a local and healthy diet, which is a relatively simple and cost-effective strategy to improve CHW motivation and retention [47]. The impact of organic food remains to be determined; it helps reduce food safety risks such as pesticide residue and excessive additives [48].

While there is a strong correlation between nutrition and job satisfaction, few studies, especially in health-related fields, have explored this link. The job satisfaction and food habits of CHWs are largely influenced by their socioeconomic conditions and social and cultural practices. For Indigenous CHWs, local food products play a crucial role in maintaining their health and job satisfaction, which significantly impact their retention [49]. A balanced diet contributes to sustained energy and reduces feelings of fatigue and burnout, allowing workers to perform effectively, which enhances their satisfaction with their jobs. A study on nutra-ergonomics explores the relationship between workers, their work environment, and job satisfaction in connection to their nutritional status. It highlights nutrition as a key component of a safe and productive workplace, influencing physical and mental health, and contributing to long-term retention in their current roles [50].

Cultural and Community Ties

Indigenous peoples typically share a deep ancestral connection to their lands and natural resources. They possess distinct cultures, languages, beliefs, and knowledge systems and maintain strong bonds with their land, properties, and territories. Their unique heritage and traditions are central to their identity and way of life. Culturally and politically, they will find themselves out of place from the rest of society [48].

Impact of Nutritional Support

Nutrition contributes to many indicators of well-being, including maternal health, birth weight, child development, and oral health, and is an important determinant of chronic disease, which reduces life expectancy [51]. Inadequate nutritional intake is a major factor contributing to the burden of disease, and when individuals develop chronic conditions as a result, it often leads to significant out-of-pocket expenses for treatment [52,53].

Government Policy

To attract and retain health workers in rural areas, both the state and central governments have implemented several monetary and nonmonetary benefits:

- Monetary incentives: (1) Hard area allowances and provision of residential facilities; (2) flexible salary schemes, such as the "You Quote, We Pay" strategy, ensuring competitive compensation; and (3) performance-based increments of up to 50% [35,54,55]
- Nonmonetary benefits: (1) Professional development opportunities for doctors, nurses, and allied health workers, including upskilling programs; (2) educational incentives, such as additional National Eligibility cum Entrance Test (Postgraduate) marks—10% for each year of service in remote or difficult areas, up to a maximum of 30%; (3) special honorariums to encourage rural practice among specialists; and (4) reservation of 50% of medical diploma seats for in-service state government doctors who have served in remote or challenging areas

These policies address financial and professional needs, making rural health care roles more attractive and sustainable [35,54]

Implications of the Study

The study revealed several significant implications for the retention and motivation of CHWs in rural settings. It underscored that CHWs with access to nutritious and diverse local food products demonstrated higher motivation and retention rates.

First, enhancing the nutrition of CHWs leads to improved health outcomes within the communities they serve. Given their pivotal role in delivering primary health care services in resource-limited rural areas, ensuring the health and motivation of CHWs directly correlated with the quality of care provided to their communities. Second, addressing the nutritional requirements of CHWs assisted in mitigating the challenge of high turnover rates prevalent in rural areas. CHWs often encounter numerous obstacles that contribute to burnout and turnover, such as long working hours, inadequate remuneration, and inadequate support. Third, the study underscored the significance of tackling social determinants of health, including access to nutritious food, to enhance health care outcomes in underserved communities. By addressing these determinants, health disparities can be reduced, thereby fostering overall community health improvement.

Limitations of the Study

The study was conducted in a specific geographic area and focused on a particular group of CHWs. The study's lack of

robust statistical representation may affect the reliability and generalizability of the results.

Conclusion

The research investigated the relationship between rural alimentation and the motivation of Indigenous CHWs in Jharkhand, India. The findings demonstrated that the retention rates of Indigenous health care workers are positively influenced by their local cuisines and nutrition. Moreover, CHWs with access to organic and locally sourced food exhibited superior retention rates compared to Indigenous CHWs deployed in urban areas. This study also indicated that individuals often

exhibit loyalty to their culinary preferences and dietary habits, which drives them to opt for local assignments. Consequently, rural sustenance plays a pivotal role in CHW retention, thereby enhancing the health outcomes of rural residents. In essence, the study underscored the significance of addressing the local diet requirements of CHWs to bolster their motivation and retention rates, consequently elevating the standard of health care services in rural settings. The implications drawn from the study hold crucial insights for policy makers and health care practitioners operating in similar contexts, offering valuable strategies for enhancing the retention and motivation of CHWs in rural areas.

Acknowledgments

The authors extend their heartfelt gratitude to Paul Lelen Hoakip, research scholar, for his invaluable insights and thoughtful contributions, which has greatly enhanced the quality of this paper. Special thanks also to Rev. Dr Ayres Fernandez and Rev. John Thekekara, PhD, for their support and guidance throughout the development of this work. Their expertise and encouragement were instrumental in refining the manuscript and ensuring its academic rigor.

Multimedia Appendix 1

Interview guidelines and questionnaire.

[[DOCX File, 19 KB - xmed_v6i1e48346_app1.docx](#)]

References

1. Bitton A, Ratcliffe HL, Veillard JH, et al. Primary health care as a foundation for strengthening health systems in low- and middle-income countries. *J Gen Intern Med* 2017 May;32(5):566-571. [doi: [10.1007/s11606-016-3898-5](https://doi.org/10.1007/s11606-016-3898-5)] [Medline: [27943038](https://pubmed.ncbi.nlm.nih.gov/27943038/)]
2. van Iseghem T, Jacobs I, Vanden Bossche D, et al. The role of community health workers in primary healthcare in the WHO-EU region: a scoping review. *Int J Equity Health* 2023 Jul 20;22(1):134. [doi: [10.1186/s12939-023-01944-0](https://doi.org/10.1186/s12939-023-01944-0)] [Medline: [37474937](https://pubmed.ncbi.nlm.nih.gov/37474937/)]
3. di Renzo L, Gualtieri P, Pivari F, et al. Eating habits and lifestyle changes during COVID-19 lockdown: an Italian survey. *J Transl Med* 2020 Jun 8;18(1):229. [doi: [10.1186/s12967-020-02399-5](https://doi.org/10.1186/s12967-020-02399-5)] [Medline: [32513197](https://pubmed.ncbi.nlm.nih.gov/32513197/)]
4. Aguilera JM. The concept of alimentation and transdisciplinary research. *J Sci Food Agric* 2021 Mar 30;101(5):1727-1731. [doi: [10.1002/jsfa.10823](https://doi.org/10.1002/jsfa.10823)] [Medline: [32949020](https://pubmed.ncbi.nlm.nih.gov/32949020/)]
5. Warra AA, Prasad MNV. Chapter 16 - African perspective of chemical usage in agriculture and horticulture—their impact on human health and environment. In: Prasad MNV, editor. *Agrochemicals Detection, Treatment and Remediation: Pesticides and Chemical Fertilizers*: Butterworth-Heinemann; 2020:401-436. [doi: [10.1016/B978-0-08-103017-2.00016-7](https://doi.org/10.1016/B978-0-08-103017-2.00016-7)]
6. de Marco M, Thorburn S, Kue J. In a country as affluent as America, people should be eating: experiences with and perceptions of food insecurity among rural and urban Oregonians. *Qual Health Res* 2009 Jul;19(7):1010-1024. [doi: [10.1177/1049732309338868](https://doi.org/10.1177/1049732309338868)] [Medline: [19556404](https://pubmed.ncbi.nlm.nih.gov/19556404/)]
7. Asfaw A, Simane B, Hassen A, Bantider A. Variability and time series trend analysis of rainfall and temperature in northcentral Ethiopia: a case study in Woleka sub-basin. *Weather Clim Extrem* 2018 Mar;19:29-41. [doi: [10.1016/j.wace.2017.12.002](https://doi.org/10.1016/j.wace.2017.12.002)]
8. Palmer MA, Menninger HL, Bernhardt E. River restoration, habitat heterogeneity and biodiversity: a failure of theory or practice? *Freshw Biol* 2010 Jan 15;55(s1):205-222. [doi: [10.1111/j.1365-2427.2009.02372.x](https://doi.org/10.1111/j.1365-2427.2009.02372.x)]
9. National Health Mission. 13th common review mission 2019. National Health Systems Resource Centre. 2019 URL: https://nhsrcindia.org/sites/default/files/2021-04/13th_common_review_mission-Report_2019_Revise.pdf
10. Government of India. Census of India 2011. 2011. URL: <https://censusindia.gov.in/nada/index.php/catalog/1366/download/4478/Pesreport.pdf> [accessed 2025-01-15]
11. Rural health statistics 2018-19. Ministry of Health and Family Welfare. 2019. URL: <https://mohfw.gov.in/?q=reports/rural-health-statistics-2018-19> [accessed 2025-01-15]
12. Kok MC, Kane SS, Tulloch O, et al. How does context influence performance of community health workers in low- and middle-income countries? evidence from the literature. *Health Res Policy Sys* 2015 Mar 7;13:13. [doi: [10.1186/s12961-015-0001-3](https://doi.org/10.1186/s12961-015-0001-3)] [Medline: [25890229](https://pubmed.ncbi.nlm.nih.gov/25890229/)]
13. Jharkhand urban population. Population Census Data (india). 2011. URL: <https://www.census2011.co.in/census/state/jharkhand>.

- [html#:~:text=As%20per%20details%20from%20Census,are%2016%2C930%2C315%20and%2016%2C057%2C819%20respectively](#) [accessed 2025-01-17]
14. Fossey E, Harvey C, McDermott F, Davidson L. Understanding and evaluating qualitative research. *Aust N Z J Psychiatry* 2002 Dec;36(6):717-732. [doi: [10.1046/j.1440-1614.2002.01100.x](#)] [Medline: [12406114](#)]
 15. Creswell JW, Creswell JD. *Research Design: Qualitative, Quantitative and Mixed Methods Approaches*: Sage Publications, Inc; 2009. URL: <https://collegepublishing.sagepub.com/products/research-design-6-270550> [accessed 2025-01-17]
 16. Sutton J, Austin Z. Qualitative research: data collection, analysis, and management. *Can J Hosp Pharm* 2015;68(3):226-231. [doi: [10.4212/cjhp.v68i3.1456](#)] [Medline: [26157184](#)]
 17. de Leeuw JA, Woltjer H, Kool RB. Identification of factors influencing the adoption of health information technology by nurses who are digitally lagging: in-depth interview study. *J Med Internet Res* 2020 Aug 14;22(8):e15630. [doi: [10.2196/15630](#)] [Medline: [32663142](#)]
 18. Bebbington P, Wilkins S, Sham P, et al. Life events before psychotic episodes: do clinical and social variables affect the relationship? *Soc Psychiatry Psychiatr Epidemiol* 1996 Jun;31(3-4):122-128. [doi: [10.1007/BF00785758](#)] [Medline: [8766457](#)]
 19. Ghai S, Dutta M, Garg A. Perceived level of stress, stressors and coping behaviours in nursing students. *Indian J Posit Psychol* 2014;5(1):60-65 [FREE Full text]
 20. Jacobs K. Discourse analysis. In: Baum S, editor. *Methods in Urban Analysis*: Springer; 2021:151-172. [doi: [10.1007/978-981-16-1677-8_9](#)]
 21. Castleberry A, Nolen A. Thematic analysis of qualitative research data: is it as easy as it sounds? *Curr Pharm Teach Learn* 2018 Jun;10(6):807-815. [doi: [10.1016/j.cptl.2018.03.019](#)] [Medline: [30025784](#)]
 22. Tirandaz Z, Akbarizadeh G, Kaabi H. PolSAR image segmentation based on feature extraction and data compression using weighted neighborhood filter bank and hidden Markov random field-expectation maximization. *Measurement (Lond)* 2020 Mar;153:107432. [doi: [10.1016/j.measurement.2019.107432](#)]
 23. Bradley EH, Curry LA, Devers KJ. Qualitative data analysis for health services research: developing taxonomy, themes, and theory. *Health Serv Res* 2007 Aug;42(4):1758-1772. [doi: [10.1111/j.1475-6773.2006.00684.x](#)] [Medline: [17286625](#)]
 24. Thomas J, Harden A. Methods for the thematic synthesis of qualitative research in systematic reviews. *BMC Med Res Methodol* 2008 Jul 10;8:45. [doi: [10.1186/1471-2288-8-45](#)] [Medline: [18616818](#)]
 25. Baškarada S. Qualitative case study guidelines. *The Qualitative Report* 2014;19(40):1-18. [doi: [10.46743/2160-3715/2014.1008](#)]
 26. Mbindyo P, Gilson L, Blaauw D, English M. Contextual influences on health worker motivation in district hospitals in Kenya. *Implementation Sci* 2009 Jul 23;4:43. [doi: [10.1186/1748-5908-4-43](#)] [Medline: [19627590](#)]
 27. Coventry PA, Fisher L, Kenning C, Bee P, Bower P. Capacity, responsibility, and motivation: a critical qualitative evaluation of patient and practitioner views about barriers to self-management in people with multimorbidity. *BMC Health Serv Res* 2014 Oct 31;14(1):536. [doi: [10.1186/s12913-014-0536-y](#)] [Medline: [25367263](#)]
 28. Cavaye ALM. Case study research: a multi - faceted research approach for IS. *Information Systems Journal* 1996;6(3):227-242. [doi: [10.1111/j.1365-2575.1996.tb00015.x](#)]
 29. Peterson BL. Thematic analysis/interpretive thematic analysis. In: *The International Encyclopedia of Communication Research Methods*: Wiley; 2017:1-9. [doi: [10.1002/9781118901731.iecrm0249](#)]
 30. Willan J. Doing early childhood research. In: *Early Childhood Studies: A Multidisciplinary Approach*: Bloomsbury Publishing; 2017:353-371 URL: <https://www.bloomsbury.com/ca/early-childhood-studies-9781137274021/> [accessed 2025-01-23] [doi: [10.1057/978-1-137-27402-1_18](#)]
 31. Coates J. In: Gray M, editor. *Indigenous Social Work around the World: Towards Culturally Relevant Education and Practice*: Routledge; 2016:1-5. [doi: [10.4324/9781315588360](#)]
 32. Smith-Morris C. *Indigenous Communalism: Belonging, Healthy Communities, and Decolonizing the Collective*: Rutgers University Press; 2020:25. [doi: [10.2307/j.ctvscxrb6.5](#)]
 33. Akintola O, Chikoko G. Factors influencing motivation and job satisfaction among supervisors of community health workers in marginalized communities in South Africa. *Hum Resour Health* 2016 Sep 6;14(1):54. [doi: [10.1186/s12960-016-0151-6](#)] [Medline: [27601052](#)]
 34. Weber EU. Climate change demands behavioral change: what are the challenges? *Social Research: An International Quarterly* 2015;82(3):561-580. [doi: [10.1353/sor.2015.0050](#)]
 35. Ghosh K. Why we don't get doctors for rural medical service in India? *Natl Med J India* 2018;31(1):44-46. [doi: [10.4103/0970-258X.243416](#)] [Medline: [30348926](#)]
 36. Agyapong VIO, Osei A, Farren CK, McAuliffe E. Factors influencing the career choice and retention of community mental health workers in Ghana. *Hum Resour Health* 2015 Jul 9;13(1):56. [doi: [10.1186/s12960-015-0050-2](#)] [Medline: [26156234](#)]
 37. Schrank Z, Running K. Individualist and collectivist consumer motivations in local organic food markets. *J Cons Cult* 2018 Feb;18(1):184-201. [doi: [10.1177/1469540516659127](#)]
 38. Musaiger AO. Socio-cultural and economic factors affecting food consumption patterns in the Arab countries. *J R Soc Health* 1993 Apr;113(2):68-74. [doi: [10.1177/146642409311300205](#)] [Medline: [8478894](#)]

39. Chakona G. Social circumstances and cultural beliefs influence maternal nutrition, breastfeeding and child feeding practices in South Africa. *Nutr J* 2020 May 20;19(1):47. [doi: [10.1186/s12937-020-00566-4](https://doi.org/10.1186/s12937-020-00566-4)] [Medline: [32434557](https://pubmed.ncbi.nlm.nih.gov/32434557/)]
40. Karaferis D, Aletras V, Raikou M, Niakas D. Factors influencing motivation and work engagement of healthcare professionals. *Mater Sociomed* 2022 Sep;34(3):216-224. [doi: [10.5455/msm.2022.34.216-224](https://doi.org/10.5455/msm.2022.34.216-224)] [Medline: [36310751](https://pubmed.ncbi.nlm.nih.gov/36310751/)]
41. Verbeke W, Poquiqui LÓpez G. Ethnic food attitudes and behaviour among Belgians and Hispanics living in Belgium. *British Food Journal* 2005 Dec 1;107(11):823-840. [doi: [10.1108/00070700510629779](https://doi.org/10.1108/00070700510629779)]
42. Wronska MD, Coffey M, Robins A. Determinants of nutrition practice and food choice in UK construction workers. *Health Promot Int* 2022 Oct 1;37(5):daac129. [doi: [10.1093/heapro/daac129](https://doi.org/10.1093/heapro/daac129)] [Medline: [36166265](https://pubmed.ncbi.nlm.nih.gov/36166265/)]
43. Ozano K, Simkhada P, Thann K, Khatri R. Improving local health through community health workers in Cambodia: challenges and solutions. *Hum Resour Health* 2018 Jan 6;16(1):2. [doi: [10.1186/s12960-017-0262-8](https://doi.org/10.1186/s12960-017-0262-8)] [Medline: [29304869](https://pubmed.ncbi.nlm.nih.gov/29304869/)]
44. Greenspan JA, McMahon SA, Chebet JJ, Mpunga M, Urassa DP, Winch PJ. Sources of community health worker motivation: a qualitative study in Morogoro Region, Tanzania. *Hum Resour Health* 2013 Oct 10;11:52. [doi: [10.1186/1478-4491-11-52](https://doi.org/10.1186/1478-4491-11-52)] [Medline: [24112292](https://pubmed.ncbi.nlm.nih.gov/24112292/)]
45. Steinman L, Heang H, van Pelt M, et al. Facilitators and barriers to chronic disease self-management and mobile health interventions for people living with diabetes and hypertension in Cambodia: qualitative study. *JMIR Mhealth Uhealth* 2020 Apr 24;8(4):e13536. [doi: [10.2196/13536](https://doi.org/10.2196/13536)] [Medline: [32329737](https://pubmed.ncbi.nlm.nih.gov/32329737/)]
46. Cena H, Calder PC. Defining a healthy diet: evidence for the role of contemporary dietary patterns in health and disease. *Nutrients* 2020 Jan 27;12(2):334. [doi: [10.3390/nu12020334](https://doi.org/10.3390/nu12020334)] [Medline: [32012681](https://pubmed.ncbi.nlm.nih.gov/32012681/)]
47. Mozaffarian D, Angell SY, Lang T, Rivera JA. Role of government policy in nutrition-barriers to and opportunities for healthier eating. *BMJ* 2018 Jun 13;361:k2426. [doi: [10.1136/bmj.k2426](https://doi.org/10.1136/bmj.k2426)] [Medline: [29898890](https://pubmed.ncbi.nlm.nih.gov/29898890/)]
48. Chai D, Meng T, Zhang D. Influence of food safety concerns and satisfaction with government regulation on organic food consumption of Chinese urban residents. *Foods* 2022 Sep 22;11(19):2965. [doi: [10.3390/foods11192965](https://doi.org/10.3390/foods11192965)] [Medline: [36230045](https://pubmed.ncbi.nlm.nih.gov/36230045/)]
49. Kendirkiran G, Batur B. The relationship between eating behavior and job satisfaction of academic staff. *Int J Caring Sci* 2022 Aug;15(2):825-836 [FREE Full text]
50. Shearer J, Graham TE, Skinner TL. Nutra-ergonomics: influence of nutrition on physical employment standards and the health of workers. *Appl Physiol Nutr Metab* 2016 Jun;41(6 Suppl 2):S165-S174. [doi: [10.1139/apnm-2015-0531](https://doi.org/10.1139/apnm-2015-0531)] [Medline: [27277565](https://pubmed.ncbi.nlm.nih.gov/27277565/)]
51. Alfred T, Corntassel J. Being Indigenous: resurgences against contemporary colonialism. *Government and Opposition* 2005;40(4):597-614. [doi: [10.1111/j.1477-7053.2005.00166.x](https://doi.org/10.1111/j.1477-7053.2005.00166.x)]
52. Browne J, Hayes R, Gleeson D. Aboriginal health policy: is nutrition the 'gap' in 'Closing the Gap'? *Aust N Z J Public Health* 2014 Aug;38(4):362-369. [doi: [10.1111/1753-6405.12223](https://doi.org/10.1111/1753-6405.12223)] [Medline: [25091077](https://pubmed.ncbi.nlm.nih.gov/25091077/)]
53. Schembri L, Curran J, Collins L, et al. The effect of nutrition education on nutrition - related health outcomes of Aboriginal and Torres Strait Islander people: a systematic review. *Aust N Z J Public Health* 2016 Apr;40(Suppl 1):S42-S47. [doi: [10.1111/1753-6405.12392](https://doi.org/10.1111/1753-6405.12392)] [Medline: [26123037](https://pubmed.ncbi.nlm.nih.gov/26123037/)]
54. Press Information Bureau, Ministry of Information and Broadcasting, Government of India. Revolutionizing healthcare: digital innovations in India's health sector. Press Information Bureau. 2024 Jan 15. URL: <https://static.pib.gov.in/WriteReadData/specificdocs/documents/2024/jan/doc2024115298601.pdf> [accessed 2025-01-15]
55. Silvestri DM, Blevins M, Afzal A, et al. Medical and nursing students' intentions to work abroad or in rural areas: an eight-country cross-sectional survey in Asia and Africa. *Ann Glob Health* 2015 Mar 12;81(1):52. [doi: [10.1016/j.aogh.2015.02.627](https://doi.org/10.1016/j.aogh.2015.02.627)]

Abbreviations

CHC: Community Health Centre

CHW: community health worker

Edited by A Schwartz; submitted 20.04.23; peer-reviewed by SK Thalari; revised version received 28.11.24; accepted 13.12.24; published 23.01.25.

Please cite as:

Kerketta A, A N R

The Impact of Rural Alimentation on the Motivation and Retention of Indigenous Community Health Workers in India: A Qualitative Study

JMIRx Med 2025;6:e48346

URL: <https://xmed.jmir.org/2025/1/e48346>

doi: [10.2196/48346](https://doi.org/10.2196/48346)

© Ajit Kerketta, Raghavendra AN. Originally published in JMIRx Med (<https://med.jmirx.org>), 23.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review

Feryal Kurdi*, MD; Yahya Kurdi*, MD; Igor Vladimirovich Reshetov, MD, PhD

Department of Oncology, Radiotherapy and Plastic and Reconstructive Surgery, Sechenov University, Bolshaya Pirogovskaya, 6c1, Moscow, Russian Federation

*these authors contributed equally

Corresponding Author:

Feryal Kurdi, MD

Department of Oncology, Radiotherapy and Plastic and Reconstructive Surgery, Sechenov University, Bolshaya Pirogovskaya, 6c1, Moscow, Russian Federation

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.07.30.24311256v1>

Companion article: <https://med.jmirx.org/2025/1/e69705>

Companion article: <https://med.jmirx.org/2025/1/e68769>

Abstract

Introduction: Breast cancer is the leading cause of morbidity and mortality worldwide. Accurate sentinel lymph node (SLN) mapping is crucial for staging and treatment planning in early-stage breast cancer. Indocyanine green (ICG) has emerged as a promising agent for fluorescence imaging in SLN mapping. However, comprehensive assessment of its clinical utility, including accuracy and adverse effects, remains limited. This scoping review aims to consolidate evidence on the use of ICG in breast cancer SLN mapping.

Objective: The objective of this scoping review is to evaluate the current literature on the use of ICG in SLN mapping for patients with breast cancer. This review aims to assess the accuracy, efficacy, and safety of ICG in this context and to identify gaps in the existing research. The outcomes will contribute to the development of further research as part of a PhD project.

Methods: Five electronic databases will be searched (PubMed, Embase, MEDLINE, Web of Science, and Scopus) using search strategies developed in consultation with an academic supervisor. The search strategy is set to human studies published in English within the last 11 years. All retrieved citations will be imported to Zotero and then uploaded to Covidence for the screening of titles, abstracts, and full text according to prespecified inclusion criteria. Patients with early-stage breast cancer (T1 and T2), selected T3 cases where the SLN biopsy is accurate, and those with clinically node-negative breast cancer will be included. The intervention criterion includes studies using ICG for SLN mapping and studies on the assessment of fluorescence imaging cameras. Citations meeting the inclusion criteria for full-text review will have their data extracted by 2 independent reviewers, with disagreements resolved by discussion. A data extraction tool will be developed to capture full details about the participants, concept, and context, and findings relevant to the scoping review will be summarized.

Results: The preliminary search began in December 2023. As of September 2024, papers have been screened and data are currently being extracted. Out of the 2130 references initially imported, 126 studies met the inclusion criteria after screening. The scoping review is expected to be published in January 2025.

Conclusions: Although ICG technology has been used for SLN mapping in patients with breast cancer, initial searches in 2022 revealed limited data on this technique's feasibility, safety, and effectiveness. At that time, preliminary search of Scopus, MEDLINE, Embase, and PubMed identified no current or forthcoming systematic reviews or scoping reviews on the topic. However, recent searches indicate a substantial increase in research and reviews, reflecting a growing interest and evidence in this area.

(*JMIRx Med* 2025;6:e66213) doi:[10.2196/66213](https://doi.org/10.2196/66213)

KEYWORDS

indocyanine green; ICG; sentinel lymph node; breast cancer; fluorescence; axillary lymph node mapping; NIR; surgical planning; near-infrared

Introduction

Sentinel lymph node (SLN) biopsy plays a crucial role in staging and prognosis in breast cancer management. The SLN is the initial lymph node to which breast cancer cells are likely to metastasize, and the presence of cancer cells in the SLN indicates a higher likelihood of further metastasis to other lymph nodes and distant organs [1].

SLN biopsy involves injecting a tracer substance into the breast, which then migrates to the SLN. The SLN is then identified, excised, and examined for cancer cells. If the SLN is free of cancer cells, it suggests that the cancer has not spread to other lymph nodes, eliminating the need for additional lymph node dissection. Conversely, if the SLN contains metastases, further dissection is typically required [2].

Over the past 2 decades, SLN biopsy using blue dye and radiotracers has been established as the diagnostic standard of care for patients with early-stage breast cancer who have clinically negative lymph nodes [3,4].

However, these methods come with certain drawbacks, including the potential for allergic reactions to the blue dye and the necessity of nuclear medicine facilities for radiotracer injection and detection. In a cohort undergoing blue dye and radiotracer injection procedures, a small number of adverse reactions, such as skin tattooing and anaphylaxis, were reported [5].

In recent years, near-infrared (NIR) fluorescence imaging using indocyanine green (ICG) has emerged as an alternative approach for SLN mapping in patients with breast cancer. ICG, a fluorescent dye, is injected into the breast, which then migrates to the SLNs. A NIR camera detects the fluorescence emitted by ICG, enabling the surgeon to identify and excise the SLNs [6,7].

This technology offers several advantages over traditional methods, including enhanced visualization of SLNs, a lower risk of allergic reactions, and the elimination of the need for nuclear medicine facilities. Furthermore, ICG has an excellent safety profile [8-11].

The importance of this topic stems from the potential of ICG technology to enhance the accuracy and safety of SLN mapping in patients with breast cancer. Precise identification and removal of the SLN are crucial for accurate staging and prognosis. Inaccurate SLN identification can lead to unnecessary lymph node dissection, resulting in complications such as lymphedema and impaired arm function. Sampling a larger number of SLNs may increase the risk of upper limb lymphedema, sensory deficits, and reduced shoulder function.

Landmark trials have shown a significant difference in morbidity rates when comparing SLN biopsy to axillary dissection, with rates of 25% and 70%, respectively [3,12]. Recent studies have reported excising, on average, 2 nodes per patient, likely due to advancements in NIR technology and ICG fluorescence protocols [13-17]. Nevertheless, further research is essential to assess the long-term outcomes and cost-effectiveness of ICG technology compared to traditional methods.

Methods

Overview

The proposed scoping review will be guided by the JBI methodology for scoping reviews [18]. The search strategy aims to locate both published and unpublished articles. An initial limited search of PubMed, Embase, MEDLINE, Web of Science, and Scopus was undertaken to identify relevant articles on the use of ICG for SLN mapping in breast cancer. In consultation with an academic supervisor, the keywords in the titles and abstracts of relevant articles, as well as the index terms used to describe these articles, were used to develop a comprehensive search strategy for PubMed, Embase, MEDLINE, Web of Science, and Scopus (see [Multimedia Appendix 1](#)). This strategy, including all identified keywords and index terms, will be adapted for each included database. The articles sourced from all included sources of evidence will be exported into Zotero (Corporation for Digital Scholarship).

Only articles published in English will be included due to the language proficiency of the reviewers. Articles published since January 1, 2014, will be included to ensure relevance, aligning with the project's consideration of recent data and the ongoing advancements in SLN mapping techniques using ICG.

JBI Methodology for Scoping Reviews

The outcomes of the scoping review will inform and frame three subsequent pieces of work planned as part of a PhD project:

1. Prospective cohort study on the long-term outcomes of ICG in SLN mapping
2. Systematic review and meta-analysis of ICG for SLN mapping in breast cancer
3. Development of standardized clinical guidelines and protocols for the use of ICG in SLN mapping in patients with breast cancer

The Participants-Concept-Context framework for this scoping review defines (1) the participants as patients with early-stage breast cancer, (2) the concept as the use of ICG for SLN mapping in patients with breast cancer, and (3) the context as SLN mapping that is performed as part of breast cancer staging and treatment planning.

Review Questions

The review questions are as follows:

1. What do we know about the evaluation and integration of emergent evidence on the use of ICG for SLN mapping in patients with breast cancer into clinical practice and decision-making?
2. To what extent is emergent evidence on the feasibility, safety, and effectiveness of ICG for SLN mapping integrated into clinical guidelines and decision-making processes?
3. How is emergent evidence on the use of ICG for SLN mapping evaluated and incorporated into clinical guidelines and decision-making processes?

For the purposes of this scoping review, emergent evidence refers to new research findings on ICG for SLN mapping that

have emerged after market launch and have not yet been fully integrated into clinical guidelines and practice.

Eligibility Criteria

The eligibility criteria are as follows. Participants will include patients with early-stage breast cancer (T1 and T2) and selected T3 cases where SLN biopsy has been shown to be accurate. Concept will include the use of ICG for SLN mapping in patients with breast cancer, as well as the assessment of imaging techniques and devices used in conjunction with ICG for SLN mapping. Context will include clinical settings where SLN mapping is performed as part of breast cancer staging and treatment planning.

This scoping review will consider both experimental and quasi-experimental study designs, including controlled before-and-after studies and controlled interrupted time-series studies. In addition, analytical observational studies including prospective and retrospective cohort studies, case-control studies, and analytical cross-sectional studies will be considered for inclusion. This review will also consider descriptive observational study designs such as descriptive cross-sectional studies for inclusion. Qualitative studies that focus on qualitative data will be considered for inclusion.

Following the search, all identified articles will be exported into Zotero. Then, the remaining articles will be uploaded into Covidence (Veritas Health Innovations Ltd). Titles and abstracts will then be screened by the lead author against the inclusion criteria for the scoping review. Potentially relevant articles will be retrieved in full and included in Covidence. The full text of these articles will be assessed in detail against the inclusion criteria by 2 independent reviewers. Reasons for the exclusion of sources of evidence at the full-text stage that do not meet the inclusion criteria will be recorded and reported in the scoping review. Any disagreements that arise between the reviewers at each stage of the selection process will be resolved through discussion or with an additional reviewer. The results of the search and the study inclusion process will be reported in full in the final scoping review and presented in a PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews) checklist, as extracted from Covidence (see [Multimedia Appendix 2](#)) [19].

Data will be extracted from all articles included in the scoping review by 2 independent reviewers, using a data extraction tool developed by the lead reviewer and piloted with about 15 articles to refine and improve it. The data extracted will include specific details about the participants, concept, context, study methods, and key findings relevant to the scoping review questions and will be imported into either Covidence or Microsoft Excel.

A draft extraction form is provided (see [Multimedia Appendix 3](#)). The draft data extraction tool will be modified and revised as necessary during the process of extracting data from each included article. Modifications will be detailed in the scoping review. Any disagreements that arise between the reviewers will be resolved through discussion or with an additional reviewer. If appropriate, authors of articles will be contacted to request missing or additional data, where required.

The evidence presented will directly respond to the scoping review's objective and questions. The data will be presented graphically or in diagrammatic or tabular form. A narrative summary will accompany the tabulated and/or charted results and will describe how the results relate to the scoping review's objective and questions.

Results

The preliminary search began in December 2023. As of September 2024, papers have been screened and data are currently being extracted. Out of the 2130 references initially imported, 126 studies met the inclusion criteria after screening (see [Multimedia Appendix 4](#)). The scoping review is anticipated to be published in January 2025.

Discussion

The significance of SLN mapping using ICG technology in breast cancer lies in its potential to enhance accuracy and safety, reduce complications, and improve patient outcomes [20]. Although ICG technology has been used for SLN mapping in patients with breast cancer, initial searches in 2022 revealed limited data on the feasibility, safety, and effectiveness of this technique. At that time, a preliminary search of Scopus, MEDLINE, Embase, and PubMed identified no current or forthcoming systematic reviews or scoping reviews on the topic. However, recent searches indicate a substantial increase in research and reviews, reflecting a growing interest and evidence in this area. Further studies are necessary to assess the long-term efficacy and cost-effectiveness of this technique and to identify the patient populations most likely to benefit.

The objective of this scoping review is to assess the extent of the literature on SLN mapping using ICG technology around the evaluation and integration of emergent evidence for benefits and harms; explore its feasibility, safety, and effectiveness in a larger cohort of patients with breast cancer; and provide guidance for clinical decision-making.

This scoping review could also identify specific patient populations, such as those with higher BMIs, who may benefit most from ICG technology. Additionally, patients who have undergone neoadjuvant therapy could be particularly advantageous candidates.

Factors such as the type of NIR cameras used, the learning curve for surgeons to become proficient with ICG for SLN detection, the availability of ICG and radioisotopes, the presence of nuclear medicine facilities, regional variations in ICG usage, and cost comparisons with the gold standard are also critical considerations in the broader adoption of this technology.

Limitations of this study include a lack of quantitative synthesis (ie, meta-analysis) of the results, which may limit the ability to draw strong conclusions. This scoping review serves as a foundational step toward a more comprehensive systematic review and meta-analysis guiding the clinical decision-making and the integration of ICG into standardized guidelines for SLN mapping in patients with breast cancer.

Acknowledgments

This scoping review is to contribute in part to a Doctor of Philosophy degree.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Search strategy.

[\[DOCX File, 14 KB - xmed_v6i1e66213_app1.docx \]](#)

Multimedia Appendix 2

PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews) checklist.

[\[DOCX File, 112 KB - xmed_v6i1e66213_app2.docx \]](#)

Multimedia Appendix 3

Data extraction instrument.

[\[DOCX File, 15 KB - xmed_v6i1e66213_app3.docx \]](#)

Multimedia Appendix 4

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) extraction flowchart.

[\[DOCX File, 79 KB - xmed_v6i1e66213_app4.docx \]](#)

References

1. McMasters KM, Tuttle TM, Carlson DJ, et al. Sentinel lymph node biopsy for breast cancer: a suitable alternative to routine axillary dissection in multi-institutional practice when optimal technique is used. *J Clin Oncol* 2000 Jul;18(13):2560-2566. [doi: [10.1200/JCO.2000.18.13.2560](https://doi.org/10.1200/JCO.2000.18.13.2560)] [Medline: [10893287](https://pubmed.ncbi.nlm.nih.gov/10893287/)]
2. Gradishar WJ, Moran MS, Abraham J, et al. NCCN Guidelines® Insights: Breast Cancer, version 4.2023. *J Natl Compr Canc Netw* 2023 Jun;21(6):594-608. [doi: [10.6004/jnccn.2023.0031](https://doi.org/10.6004/jnccn.2023.0031)] [Medline: [37308117](https://pubmed.ncbi.nlm.nih.gov/37308117/)]
3. Mansel RE, Fallowfield L, Kissin M, et al. Randomized multicenter trial of sentinel node biopsy versus standard axillary treatment in operable breast cancer: the ALMANAC Trial. *J Natl Cancer Inst* 2006 May 3;98(9):599-609. [doi: [10.1093/jnci/djj158](https://doi.org/10.1093/jnci/djj158)] [Medline: [16670385](https://pubmed.ncbi.nlm.nih.gov/16670385/)]
4. Donker M, van Tienhoven G, Straver ME, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. *Lancet Oncol* 2014 Nov;15(12):1303-1310. [doi: [10.1016/S1470-2045\(14\)70460-7](https://doi.org/10.1016/S1470-2045(14)70460-7)] [Medline: [25439688](https://pubmed.ncbi.nlm.nih.gov/25439688/)]
5. Nguyen CL, Zhou M, Easwaralingam N, et al. Novel dual tracer indocyanine green and radioisotope versus gold standard sentinel lymph node biopsy in breast cancer: the GREENORBLUE Trial. *Ann Surg Oncol* 2023 Oct;30(11):6520-6527. [doi: [10.1245/s10434-023-13824-6](https://doi.org/10.1245/s10434-023-13824-6)] [Medline: [37402976](https://pubmed.ncbi.nlm.nih.gov/37402976/)]
6. Polom K, Murawa D, Rho YS, Nowaczyk P, Hünerbein M, Murawa P. Current trends and emerging future of indocyanine green usage in surgery and oncology: a literature review. *Cancer* 2011 Nov 1;117(21):4812-4822. [doi: [10.1002/cncr.26087](https://doi.org/10.1002/cncr.26087)] [Medline: [21484779](https://pubmed.ncbi.nlm.nih.gov/21484779/)]
7. Liberale G, Vankerckhove S, Bouazza F, et al. Systemic sentinel lymph node detection using fluorescence imaging after indocyanine green intravenous injection in colorectal cancer: protocol for a feasibility study. *JMIR Res Protoc* 2020 Aug 14;9(8):e17976. [doi: [10.2196/17976](https://doi.org/10.2196/17976)] [Medline: [32554370](https://pubmed.ncbi.nlm.nih.gov/32554370/)]
8. van der Vorst JR, Schaafsma BE, Hutteman M, et al. Near-infrared fluorescence-guided resection of colorectal liver metastases. *Cancer* 2013 Sep 15;119(18):3411-3418. [doi: [10.1002/cncr.28203](https://doi.org/10.1002/cncr.28203)] [Medline: [23794086](https://pubmed.ncbi.nlm.nih.gov/23794086/)]
9. Hope-Ross M, Yannuzzi LA, Gragoudas ES, et al. Adverse reactions due to indocyanine green. *Ophthalmology* 1994 Mar;101(3):529-533. [doi: [10.1016/s0161-6420\(94\)31303-0](https://doi.org/10.1016/s0161-6420(94)31303-0)] [Medline: [8127574](https://pubmed.ncbi.nlm.nih.gov/8127574/)]
10. Griffiths M, Chae MP, Rozen WM. Indocyanine green-based fluorescent angiography in breast reconstruction. *Gland Surg* 2016 Apr;5(2):133-149. [doi: [10.3978/j.issn.2227-684X.2016.02.01](https://doi.org/10.3978/j.issn.2227-684X.2016.02.01)] [Medline: [27047782](https://pubmed.ncbi.nlm.nih.gov/27047782/)]
11. Benya R, Quintana J, Brundage B. Adverse reactions to indocyanine green: a case report and a review of the literature. *Cathet Cardiovasc Diagn* 1989 Aug;17(4):231-233. [doi: [10.1002/ccd.1810170410](https://doi.org/10.1002/ccd.1810170410)] [Medline: [2670244](https://pubmed.ncbi.nlm.nih.gov/2670244/)]
12. Giuliano AE, Hunt KK, Ballman KV, et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA* 2011 Feb 9;305(6):569-575. [doi: [10.1001/jama.2011.90](https://doi.org/10.1001/jama.2011.90)] [Medline: [21304082](https://pubmed.ncbi.nlm.nih.gov/21304082/)]

13. Fleissig A, Fallowfield LJ, Langridge CI, et al. Post-operative arm morbidity and quality of life. results of the ALMANAC randomised trial comparing sentinel node biopsy with standard axillary treatment in the management of patients with early breast cancer. *Breast Cancer Res Treat* 2006 Feb;95(3):279-293. [doi: [10.1007/s10549-005-9025-7](https://doi.org/10.1007/s10549-005-9025-7)] [Medline: [16163445](#)]
14. Ahmed M, Purushotham AD, Douek M. Novel techniques for sentinel lymph node biopsy in breast cancer: a systematic review. *Lancet Oncol* 2014 Jul;15(8):e351-e362. [doi: [10.1016/S1470-2045\(13\)70590-4](https://doi.org/10.1016/S1470-2045(13)70590-4)] [Medline: [24988938](#)]
15. Schaafsma BE, Verbeek FPR, Rietbergen DDD, et al. Clinical trial of combined radio- and fluorescence-guided sentinel lymph node biopsy in breast cancer. *Br J Surg* 2013 Jul;100(8):1037-1044. [doi: [10.1002/bjs.9159](https://doi.org/10.1002/bjs.9159)] [Medline: [23696463](#)]
16. Ballardini B, Santoro L, Sangalli C, et al. The indocyanine green method is equivalent to the 99mTc-labeled radiotracer method for identifying the sentinel node in breast cancer: a concordance and validation study. *Eur J Surg Oncol* 2013 Dec;39(12):1332-1336. [doi: [10.1016/j.ejso.2013.10.004](https://doi.org/10.1016/j.ejso.2013.10.004)] [Medline: [24184123](#)]
17. Abe H, Yamazaki K, Tokuda A, Ogawa M, Kawasaki M, Kameyama M. A novel approach for sentinel lymph node identification using fluorescence imaging and computed tomography lymphography in early-stage breast cancer patients. *J Clin Oncol* 2014 May 20;32(15_suppl):e12025-e12025. [doi: [10.1200/jco.2014.32.15_suppl.e12025](https://doi.org/10.1200/jco.2014.32.15_suppl.e12025)]
18. Peters MDJ, Marnie C, Tricco AC, et al. Updated methodological guidance for the conduct of scoping reviews. *JBI Evid Synth* 2020 Oct;18(10):2119-2126. [doi: [10.11124/JBIES-20-00167](https://doi.org/10.11124/JBIES-20-00167)] [Medline: [33038124](#)]
19. Peters MDJ, Godfrey CM, Khalil H, McInerney P, Parker D, Soares CB. Guidance for conducting systematic scoping reviews. *Int J Evid Based Healthc* 2015 Sep;13(3):141-146. [doi: [10.1097/XEB.000000000000050](https://doi.org/10.1097/XEB.000000000000050)] [Medline: [26134548](#)]
20. Sugie T, Ikeda T, Kawaguchi A, Shimizu A, Toi M. Sentinel lymph node biopsy using indocyanine green fluorescence in early-stage breast cancer: a meta-analysis. *Int J Clin Oncol* 2017 Feb;22(1):11-17. [doi: [10.1007/s10147-016-1064-z](https://doi.org/10.1007/s10147-016-1064-z)] [Medline: [27864624](#)]

Abbreviations

ICG: indocyanine green

NIR: near-infrared

PRISMA-ScR: Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews

SLN: sentinel lymph node

Edited by S Tungjitviboonkun; submitted 06.09.24; peer-reviewed by Anonymous; revised version received 20.10.24; accepted 21.10.24; published 06.01.25.

Please cite as:

Kurdi F, Kurdi Y, Reshetov IV

Applications of Indocyanine Green in Breast Cancer for Sentinel Lymph Node Mapping: Protocol for a Scoping Review

JMIRx Med 2025;6:e66213

URL: <https://xmed.jmir.org/2025/1/e66213>

doi: [10.2196/66213](https://doi.org/10.2196/66213)

© Feryal Kurdi, Yahya Kurdi, Igor Vladimirovich Reshetov. Originally published in JMIRx Med (<https://med.jmirx.org/>), 6.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study

Abdul Aziz Tayoun, MPH

School of Medicine, Department of Family and Community Medicine, Jordan University, Queen Rania Street, Amman, Jordan

Corresponding Author:

Abdul Aziz Tayoun, MPH

School of Medicine, Department of Family and Community Medicine, Jordan University, Queen Rania Street, Amman, Jordan

Related Articles:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.02.03.24302286v1>

Companion article: <https://med.jmirx.org/2025/1/e71529>

Companion article: <https://med.jmirx.org/2025/1/e71531>

Companion article: <https://med.jmirx.org/2025/1/e71528>

Abstract

Background: Routine periodic health examinations (PHEs) for adults who are asymptomatic are included in clinical preventive services. They aim to prevent morbidity and mortality by identifying modifiable risk factors and early signs of treatable diseases. PHEs are a standard procedure in primary health care worldwide, including in Jordan. The country is undergoing an epidemiological transition toward noncommunicable diseases, which are the leading causes of morbidity and mortality. The prevalence of smoking is among the highest in the world, with escalating rates of obesity and physical inactivity. Notably, hypertension and diabetes are the most prevalent diseases.

Objective: This study aims to determine the extent to which individuals in Jordan participate in PHEs and to evaluate the various factors related to sociodemographics, health, knowledge, and behavior that influence this participation.

Methods: This study used a cross-sectional design and includes 362 participants 18 years or older residing in Jordan. A convenience sampling method was used, and data were collected through a hybrid web-based and face-to-face questionnaire. The analysis involved the application of logistic regression through SPSS to investigate the relationship between various influencing factors and the uptake of PHEs.

Results: Our study indicated that only 98 of the 362 (27.1%, 95% CI 22.8%-31.9%) participants underwent PHEs within the last 2 years. Noteworthy predictors of PHE uptake among Jordanians included recent visits to a primary health care facility within the previous year (adjusted odds ratio [AOR] 4.32, 95% CI 2.40 - 7.76; $P < .001$), monthly income ($P = .02$; individuals with a monthly income of 1500 - 2000 JD displayed more than five times the odds of undertaking PHEs than those with a monthly income < 500 JD; AOR 5.74, 95% CI 1.32 - 24.90; $P = .02$; those with a monthly income of more than 2000 JD exhibited even higher odds; AOR 9.81, 95% CI 1.73 - 55.55; $P = .02$; a currency exchange rate of 1 JD=US \$1.43 is applicable), and knowledge levels regarding PHEs and preventive health measures (AOR 1.23, 95% CI 1.03 - 1.47; $P = .007$). These variables emerged as the strongest predictors in our analysis, shedding light on key factors influencing PHE uptake in the population. Contrary to other research, our study did not find any statistically significant association between gender ($P = .33$), smoking status ($P = .76$), marital status ($P = .52$), health status self-evaluation ($P = .18$), seasonal influenza vaccination ($P = .07$), combined health behavior factors ($P = .34$), and BMI ($P = .76$) and PHE uptake.

Conclusions: PHE uptake is notably low in Jordan. Critical determinants of this uptake include recent visits to a primary health care facility within the previous year, monthly income, and knowledge levels regarding PHEs and preventive health services. To enhance PHE uptake, there is a critical need to integrate PHEs with primary health care services, increase awareness about PHEs, and offer free preventive services, particularly for those at high risk.

(*JMIRx Med* 2025;6:e57597) doi:[10.2196/57597](https://doi.org/10.2196/57597)

KEYWORDS

periodic health examination; PHE; preventive health services; routine health checkups; Jordan; cross-sectional study

Introduction

Background

Routine periodic health examinations (PHEs) for adults who are asymptomatic are integral to primary health care practice. These examinations involve clinical preventive services administered by primary health care clinicians to individuals without signs or symptoms of illness, constituting a routine health care process. The goal of these examinations is to prevent morbidity and mortality proactively, this is achieved by identifying modifiable risk factors and detecting early signs of treatable diseases [1].

The health belief model (HBM) was conceptualized to elucidate why individuals are reluctant to engage in disease prevention programs and health checkups. As a crucial predictive framework, the HBM aids in understanding various health-related behaviors, including smoking, exercise, patient roles, and use of medical services [2].

Integrating with the HBM, health beliefs are defined as personal convictions associated with perceiving and managing specific diseases. These beliefs encompass key elements: perceived sensitivity, perceived severity, perceived benefit, perceived barrier, and cue to action [3].

Literature Review

A systematic review recently published in the *Canadian Family Physician Journal* aimed to assess the reasons for visits to primary health care clinics. Clinicians participating in the review identified routine health maintenance as the third most prevalent reason for individuals seeking consultations with primary health care physicians. This ranking positioned routine health maintenance after upper respiratory tract infections and hypertension, highlighting the significant role of primary health care practitioners in motivating individuals to engage with preventive health services [4].

A study conducted among undergraduate students in a Nigerian health science college found that 91.2% of participants demonstrated awareness of PHEs. However, the actual participation in PHEs was notably low at 28.4%. The primary obstacles to uptake were identified as insufficient time, religious considerations, duration of education, perceived susceptibility to diseases, financial constraints, apprehension about the results, and a general lack of interest [5].

A nationwide study in Saudi Arabia revealed that 22.9% of participants 15 years or older had undergone a PHE in the preceding 2 years. The probability of receiving a PHE during this period exhibited positive correlations with various factors—including age; educational attainment; marital status; regular consumption of five servings of fruits and vegetables daily; and diagnoses such as prediabetes, diabetes, or hypercholesterolemia—visit to a health care setting within the last 2 years due to illness or injury [6].

Rationale and Significance of the Study

Jordan, classified as an upper middle-income country, spans an area of 89,318 square kilometers and is divided into four provinces and 12 governorates. The population has grown

substantially, increasing from 5.4 million in 2003 to over 11.5 million in 2023. This demographic shift can be attributed mainly to the influx of refugees and a relatively high birth rate [7,8].

The country is undergoing a notable epidemiological transition characterized by a rising prevalence of noncommunicable diseases (NCDs). These diseases are responsible for approximately 78% of deaths, establishing themselves as the primary cause of mortality and morbidity among the Jordanian population. Key risk factors contributing to the burden of NCDs include tobacco use, with a prevalence of about 50% (including e-cigarettes and shisha). One-quarter of the population reports insufficient physical activity and approximately 60% are classified as overweight or obese. Additionally, 22% of the population has hypertension, 14% has diabetes, and about 18% has depression [9].

Goals of This Study

This profile underscores a pressing concern regarding the country's high risk of NCDs. There is a need for evidence-based preventive health measures to curb the progression of NCDs and their associated risk factors. If conducted according to evidence-based guidelines, PHEs can effectively control communicable diseases and NCDs. Recognizing the urgency of the situation, gathering data on the uptake rate of PHEs, and identifying the factors influencing this uptake is imperative. The absence of previous studies on the uptake of PHEs in Jordan underscores the necessity for comprehensive research. Our study aims to estimate the uptake of PHEs among Jordanians while concurrently investigating various sociodemographic, health status, knowledge, and behavioral factors that play a role in influencing this uptake. The findings from this research will not only contribute valuable insights into the current scenario but also guide educational and promotional activities to encourage citizens to use preventive health services. In doing so, we strive to fill a crucial gap in existing knowledge and provide a foundation for evidence-based strategies to enhance public health in the country.

Methods

Recruitment

This descriptive cross-sectional study was conducted using an anonymous web-based Google Forms questionnaire between March 15 and May 1, 2023. Due to the lack of resources, a convenience sampling method was used to recruit participants. Jordanian residents aged ≥ 18 years who agreed to participate in our study were considered eligible. The research uses a questionnaire with five key domains: sociodemographic, health status, PHE uptake history, knowledge about PHEs, and health behaviors based on the HBM. This questionnaire was sent through the WhatsApp and Facebook platforms to participants, who were encouraged to share them with their family members. In addition, collecting data through face-to-face interviews targeted clients of grand malls, mosques, and pharmacies, supplementing the web-based data collection.

The study adopted a stratified proportional sampling strategy across four provinces of Jordan. This approach is carefully extended to maintain a balance in gender and nationality among

participants. The initial page of the web-based questionnaire explicitly outlines the study's objectives and provides detailed instructions on how to complete the questionnaire. This effort was complemented by the researcher's availability to answer questions, ensuring participants' queries or doubts were promptly addressed.

Sampling Method

The following inclusion and exclusion criteria were used:

- Inclusion criteria: any citizen regardless of nationality, 18 years or older, and residing in Jordan
- Exclusion criteria: persons younger than 18 years and individuals who declined to participate in the study

We recruited 362 respondents, aiming to provide a representative sample that reflects the entire population of Jordan in terms of district, age, sex, and nationality. The convenience sample size of 362 was calculated using the sample size formula for proportions:

$$N = Z_{\alpha/2}^2 P(1-P) / D^2$$

This calculation considered a study conducted in Saudi Arabia, where approximately 34% of the population underwent PHEs [10]. The chosen values for statistical significance (α error) and margin of error (D) were .05% and 5%, respectively. As a result, the calculated sample size required for the survey was 345 respondents.

Questionnaire Development

The PHE questionnaire ([Multimedia Appendix 1](#)), comprising 36 questions across five domains, was developed following an extensive literature review [10-14]. The questionnaire's five domains are as follows:

1. Sociodemographic (9 items): inquires about relevant sociodemographic variables of participants
2. Health status and risk factors (7 items): explores participants' health status and associated risk factors
3. PHE uptake (4 items): focuses on the outcome variable of PHE uptake
4. Knowledge about PHE and preventive health services (8 items): assesses knowledge using a 3-option scale (agree, don't agree, I don't know). The items are scored, with correct answers receiving a score of 1 and incorrect or I don't know responses scoring 0. The total score ranges from 0 to 8, with higher scores indicating more significant knowledge of health checkups and preventive measures. The Cronbach α , estimated during the pilot phase with 25 participants, was 0.68.
5. Health behaviors toward PHE based on the HBM (6 items): measures health behaviors using a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The total score ranges from 6 to 30, with higher scores indicating more positive health beliefs for each item. The Cronbach α for health behaviors toward PHEs during the pilot testing phase was 0.74, demonstrating acceptable internal consistency.

The questionnaire was translated into Arabic for comprehensibility and then back to English with the assistance

of an expert translator. This rigorous process ensures the questionnaire's clarity and accuracy across languages.

Statistical Analysis

The primary outcome variable is the uptake of PHEs in Jordan, categorized as a dichotomous (yes or no) variable. The independent variables encompass sociodemographics, health status, knowledge, and health behavioral factors. Records with missing data were excluded to ensure the integrity of the analysis. Data was analyzed using SPSS, version 26.0 (IBM Corp).

Participant characteristics were examined using counts, percentages, means, and SDs through descriptive statistics. Graphs and tables were used as needed for visual representation. A 95% CI was calculated using appropriate methods, and a 2-sided P value $<.05$ was considered statistically significant.

A binary logistic regression test was used to study the association between the binary outcome variable and the various continuous and nominal predictor variables. Multivariate logistic regression analysis was used to examine the relationship between the uptake of PHEs and various independent covariables to adjust for confounding.

A hierarchical block-wise logistic regression model was also constructed to identify the most potent predictor variables. This comprehensive approach blends descriptive, inferential, and multivariate statistical techniques to provide a thorough understanding of the factors influencing the uptake of PHEs in Jordan.

Ethical Considerations

Before the formal survey, the study protocol was approved by the Jordan University Ethics Committee (approval 13 - 2023) and the Jordan University Hospital Ethics Committee (approval 10/2023/4560). The questionnaire was designed to be anonymous and voluntary, and respondents were informed that submission of the questionnaire implied informed consent. The data were kept confidential, and the results did not identify the respondents personally. Contact information for the researcher was provided for clarification purposes. No compensation was provided to participants.

Results

A total of 365 individuals participated in the study between March and April 2023, with a response rate of 99%; 3 participants were excluded (one was younger than 18 years, and the other two did not complete the questionnaire), leaving 362 participants for analysis.

Descriptive Statistics

The demographic characteristics of participants are summarized in [Table 1](#). The mean age was 38.2 (SD 14.6, range 18-88) years. Of the 362 participants, there were slightly more male ($n=185$, 51.1%) than female participants. Approximately 230 (63.5%) were married, 270 (74.6%) were Jordanians, and 202 (55.8%) held a university degree. Most participants ($n=225$, 62.1%) reported a monthly income of less than 500 JD (a currency

exchange rate of 1 JD=US \$1.43 is applicable), with half lacking health insurance.

Regarding health status, [Table 2](#) shows that 240 (66.3%) participants reported good or excellent health, 78 (21.5%) had a chronic disease, and 200 (55.2%) visited a primary health care

clinic in the past year. Additionally, 191 (52.8%) participants were current smokers.

Regarding PHEs, only 98 of the 362 (27.1%, 95% CI 22.8% - 31.9%) participants underwent a medical checkup in the last 2 years.

Table . Sociodemographic characteristics of participants (N=362).

Characteristic	Participants, n (%)
Gender	
Male	185 (51.1)
Age group (years)	
18 - 29	122 (33.7)
30 - 39	90 (24.9)
40 - 49	70 (19.3)
50 - 59	41 (11.3)
≥60	39 (10.8)
Marital status	
Married	230 (63.5)
Single	101 (27.9)
Divorced	14 (3.9)
Widowed	17 (4.7)
Monthly income (JD) ^a	
<500	225 (62.1)
500 - 999	93 (25.7)
1000 - 1499	26 (7.2)
1500 - 1999	10 (2.8)
≥2000	8 (2.2)
Educational level	
Elementary school	42 (11.6)
Secondary school	118 (32.6)
University	166 (45.9)
Postgraduate	36 (9.9)
Province of residence	
Amman	151 (41.7)
Central Jordan	82 (22.7)
North Jordan	100 (27.6)
South Jordan	29 (8.0)
Nationality	
Jordanians	270 (74.6)
Syrians	47 (13.0)
Palestinians	22 (6.1)
Egyptians	18 (5.0)
Iraqis	5 (1.4)

^aA currency exchange rate of 1 JD=US \$1.43 is applicable.

Table . Health characteristics of participants in the study.

Variable	Participants, n (%)
Visiting a primary health care facility within the previous year	
Yes	200 (55.2)
No	162 (44.8)
Noncommunicable diseases	
Yes	78 (21.5)
No	284 (78.5)
Smoking	
Smoker	191 (52.8)
Not smoker	171 (47.2)
Health insurance	
Insured	183 (50.6)
Not insured	179 (49.4)
Seasonal flu vaccination	
Yes	60 (16.6)
No	302 (83.4)
Health status self-evaluation	
Poor	9 (2.5)
Fair	25 (6.9)
Good	88 (24.3)
Very good	136 (37.6)
Excellent	104 (28.7)
BMI \geq 25	
Yes	223 (61.6)
No	139 (38.4)
Physical activity	
Yes	108 (29.8)
No	254 (70.2)

Logistic Regression Analysis

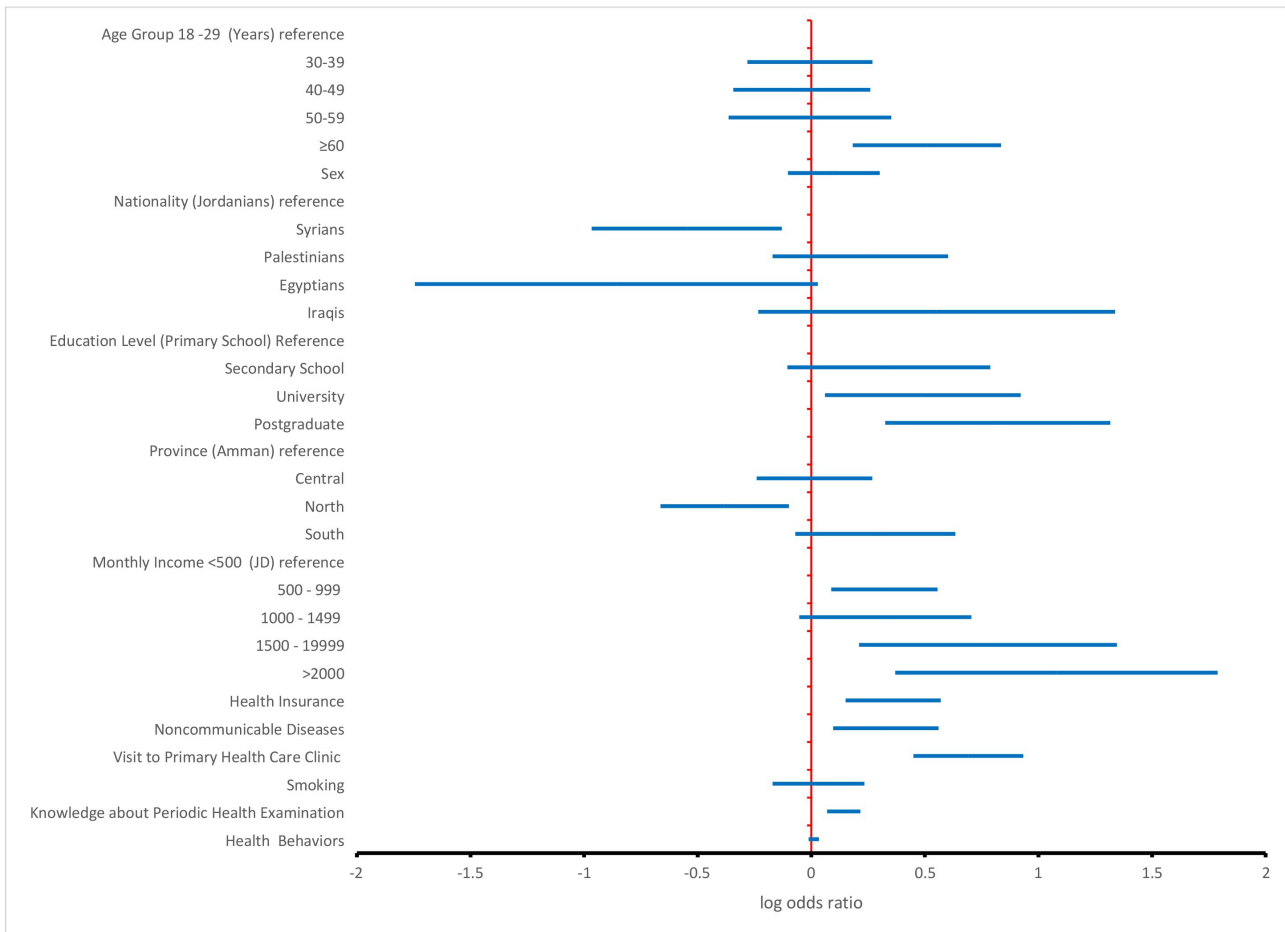
The forest plot in [Figure 1](#) highlights several significant findings from the analysis of the predicting factors' association with PHE uptake.

Age was found to be a significant determinant of PHE uptake: with each additional year of age there, is a 2.2% increase in the odds of undertaking PHEs (odds ratio [OR] 1.022, 95% CI 1.006 - 1.038; $P=.006$). Nationality also proved to be a factor, with Syrians demonstrating a lower frequency of PHE uptake. The odds of Syrians undergoing PHEs were 0.283 compared to Jordanians (OR 0.28, 95% CI 0.11 - 0.74; $P=.01$). Education level exhibited a strong association, with postgraduates displaying more than 6 times the odds of undertaking PHE than individuals with only primary school education (OR 6.62, 95% CI 2.12 - 20.71; $P=.001$). Health care workers displayed more than 12 times the odds of undergoing PHEs than general employees (OR 12.28, 95% CI 4.69 - 32.19; $P<.001$). Individuals earning more than 2000 JD monthly had 12 times

greater odds of receiving PHEs compared to those with a monthly income of less than 500 JD (OR 12.00, 95% CI 2.34 - 61.45; $P=.003$). Health insurance emerged as a significant facilitator of PHE uptake. Insured participants demonstrated more than 2 times the odds of undertaking PHEs than noninsured individuals (OR 2.30, 95% CI 1.42 - 3.71; $P=.001$). People with chronic diseases have more than twice the odds of undertaking PHEs than those without chronic diseases (OR 2.3, 95% CI 1.258 - 3.629; $P=.005$). Visits to a primary health care clinic in the past year significantly impacted PHE uptake. Those who had visited had 5 times the odds of PHE uptake compared to those who did not visit a primary health care facility in the past year (OR 4.91, 95% CI 2.82 - 8.57; $P<.001$). Participants who were physically active had 1.65 times the odds of undertaking PHEs than those without enough physical activity (OR 1.65, 95% CI 1.01-2.69; $P=.046$). Finally, for every extra point in knowledge about PHEs, there is a 39% increase in PHE uptake (OR 1.39, 95% CI 1.18 - 1.64; $P<.001$).

On the other hand, several variables were not associated with PHE uptake. These included gender ($P=.33$), smoking status ($P=.18$), seasonal influenza vaccination ($P=.07$), combined health behavior factors ($P=.34$), and BMI ($P=.76$), marital status ($P=.52$), health status self-evaluation

Figure 1. Univariate logistic regression analysis for predictor factors of periodic health examination uptake, Jordan 2023. A currency exchange rate of 1 JD=US \$1.43 is applicable.



Adjusted Logistic Regression Model

After meticulously adjusting for confounding variables and carefully selecting clinically and statistically significant factors,

we successfully constructed a logistic regression model using the hierarchical block-wise method. This refined model, depicted in Table 3, encapsulates three variables that significantly influence the uptake of PHEs.

Table . Logistic regression model for most significant predictor factors for periodic health examination uptake, Jordan 2023.

Variable	P value	Adjusted odds ratio (95% CI)
Visiting a primary health care facility	<.001	4.315 (2.40-7.76)
Knowledge about periodic health examinations	.02	1.230 (1.03-1.47)
Monthly income (JD) ^a	.07	
<500 (reference)	— ^b	1.00
500 - 999	.07	1.71 (0.96-3.02)
1000 - 1499	.11	2.18 (0.84-5.66)
1500 - 1999	.02	5.74 (1.32-24.90)
≥2000	.01	9.81 (1.73-55.55)

^aA currency exchange rate of 1 JD=US \$1.43 is applicable.

^bNot applicable.

Visit to Primary Health Care Facilities in the Past Year

Visiting primary health care facilities within the past year exhibited a substantial impact on PHE uptake. These individuals demonstrated more than 4 times the odds of undertaking PHEs compared to those who did not visit a primary health care facility within the same time frame (adjusted OR [AOR] 4.32, 95% CI 2.40 - 7.76; $P < .001$).

Income Level

Individuals with a monthly income of 1500 - 2000 JD displayed more than five times the odds of undertaking PHEs than those with a monthly income of less than 500 JD (AOR 5.74, 95% CI 1.32 - 24.90; $P = .02$). Furthermore, those with a monthly income of more than 2000 JD exhibited even higher odds (AOR 9.81, 95% CI 1.73 - 55.55; $P = .02$).

Health Knowledge

The analysis indicates that for every point increase in PHE knowledge, the likelihood of individuals opting for PHEs increases by 23% (AOR 1.23, 95% CI 1.03-1.47; $P = .02$).

Discussion

Principal Findings and Comparison With Other Studies

Of the 362 participants, only 98 (27.1%, 95% CI 22.8%-31.9%) had undergone a PHE in the past 2 years. Some significant predictors included recent visits to a primary health care facility the previous year, monthly income, knowledge about PHEs, and preventive health measures. Other nonsignificant factors were gender, marital status, smoking status, and BMI, which did not emerge as being significantly associated with the uptake of PHEs.

Interestingly, the uptake rate observed in our study is comparable to that reported in studies conducted in Saudi Arabia [6,10] and Nigeria [12]. In contrast, this rate notably fell below those reported in studies conducted in the United States [1], the United Kingdom [13], and Switzerland [15].

The most influential determinant for the uptake of PHEs found in our study was a visit to a primary health care facility in the past year. Our findings again were consistent with those from several other studies [6,16,17]. Notably, those who had visited any primary health care clinic in the previous year were found to be five times more likely to undertake PHEs compared to those who had not visited such clinics in the same time frame. This association was statistically significant even after adjusting for other relevant factors, thus underlining its strength. The second most important factor influencing the uptake of PHEs was monthly income. This finding agrees with results from other sources [1,12,14,17-21]. The influence of monthly income on the uptake of PHEs reflects how socioeconomic issues can affect health care-seeking behavior. There is a great need for focused efforts or an intervention policy that addresses these issues. Knowledge about PHEs was the third most influential factor. The findings are in agreement with those of previous studies [22-24] and underline the role of informed choice in health care use. This paper should, however, state that knowledge of PHEs was associated with other factors such as

educational level and occupation. However, adjustment for these factors associated with knowledge of PHEs did not reduce the strength of the association with knowledge and PHE uptake.

More variables were positively associated with the uptake of PHEs. The older the age, the better the PHE uptake, which agrees with other studies' findings [13,17,19]. This may indicate that with increased age, people are likely to undergo regular health checkups, either because of the higher burden of NCDs in older age or maybe because more attention is paid to preventive measures with increased age. Individuals of Syrian nationality were found to be less likely to undergo PHEs than Jordanians. Economic factors may explain this difference, emphasizing the need for targeted interventions to ensure equitable access to preventive health care services among diverse populations. There was a strong association between education and PHE uptake, evidenced by a substantial increase in PHE uptake corresponding to higher levels of education. This finding is similar to results from other studies [17,21,25]. Compared to employees in general, health workers and retirees were more likely to undergo PHEs. This may be because health care workers are more aware of the importance of preventive health. Age can serve as a confounder for retired people because it may affect retired status and PHE uptake.

The health-related factors identified to be associated with PHE uptake in our study, and supported by other studies, include the presence of chronic diseases [6,14,18,22,26], being insured [17,21,25,27,28], and engagement in physical activity [1].

Other factors showed no significant association with the uptake of PHEs. For example, one nonsignificant factor was sex, which contrasts many studies indicating that females are more willing to participate in PHEs than males [6,13,15,20]. Being married has often been linked to higher PHE uptake in previous studies but not in our study [1,13-15,19,29,30]. Surprisingly, smoking status was not associated with the uptake of PHEs; several studies in the past have argued that smokers are less likely than nonsmokers to undergo PHEs [11,13,15,20,29]. Our study did not find any clear association between combined behavioral factors and the uptake of PHEs, although many studies identify such associations [3,11,14,20,30,31]. This is possibly because of the suitability of the questionnaires to the Jordanian population or problems with participants understanding.

Strengths of the Study

This study is the first of its kind to investigate the uptake of PHEs in Jordan and hence addresses an important gap in existing knowledge. Given that this is the first study on this topic, it has contributed quite substantially to the understanding of PHE uptake in the country. The statistical analysis approach adopted in this study is broad and solid, using descriptive, inference, and multivariate statistical techniques. This approach leads to a deeper analysis and more reliable findings. The study also managed to identify the significant predictors of PHE uptake.

Limitations of the Study

One of the primary limitations is its cross-sectional design, which restricts the ability to establish causality between the different predictor factors and PHE uptake. To address this issue, future research could adopt a longitudinal approach,

providing a better understanding of how these predictors influence PHE uptake. Another limitation relates to the sampling method. The study used a convenience sampling strategy, which may have introduced selection bias, and the web-based survey format could lead to measurement bias. To decrease the chances of bias, we used a stratified sampling method, taking into account population size and stratifying participants by gender, age group, and nationality across the four provinces of Jordan. Additionally, a hybrid approach integrating both web-based and face-to-face interviews, and collecting data from various settings such as social media platforms, grand malls, mosques, and pharmacies helped ensure a more representative sample. The author's availability for clarifications via WhatsApp and email also aimed to reduce potential measurement biases during data collection. The third limitation concerns the survey instrument itself. The comprehensiveness and relevance of the questionnaire to the Jordanian population might not have been fully ensured. To address this issue, a pilot study with 25 participants was conducted, and the questionnaire was revised based on their feedback and reliability measures. Lastly, the study's results may have limited generalizability beyond the population of Jordan. To enhance the applicability of the findings to broader populations, future research should consider a more diverse sample by including other countries. This would provide a more comprehensive understanding of PHE uptake within and outside Jordan.

Future Directions

First, we established that recent visits to primary health care facilities were the strongest predictor of PHE uptake. From this, we recommend incorporating preventive health services into existing primary health care services to enhance accessibility and efficiency. This may take the form of incentivizing both health caregivers and patients. Second, economic issues can be

resolved by suggesting the provision of all preventive services free of cost at primary health care centers. Private health insurance companies can also facilitate this endeavor by covering preventive services like PHEs within the realm of their service provision so that people can have better access to these services. More importantly, public awareness will have to increase. The positive correlation between knowledge of PHEs and their uptake points to a need for more organized and evidence-based awareness campaigns. Another issue involves the study's findings on behavioral factors. The study did not find a significant relationship between behavioral factors and PHE uptake, contradicting findings from other contexts. To better understand these results, future research could involve a more detailed investigation into the cultural and societal influences on health behaviors in Jordan, which may help clarify why these factors did not show the expected association. It is also recommended that further studies, especially on smoking as a predictor factor for PHE uptake, be done in detail to understand how to best address these areas in future studies.

Conclusion

Our study has highlighted the low level of PHE uptake in Jordan. This paper identified visitation to primary health care facilities in the past year, monthly income, and knowledge about PHEs and preventive health services as the major predictors influencing the likelihood of undergoing PHEs. The association of regular visits to primary health care facilities with higher uptake of PHEs suggests that PHEs should be integrated with the available services at primary health care facilities. These findings also suggest that targeted interventions should be implemented to enhance awareness and knowledge of the value of preventive health practices among the Jordanian population, particularly for patients with lower income status.

Acknowledgments

We conducted this review using the ChatGPT-4 model, 2023, developed by OpenAI, which only helped create text. The author reviewed and edited ChatGPT's draft for accuracy and coherence ([Multimedia Appendix 2](#)). We are grateful to all the survey participants, and the Jordan University and Jordan University Hospital ethical committees.

Data Availability

The datasets generated or analyzed during this study are available from the corresponding author upon reasonable request.

Authors' Contributions

AAT analyzed the data, drafted the manuscript, and devised the study concept and design. Furthermore, AAT interpreted the results and is responsible for the decision to submit for publication.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Questionnaire in English.

[\[DOCX File, 20 KB - xmed_v6i1e57597_app1.docx \]](#)

Multimedia Appendix 2

ChatGPT's draft.

[[DOCX File, 32 KB - xmed_v6i1e57597_app2.docx](#)]

Checklist 1

STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist.

[[DOCX File, 34 KB - xmed_v6i1e57597_app3.docx](#)]

References

1. Culica D, Rohrer J, Ward M, Hilsenrath P, Pomrehn P. Medical checkups: who does not get them? *Am J Public Health* 2002 Jan;92(1):88-91. [doi: [10.2105/ajph.92.1.88](#)] [Medline: [11772768](#)]
2. Cho MK, Cho YH. Role of perception, health beliefs, and health knowledge in intentions to receive health checkups among young adults in Korea. *Int J Environ Res Public Health* 2022 Oct 24;19(21):13820. [doi: [10.3390/ijerph192113820](#)] [Medline: [36360698](#)]
3. Andersen RM. Revisiting the behavioral model and access to medical care: does it matter? *J Health Soc Behav* 1995 Mar;36(1):1-10. [Medline: [7738325](#)]
4. Finley CR, Chan DS, Garrison S, et al. What are the most common conditions in primary care? Systematic review. *Can Fam Physician* 2018 Nov;64(11):832-840. [Medline: [30429181](#)]
5. Esan O, Akinyemi A, Ayegbusi O, Bakare T, Balogun Y, Ogunwusi A. Determinants of uptake of periodic medical examination among students of college of health sciences, Obafemi Awolowo University Ile-Ife, South-West Nigeria. *Nigerian J Med* 2020;29(4):575-581. [doi: [10.4103/NJM.NJM_150_20](#)]
6. El Bcheraoui C, Tuffaha M, Daoud F, et al. Low uptake of periodic health examinations in the Kingdom of Saudi Arabia, 2013. *J Family Med Prim Care* 2015;4(3):342-346. [doi: [10.4103/2249-4863.161313](#)] [Medline: [26288771](#)]
7. Department of Statistics Jordan. URL: <https://dosweb.dos.gov.jo/> [accessed 2023-01-14]
8. Jordan Ministry of Health. URL: <https://moh.gov.jo/Default/En> [accessed 2023-01-14]
9. Country cooperation strategy for WHO and Jordan 2021–2025: CCS Jordan. World Health Organization. 2021 Jun 21. URL: <https://www.who.int/publications/i/item/9789290227014> [accessed 2023-01-14]
10. Al-Kahil AB, Khawaja RA, Kadri AY, Abbarh Mbbs SM, Alakhras JT, Jaganathan PP. Knowledge and practices toward routine medical checkup among middle-aged and elderly people of Riyadh. *J Patient Exp* 2020 Dec;7(6):1310-1315. [doi: [10.1177/2374373519851003](#)] [Medline: [33457580](#)]
11. Zhang Z, Yin AT, Bian Y. Willingness to receive periodic health examination based on the health belief model among the elderly in rural China: a cross-sectional study. *Patient Prefer Adherence* 2021 Jun 21;15:1347-1358. [doi: [10.2147/PPA.S312806](#)] [Medline: [34188452](#)]
12. Ofoli JNT, Ashau-Oladipo T, Hati SS, Ati L, Ede V. Preventive healthcare uptake in private hospitals in Nigeria: a cross-sectional survey (Nisa Premier Hospital). *BMC Health Serv Res* 2020 Apr 1;20(1):273. [doi: [10.1186/s12913-020-05117-5](#)] [Medline: [32238153](#)]
13. Labeit A, Peinemann F, Baker R. Utilisation of preventative health check-ups in the UK: findings from individual-level repeated cross-sectional data from 1992 to 2008. *BMJ Open* 2013 Dec 23;3(12):e003387. [doi: [10.1136/bmjopen-2013-003387](#)] [Medline: [24366576](#)]
14. Liu X, Li N, Liu C, et al. Urban-rural disparity in utilization of preventive care services in China. *Medicine (Baltimore)* 2016 Sep;95(37):e4783. [doi: [10.1097/MD.0000000000004783](#)] [Medline: [27631229](#)]
15. Diaz Hernandez L, Giezendanner S, Fischer R, Zeller A. Expectations about check-up examinations among Swiss residents: a nationwide population-based cross-sectional survey. *PLoS One* 2021 Jul 21;16(7):e0254700. [doi: [10.1371/journal.pone.0254700](#)] [Medline: [34288961](#)]
16. Alzahrani AMA, Felix HC, Stewart MK, Selig JP, Swindle T, Abdeldayem M. Utilization of routine medical checkup and factors influencing use of routine medical checkup among Saudi students studying in the USA in 2019. *Saudi J Health Syst Res* 2021 Mar 11;1(1):16-25. [doi: [10.1159/000514178](#)]
17. Okoli GN, Abou-Setta AM, Neilson CJ, Chit A, Thommes E, Mahmud SM. Determinants of seasonal influenza vaccine uptake among the elderly in the United States: a systematic review and meta-analysis. *Gerontol Geriatr Med* 2019 Aug 17;5:2333721419870345. [doi: [10.1177/2333721419870345](#)] [Medline: [31453267](#)]
18. Getahun GK, Arega M, Keleb G, Shiferaw A, Bezabih D. Assessment of routine medical checkups for common noncommunicable diseases and associated factors among healthcare professionals in Addis Ababa, Ethiopia, in 2022 a cross-sectional study. *Ann Med Surg (Lond)* 2023 Apr 1;85(5):1633-1641. [doi: [10.1097/MS9.0000000000000558](#)] [Medline: [37229001](#)]
19. Dryden R, Williams B, McCowan C, Themessl-Huber M. What do we know about who does and does not attend general health checks? Findings from a narrative scoping review. *BMC Public Health* 2012 Aug 31;12:723. [doi: [10.1186/1471-2458-12-723](#)] [Medline: [22938046](#)]
20. Bjerregaard AL, Maindal HT, Bruun NH, Sandbæk A. Patterns of attendance to health checks in a municipality setting: the Danish “Check Your Health Preventive Program”. *Prev Med Rep* 2016 Dec 21;5:175-182. [doi: [10.1016/j.pmedr.2016.12.011](#)] [Medline: [28050340](#)]

21. Obi IR, Obi KM, Seer-Uke EN, Onuorah SI, Okafor NP. Preventive health care services utilization and its associated factors among older adults in rural communities in Anambra State, Nigeria. *Pan Afr Med J* 2021 May 28;39:83. [doi: [10.11604/pamj.2021.39.83.26997](https://doi.org/10.11604/pamj.2021.39.83.26997)] [Medline: [34466185](https://pubmed.ncbi.nlm.nih.gov/34466185/)]
22. Alzahrani AM, Quronfulah BS, Felix HC, Khogeer AA. Barriers to routine checkups use among Saudis from the perspective of primary care providers: a qualitative study. *Saudi Med J* 2022 Jun;43(6):618-625. [doi: [10.15537/smj.2022.43.6.20220090](https://doi.org/10.15537/smj.2022.43.6.20220090)] [Medline: [35675932](https://pubmed.ncbi.nlm.nih.gov/35675932/)]
23. Laz TH, Rahman M, Berenson AB. An update on human papillomavirus vaccine uptake among 11-17 year old girls in the United States: National Health Interview Survey, 2010. *Vaccine (Auckl)* 2012 May 21;30(24):3534-3540. [doi: [10.1016/j.vaccine.2012.03.067](https://doi.org/10.1016/j.vaccine.2012.03.067)] [Medline: [22480927](https://pubmed.ncbi.nlm.nih.gov/22480927/)]
24. Sommer I, Titscher V, Gartlehner G. Participants' expectations and experiences with periodic health examinations in Austria - a qualitative study. *BMC Health Serv Res* 2018 Oct 30;18(1):823. [doi: [10.1186/s12913-018-3640-6](https://doi.org/10.1186/s12913-018-3640-6)] [Medline: [30376830](https://pubmed.ncbi.nlm.nih.gov/30376830/)]
25. AshaRani PV, Devi F, Wang P, et al. Factors influencing uptake of diabetes health screening: a mixed methods study in Asian population. *BMC Public Health* 2022 Aug 9;22(1):1511. [doi: [10.1186/s12889-022-13914-2](https://doi.org/10.1186/s12889-022-13914-2)] [Medline: [35941579](https://pubmed.ncbi.nlm.nih.gov/35941579/)]
26. Gosadi IM, Ayoub RA, Albrahim HT, et al. An assessment of the knowledge and practices of adults in Jazan, Saudi Arabia, concerning routine medical checkups. *Patient Prefer Adherence* 2022 Aug 5;16:1955-1969. [doi: [10.2147/PPA.S376345](https://doi.org/10.2147/PPA.S376345)] [Medline: [35958888](https://pubmed.ncbi.nlm.nih.gov/35958888/)]
27. Leung JKF, Wong MCS, Wong ELY. Unseen threats of chronic diseases among the middle-aged: examining the feasibility of well-defined healthcare vouchers in encouraging uptake of general checkups. *Int J Environ Res Public Health* 2022 Sep 17;19(18):18. [doi: [10.3390/ijerph191811751](https://doi.org/10.3390/ijerph191811751)] [Medline: [36142023](https://pubmed.ncbi.nlm.nih.gov/36142023/)]
28. Cherrington A, Corbie-Smith G, Pathman DE. Do adults who believe in periodic health examinations receive more clinical preventive services? *Prev Med* 2007 Oct;45(4):282-289. [doi: [10.1016/j.ypmed.2007.05.016](https://doi.org/10.1016/j.ypmed.2007.05.016)] [Medline: [17692368](https://pubmed.ncbi.nlm.nih.gov/17692368/)]
29. Mori Y, Matsushita K, Inoue K, Fukuma S. Patterns and predictors of adherence to follow-up health guidance invitations in a general health check-up program in Japan: a cohort study with an employer-sponsored insurer database. *PLoS One* 2023 May 25;18(5):e0286317. [doi: [10.1371/journal.pone.0286317](https://doi.org/10.1371/journal.pone.0286317)] [Medline: [37228080](https://pubmed.ncbi.nlm.nih.gov/37228080/)]
30. Zhang J, Oldenburg B, Turrell G. Measuring factors that influence the utilisation of preventive care services provided by general practitioners in Australia. *BMC Health Serv Res* 2009 Dec 3;9:218. [doi: [10.1186/1472-6963-9-218](https://doi.org/10.1186/1472-6963-9-218)] [Medline: [19954549](https://pubmed.ncbi.nlm.nih.gov/19954549/)]
31. Oboler SK, Prochazka AV, Gonzales R, Xu S, Anderson RJ. Public expectations and attitudes for annual physical examinations and testing. *Ann Intern Med* 2002 May 7;136(9):652-659. [doi: [10.7326/0003-4819-136-9-200205070-00007](https://doi.org/10.7326/0003-4819-136-9-200205070-00007)] [Medline: [11992300](https://pubmed.ncbi.nlm.nih.gov/11992300/)]

Abbreviations

- AOR:** adjusted odds ratio
HBM: health belief model
NCD: noncommunicable disease
OR: odds ratio
PHE: periodic health examination

Edited by T Leung; submitted 20.02.24; peer-reviewed by A Ahmed, Anonymous; revised version received 17.10.24; accepted 26.10.24; published 05.02.25.

Please cite as:

Tayoun AA

Determinants of Periodic Health Examination Uptake: Insights From a Jordanian Cross-Sectional Study

JMIRx Med 2025;6:e57597

URL: <https://xmed.jmir.org/2025/1/e57597>

doi: [10.2196/57597](https://doi.org/10.2196/57597)

© Abdul Aziz Tayoun. Originally published in *JMIRx Med* (<https://med.jmirx.org>), 5.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in *JMIRx Med*, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis

Hojjat Borhany, MSc

Faculty of Environmental Science, Department of Environmental Science, Informatic, and Statistics, University of Ca' Foscari Venice, Mestre (VE), Italy

Corresponding Author:

Hojjat Borhany, MSc

Faculty of Environmental Science, Department of Environmental Science, Informatic, and Statistics, University of Ca' Foscari Venice, Mestre (VE), Italy

Related Articles:

Companion article: <https://www.biorxiv.org/content/10.1101/2023.06.21.545938v2>

Companion article: <https://med.jmirx.org/2025/1/e69895>

Companion article: <https://med.jmirx.org/2025/1/e69896>

Companion article: <https://med.jmirx.org/2025/1/e69894>

Abstract

Background: Italy can augment its profit from biorefinery products by altering the operation of digesters or different designs to obtain more precious bioproducts like volatile fatty acids (VFAs) than biogas from organic municipal solid waste. In this context, recognizing the process stability and outputs through operational interventions and its technical and economic feasibility is a critical issue. Hence, this study involves an anaerobic digester in Treviso in northern Italy.

Objective: This research compares a novel line, consisting of pretreatment, acidogenic fermentation, and anaerobic digestion, with single-step anaerobic digestion regarding financial profit and surplus energy. Therefore, a mass flow model was created and refined based on the outputs from the experimental and numerical studies. These studies examine the influence of hydraulic retention time (HRT), pretreatment, biochar addition, and fine-tuned feedstock/inoculum (FS/IN) ratio on bioproducts and operational parameters.

Methods: VFA concentration, VFA weight ratio distribution, and biogas yield were quantified by gas chromatography. A *t* test was then conducted to analyze the significance of dissimilar HRTs in changing the VFA content. Further, a feasible biochar dosage was identified for an assumed FS/IN ratio with an adequately long HRT using the first-order rate model. Accordingly, the parameters for a mass flow model were adopted for 70,000 population equivalents to determine the payback period and surplus energy for two scenarios. We also explored the effectiveness of amendments in improving the process kinetics.

Results: Both HRTs were identical concerning the ratio of VFA/soluble chemical oxygen demand (0.88 kg/kg) and VFA weight ratio distribution: mainly, acetic acid (40%), butyric acid (24%), and caproic acid (17%). However, a significantly higher mean VFA content was confirmed for an HRT of 4.5 days than the quantity for an HRT of 3 days (30.77, SD 2.82 vs 27.66, SD 2.45 g-soluble chemical oxygen demand/L), using a *t* test ($t_8=-2.68$; $P=.03$; CI=95%). In this research, 83% of the fermented volatile solids were converted into biogas to obtain a specific methane (CH₄) production of 0.133 CH₄-Nm³/kg-volatile solids. While biochar addition improved only the maximum methane content by 20% (86% volumetric basis [v/v]), the FS/IN ratio of 0.3 volatile solid basis with thermal plus fermentative pretreatment improved the hydrolysis rate substantially (0.57 vs 0.07, 1/d). Furthermore, the biochar dosage of 0.12 g-biochar/g-volatile solids with an HRT of 20 days was identified as a feasible solution. Principally, the payback period for our novel line would be almost 2 years with surplus energy of 2251 megajoules [MJ] per day compared to 45 years and 21,567 MJ per day for single-step anaerobic digestion.

Conclusions: This research elaborates on the advantage of the refined novel line over the single-step anaerobic digestion and confirms its financial and technical feasibility. Further, changing the HRT and other amendments significantly raised the VFA concentration and the process kinetics and stability.

KEYWORDS

multistep fermentation; specific methane production; anaerobic digestion; kinetics study; biochar; first-order; modified Gompertz; mass balance; waste management; environment sustainability

Introduction

The European Union annually generated about 110 million tons of organic waste in 2006, which excluded slurry and manure. This waste mainly came from the food industry (33%), agriculture and hunting (30%), and households (20%) [1]. Current Italian legislation forbids landfilling organic waste and requires treating it through biological and thermal processes like anaerobic digestion, composting, and incineration with high disposal costs for secondary waste flux (€75 - €125 per ton; a currency exchange rate of €1=US \$1.05 is applicable) [2]. Under the pressure of exhaustible natural exploitation and increasing organic waste, the European Commission approved the circular economy action plan to promote sustainable recovery methods to reduce the secondary waste flux. The techniques recommended in the circular economy context assume a “take-use-reuse” viewpoint. Such an approach wants to close the circuit of cycles, extend product life, and treat the wastes as precious recyclable materials [3,4]. In this respect, the European Union states have deployed biological processes such as anaerobic digestion to gain either platform chemicals like volatile fatty acids (VFAs) or biogas from organic wastes produced in urban areas [5-9]. These products are extremely valuable in the era of environmental disasters, which have several consequences (eg, climate change), since they are renewable, sustainable, carbon-neutral, and compatible with current fossil-based fuel infrastructures [10].

Recent studies have aimed at finding a sequential reclaiming route to obtain various bioproducts such as VFAs and biohydrogen with a higher added-value market than bio-methane at distinct steps to either redesign the existing plants or integrate them into biorefinery platforms [11,12]. Various biological processes can convert different feedstock (eg, edible sugary crops, oil-bearing crops, livestock, waste sludge [WS], and food waste) into a range of biofuels, including bioethanol, biodiesel, bio-methane, and biohydrogen [10,13,14]. Biofuel production from edible crops is quite controversial in terms of food supply, ethical quandary, and insecure supply chain. However, food waste, WS, and livestock are omnipresent in urban and rural areas without widespread deployment in a biorefinery scheme. Accordingly, this research aims to convert organic municipal solid waste (OMSW), mainly from food waste, into VFAs and biogas.

This study examines the biological recovery route for OMSW for potential beneficial bioproducts and technical feasibility. This effort includes three steps: pretreatment, mesophilic acidogenic fermentation, and anaerobic digestion. Specifically, we endeavor to conceive how to make the process more profitable and practicable through operational amendments that change the share of methanogenesis and acidogenic routes in the final products (VFAs and biogas) [9] and lower the costs of the process in terms of energy and water consumption. Hence,

determining a reasonably priced process with a desirable VFA-rich stream from acidogenic fermentation and a high methane (CH₄) yield from methanogenesis [15] could ultimately encourage full-scale commercialization. VFAs typically serve as platform chemicals for many processes (eg, biopolymer synthesis of polyhydroxyalkanoates [PHAs] [16-19]), which could be later recovered through biological processes to close the material life cycle.

The major bottleneck in anaerobic digestion of biowaste is at the hydrolysis step. Such a problem could be relieved by various methods such as pretreatment, an optimized feedstock/inoculum (FS/IN) ratio, and carbonaceous material addition, including biochar [20-22]. The latter method was recently realized to have numerous benefits to the process, such as improving the process stability, acceleration of the process rate, buffering potency and alkalinity, inhibitors adsorption, enriched microbial functionality, and electron transfer mechanism. As a result, it could improve CH₄ generation by fostering hydrolysis, acetogenesis, and methanogenesis [23]. The residual solids out of the multistep line of pretreatment followed by acidogenic fermentation plus anaerobic digestion can be used in a pyrolysis line for biochar and biofuel production to further lower the secondary waste flux [24]. This strategy provides several benefits, such as combating climate change and global soil degradation and addressing the rising energy demand.

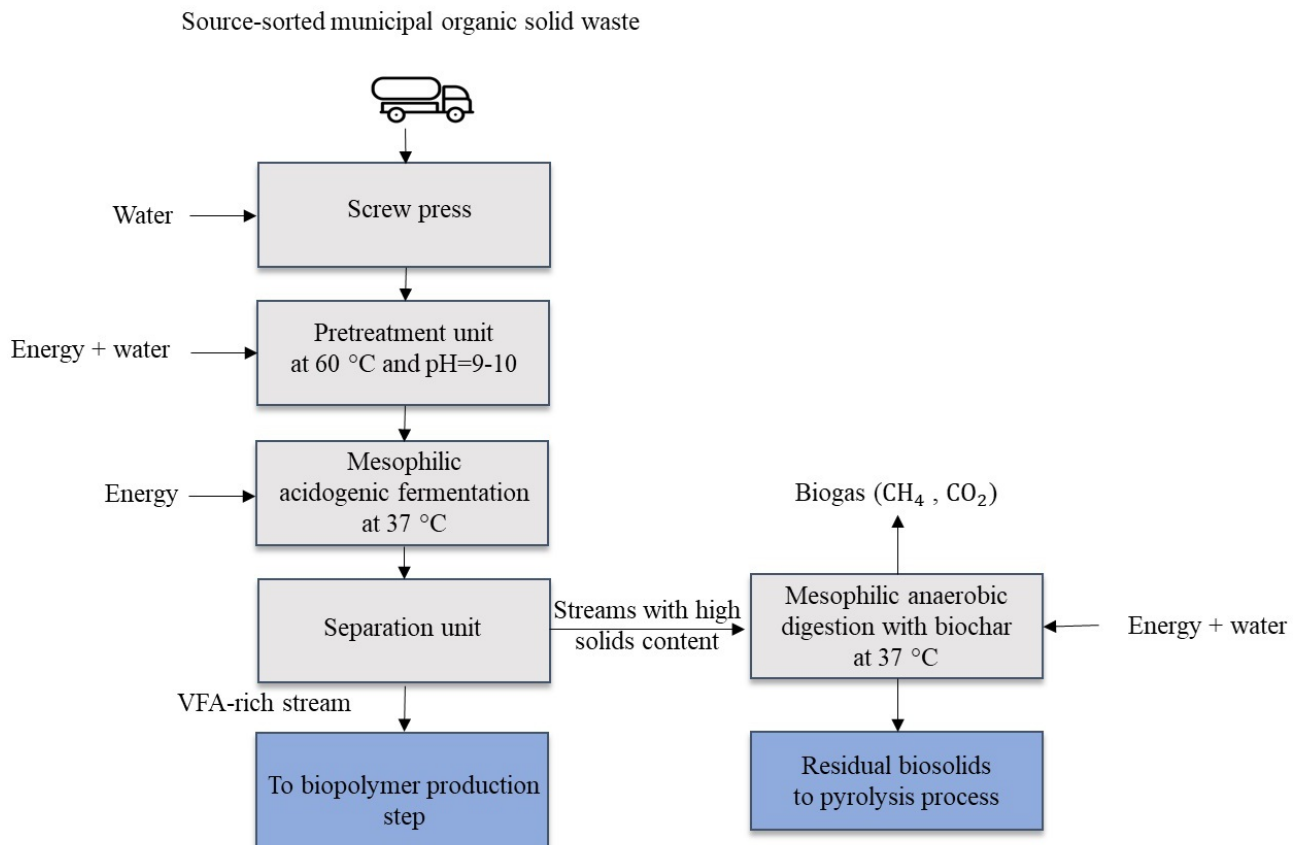
This study compares the multistep route of pretreatment, acidogenic fermentation, and anaerobic digestion with the existing method of single-step anaerobic digestion for valorizing OMSW in the Treviso wastewater treatment plant (WWTP) in terms of financial profit and technical feasibility. In this context, the present research has the ultimate goals of facilitating the entrance of the process into the market and further closure of the cycle of organic material. Accordingly, it assesses several suggestions, such as hydraulic retention time (HRT) variation, pretreatment, biochar addition, and adjusted FS/IN ratio to enhance the bioproducts and decrease the involved costs. To this end, their effects on the process were quantified through experimental tests, confirming their significance through statistical analysis. Later, the payback period, amount of surplus energy, and volatile solids (VS) destruction for the mentioned scenarios were determined using a mass balance model refined according to the laboratory studies. The boundary condition parameters for energy conversion and costs were assumed according to previous studies and experts' knowledge, respectively. To the best of the author's knowledge, this paper is novel in presenting a robust framework to assess a groundbreaking proposition for the valorization of OMSW financially and technically. Overall, we concluded that our line is viable technically and overtakes the conventional methods financially.

Methods

Biorefinery Process Scheme and Experimental Studies

Figure 1 presents the hypothesized biorefinery process line in this research. It comprises screw-pressing, a pretreatment unit,

mesophilic acidogenic fermentation, and anaerobic digestion for VFAs and biogas production from the organic municipal solid waste. CH₄: methane; CO₂: carbon dioxide; VFA: volatile fatty acid.



After and before the pretreatment, the feedstock for different parameters was characterized from time to time. These parameters include the total solids (TS), VS, chemical oxygen demand (COD), soluble COD (SCOD), total Kjeldahl nitrogen, total phosphorous (P), ammonium (N-NH₄⁺), phosphate (P-PO₄³⁻), and VFA.

The feedstock that arrived at the WWTP had already been mixed with the acidogenic fermentative inoculum, which initiated solubilizing and converting the organic solid matters into SCOD and VFAs in the transporter. Then, in the pretreatment unit, a sodium hydroxide (NaOH) solution (40% kg/kg) was added to bring the pH to 9 - 10 and heated to 60 °C for 24 hours.

Subsequently, the biomixture was fed manually into a 5 L (operational volume of 4.5 L) continuously stirred pilot acidogenic fermenter operated at the given conditions (Table 1). Its high alkalinity maintained the pH during the acidogenic fermentation in the optimal range. Further, the mixture was blended mechanically, and the whole system was kept in the oven to hold the temperature constant at 37 °C. The output was sampled frequently during the week, and the samples were centrifuged to obtain the supernatant to measure pH, SCOD, VFA, N-NH₄⁺, and P-PO₄³⁻. A tiny fraction of the residual solid part was used to characterize solids like COD, P, and total Kjeldahl nitrogen, and the rest was kept in the freezer to apply the bio-methane potential (BMP) test.

Table . The operational parameters of the mesophilic acidogenic fermenter.

Hydraulic retention time (days)	Organic loading rate (kg-volatile solids/m ³ .d)	Temperature (°C)	pH ^a , mean (SD)
4.5	6.89	37	6.56 (0.25)
3	10.33	37	6.7 (0.45)

^a13 measurements for pH.

The VS and TS characterization were performed in 105 °C and 550 °C ovens for 24 hours, respectively. Except for VFAs, all the remaining analyses (including COD measurements) followed the standard methods for examining water and wastewater [25]. The methods described in the A and D sections of No. 5220 for COD quantification were used. These methods are named “Closed Reflux, Titrimetric Method” and “Closed Reflux, Colorimetric Method” for the solid and liquid phases, respectively [25]. For the liquid, the samples were filtered after being centrifuged at 4500 rounds per minute (rpm) for 5 minutes, and before the analysis, the supernatant was filtered with a 0.45 µm cellulose filter (Whatman). For the solid, acidic digestion was performed at 220 °C with a high pressure of 2 atmospheres to destroy the 0.2 g of solid matrix for 2 hours. Afterward, the COD was measured in the solution using titration by ferrous ammonium sulfate as described in the standard methods. Our limit of detection was 50 - 500 mg-COD/L for the calorimetric method and 40 - 400 mg-COD/L for the titrimetric method. In this research, dilution was done for high-concentration values that are beyond the considered limit of detection.

In the BMP test, the effect of biochar addition was observed for 3 diverse dosages (0, 0.12, and 0.24 g-biochar/g-VS) on the bio-methane volume, content, and production kinetics in the mesophilic condition using four sets of the BMP test. The tests were conducted with a total number of 8 bottles of 250 mL (working volume of 215 mL). The anaerobic condition was ensured in bottles by sealing them after filling without any flushing with nitrogen molecules (N₂) or carbon dioxide (CO₂) since we had known that oxygen transfer at the surface of the waste stream was impossible as it contained a high TS and SCOD. This type of procedure was adopted in our laboratory and has been conducted for years. The biochar was synthesized by a local supplier, and its main physical and chemical features are reported in Table S1 in [Multimedia Appendix 1](#). It was ground into microparticles and kept under a dried condition at room temperature before being added to the bottles. Further, the inoculum for the BMP test was collected from the 2300 m³ completely stirred anaerobic digester treating thickened WS and squeezed OMSW mixture under the mesophilic condition at an organic loading rate (OLR) of 1.8 - 2.0 kg-VS/m³.d in the treatment plant. The inoculum was added to the feedstock (residual solid from acidogenic fermentation) based on the weight ratio of 0.3 FS g-VS/IN g-VS. The TS and VS contents in the bottles (ie, inoculum and feedstock) were 133 g/kg and 17.6 g/kg, respectively.

The experiments were conducted for each condition, namely, only inoculum and either with or without biochar, in 2 bottles. The test was terminated after 25 days when the cumulative biogas production reached almost 89% of the final projected value. The biogas content was characterized by gas chromatography (for days 1, 4, 6, 10, 14, 16, 18, 21, and 25). Additionally, the values for the remaining days were filled through imputation using the *k*-nearest neighbors algorithm (number of neighbors=4 and weights=distance) [26]. The imputation code is provided in the repository [27]. Then, the biogas and bio-methane volumes were subtracted from the only inoculum to correct for the endogenous methane production,

and both values were averaged for 2 bottles. Gas chromatography was performed using Agilent Technology (TM 6890N) with an HP-PLOT MoleSieve column (30 m length, 0.53 mm ID × 25 mm film thickness) and a thermal conductivity detector with argon as a carrier (79 mL/min). The hydrogen molecule (H₂), CH₄, oxygen molecule (O₂), and N₂ were analyzed using a thermal conductivity detector at 250 °C. The inlet temperature was 120 °C, with constant pressure in the injection port (ie, 70 kilopascal [kPa]). Samples were taken using a gas-type syringe (200 µL). Once the entire sample was vaporized, peak separation occurred within the column at a constant temperature of 40 °C for 8 minutes. We did not plan to monitor pH and other parameters like alkalinity, VFA, ammonia, and phosphate because the pH drop risk was negligible, and the biochar addition could provide a buffer capacity and adsorption of inhibitory compounds in the solution [28]. Moreover, a considerable part of the readily biodegradable COD of the feedstock was already converted to VFAs in the previous step. As a result, the process was easily controlled even in the transient condition when the risk of methanogenic inhibition was high [29].

Statistical Analysis and Performance Indicators

The performance indicators, including COD solubilization, VFA yield, ammonia and phosphate release, and VFA/SCOD ratio were determined. These indicators characterize the mesophilic acidogenic fermentation on the days when the data were available, and the process reached the pseudo-steady state condition. The indicators were calculated, and the data were plotted using a Microsoft Excel spreadsheet (Version 2412). In addition, the VFA weight ratio distribution was determined from the total VFA weight on the same day. The process stability was evaluated based on variations in daily VFA concentrations. The formula for the performance parameters is reported in [Multimedia Appendix 1](#). The exploratory data analysis and 2-tailed *t* test on VFA data were performed for the VFA concentration, yield, and VFA/SCOD ratio for 2 HRTs by the open source program R (version 3.5.0; The R Foundation for Statistical Computing). We assumed that the 2 datasets were paired and had a normal distribution. The code is provided in the repository [27]. The values for the 2 HRTs to increase the VFA concentration in the outlet were selected based on our experience and process knowledge. According to this information, exceeding the HRT value by more than 3 - 5 days can bring the process into an anaerobic digestion step. As a result, the VFAs with high added-value markets are converted to biogas. Hence, the 2 HRTs of 3 days and 4.5 days were tried in the pilot test, knowing that the VFA concentration would either increase or decrease linearly in this local region of operation.

For the BMP tests, two kinetic models were calibrated, namely, the first-order rate and modified Gompertz, to the biogases' cumulative yield. Additionally, the specific methane production (SMP) and specific biogas production (SGP) plus maximum volumetric methane content (v/v %) were determined. Comparing these results could reveal how the biochar addition, FS/IN ratio of 0.3, and pretreatment improved the process in terms of the rate and fostered methanogenesis. Such improvements are manifested through a higher hydrolysis rate,

a shorter lag phase, and a higher maximum volumetric methane content. Besides, the biogas yield was determined as g-biogas/g-VS.

Technical and Economic Assessment

This research sets up a mass flow analysis with parameters adopted for a municipality with 70,000 population equivalents (PEs) for the two scenarios: (1) a line with pretreatment and mesophilic acidogenic fermentation followed by mesophilic anaerobic digestion and (2) a single-stage mesophilic anaerobic digestion as currently deployed at the Treviso WWTP. This study focuses on water and energy preservation and increased profits from VFA production in our conversion line through several refinements. They were tied with the HRT identified in the previous step, integration of our process knowledge of using the fine-tuned FS/IN ratio, and biochar addition in anaerobic digestion. Detailed information and calculations regarding the mass flow analysis are available in the supplementary documents in the Excel spreadsheet named "Mass Balance" [27]. The following paragraph provides the full description of the two scenarios.

The two scenarios shared the first part of the model where the separated OMSW by a door-to-door collection system that was screw-pressed and diluted with water to reach the TS of 280 g/kg. Then, in the first scenario, adding a sodium hydroxide solution (40% kg/kg) elevated the feedstock pH to 9 - 10. Afterward, the solution was heated at 60 °C for 24 hours in the pretreatment unit. Next, it was diluted and heated further before

feeding into the mesophilic acidogenic fermenter based on the desirable HRT. The last part of the first scenario was the optimized anaerobic digestion of residual fermented solids. Specifically, the stability endowment by adding biochar to the anaerobic digestion could ultimately smooth running the process in a high OLR (low water dilution). Furthermore, an FS/IN ratio of 0.3 was applied to increase the kinetics rate with the benefit of a decrease in digester volume, energy consumption, and capital cost. This finding is of significant importance in plants and zones with limited area, water, and energy.

In the second scenario, the screw-pressed feedstock was diluted and immediately fed into a mesophilic anaerobic digester for only biogas production.

It was assumed that the reactors transfer heat from the walls with the atmosphere and earth. Further, the biogas would be consumed in the combined heat and power units for electricity production with an overall efficiency of 0.4. In this research, the mass of VFAs and the net amount of energy production were accounted for as the source of income. Meanwhile, the corresponding costs were the operational expenditure, the mass of the water process, and the final residual solids to dispose of. Reference parameters for the energy analysis and boundary conditions are given in Table 2. The price of electricity was assumed to be €130 per megawatt-hour (MWh). These two scenarios were compared to identify the most favorable one regarding surplus thermal energy and electricity or the shorter payback period.

Table 2. Reference parameters and boundary conditions for energy flow analysis.

Parameter	Heat transfer coefficient (W/(m ² .°C))	Temperature (°C)	Low heat value (MJ ^a /Nm ³)	Energy conversion efficiency
Biogas	— ^b	—	23.012	—
Thermal energy yield	—	—	—	0.5
Electrical energy yield	—	—	—	0.4
Operative temperature	—	37	—	—
Water temperature	—	15	—	—
Air temperature	—	20	—	—
Ground temperature	—	25	—	—
Outer concrete reactor wall	0.7	—	—	—
Inner concrete reactor wall	1.2	—	—	—
Floor	2.85	—	—	—

^aMJ: megajoules.

^bNot applicable.

Ethical Considerations

This research was not conducted on human or animal subjects and does not involve the collection of any new data. Therefore, it was unnecessary to obtain ethics approval.

Results

Biorefinery Process Scheme and Experimental Studies: Composition and Characteristics of the Pretreated Feedstock

The pretreated feedstock's main physical and chemical characteristics were quite stable throughout the experiment (Table 3). The feedstock had an average TS content of 45 (SD 3.15) g/kg and VS content of 32 (SD 3.28) g/kg. These values

suggest that the biodegradable solids constituted 72% of the TS, which could support the fermentation process. The chemical composition of the solid part was 12.9 g-N/kg-TS, 4 g-P/kg-TS, and 565 g-COD/kg-TS, which was in the range of the values reported for the typical OMSW in Italy [30]. The chemical composition of the liquid was 325 mg N-NH₄⁺/L, 14 mg

P-PO₄³⁻/L, and 25.8 g-SCOD/L. Further, the feedstock COD:N:P ratio was determined as 100:2.2:0.7, meaning that nutrients such as phosphor and nitrogen should not be the limiting substrates in acidogenic fermentation [31]. In this regard, the slight level of VFA concentration at the level of 3.5 g-SCOD/L was due to acidogenic fermentation, which had been happening during transportation.

Table . Main physical-chemical features of the feedstock.

Parameter	Weight ratio (g/kg)	Mass ratio (%)	Concentration (mg/L)
Total solids, mean (SD) ^a	45 (3.15)	— ^b	—
Volatile solids, mean (SD) ^a	32 (3.28)	—	—
Total Kjeldahl nitrogen ^c	12.9	—	—
Phosphorous ^c	4	—	—
Chemical oxygen demand ^c	565	—	—
Chemical oxygen demand:nitro- gen:phosphorous	—	100:2.2:0.7	—
Soluble chemical oxygen demand	—	—	25,814
N-NH ₄ ^{+d}	—	—	325
P-PO ₄ ^{3-e}	—	—	14
Volatile fatty acid ^c	—	—	3500
Volatile solids/total solids, mean (SD) ^a	—	72 (5)	—

^aBased on 3 measurements.

^bNot applicable.

^cMeasurements done for nitrogen, phosphor, and soluble chemical oxygen demand equivalents for total Kjeldahl nitrogen, phosphorous, chemical oxygen demand, and volatile fatty acid.

^dN-NH₄⁺: ammonium.

^eP-PO₄³⁻: phosphate.

Statistical Analysis and Performance Indicators

Acidogenic Fermentation

Table 4 presents the main physical and chemical characteristics of the effluent and solid cake from the acidogenic fermenters. According to Figure 2, the process reached a steady condition after 14 days, which was roughly 3 times the HRT (4.5 days).

Both HRTs were similarly stable in terms of VFA concentration variation because of a negligible difference between SDs: 2.82 g-SCOD/L versus 2.45 g-SCOD/L. These values are less than 10% of the total VFA, and the VFA production continued for more than 3 weeks without any considerable issues. The lack of any change in this process is attributed to the initial high pH of 9 - 10, which supported the process by keeping the pH variation in the optimal range of 6 - 7.5 [32].

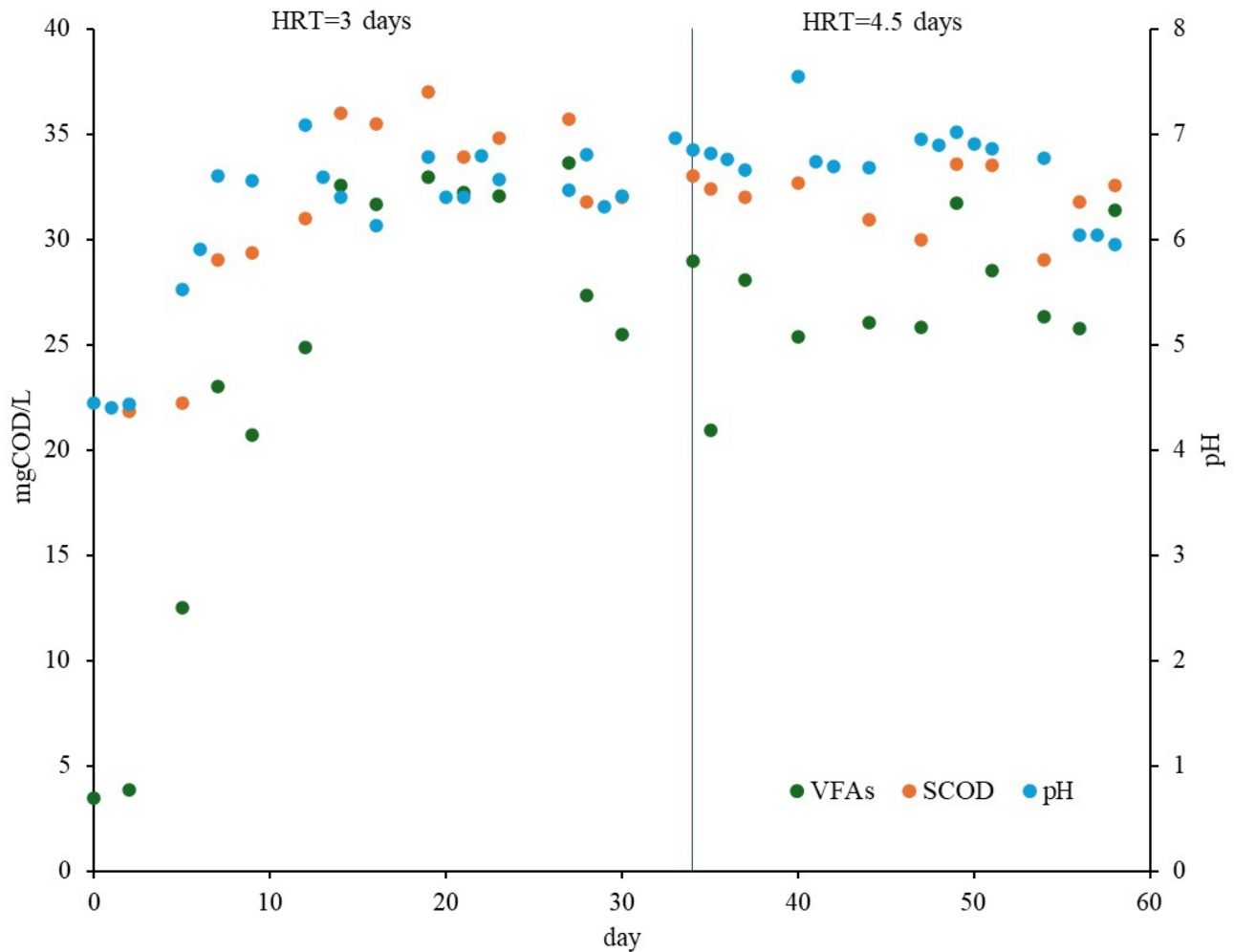
Table . Main physical-chemical features of the effluent and solid cake from mesophilic acidogenic fermentation.

Hydraulic retention time (days)	Total solids (g/kg), mean (SD)	Volatile solids (g/kg), mean (SD)	Volatile fatty acid (g-solu- ble chemical oxygen de- mand/L), mean (SD)	pH, mean (SD)
4.5 ^a	43 (5.15)	23.6 (2.07)	30.77 (2.82)	6.56 (0.25)
3 ^b	38 (4.55)	25.8 (1.5)	27.67 (2.45)	6.7 (0.45)

^a5 measurements for total solids and volatile solids; 9 measurements for volatile fatty acid; 13 measurements for pH.

^b4 measurements for total solids and volatile solids; 9 measurements for volatile fatty acid; 13 measurements for pH.

Figure 2. VFA, SCOD, and pH for mesophilic acidogenic fermentation. COD: chemical oxygen demand; HRT: hydraulic retention time; SCOD: soluble chemical oxygen demand; VFA: volatile fatty acid.



Based on the t test results ($t_8=-2.68$; $P=.03$; $CI=95\%$), it was verified that the mean VFA concentration for an HRT of 4.5 days was significantly higher than the value for 3 days (30.77 vs 27.67 g-SCOD/L). A similar statistical analysis ($t_8=-0.99$; $P=.35$; $CI=95\%$) for the VFA/SCOD ratio rejected the

significance of a higher mean value of 0.892 (SD 0.04) for an HRT of 4.5 days than 3 days, with a mean value of 0.87 (SD 0.058). The possible range of values for the VFA concentrations and VFA/SCOD, which cover 99% and 50% of the data for the 2 HRTs, are depicted by the box plots in Figures 3 and 4, respectively.

Figure 3. Box plot of volatile fatty acid concentrations for mesophilic acidogenic fermentation. HRT: hydraulic retention time; SCOD: soluble chemical oxygen demand.

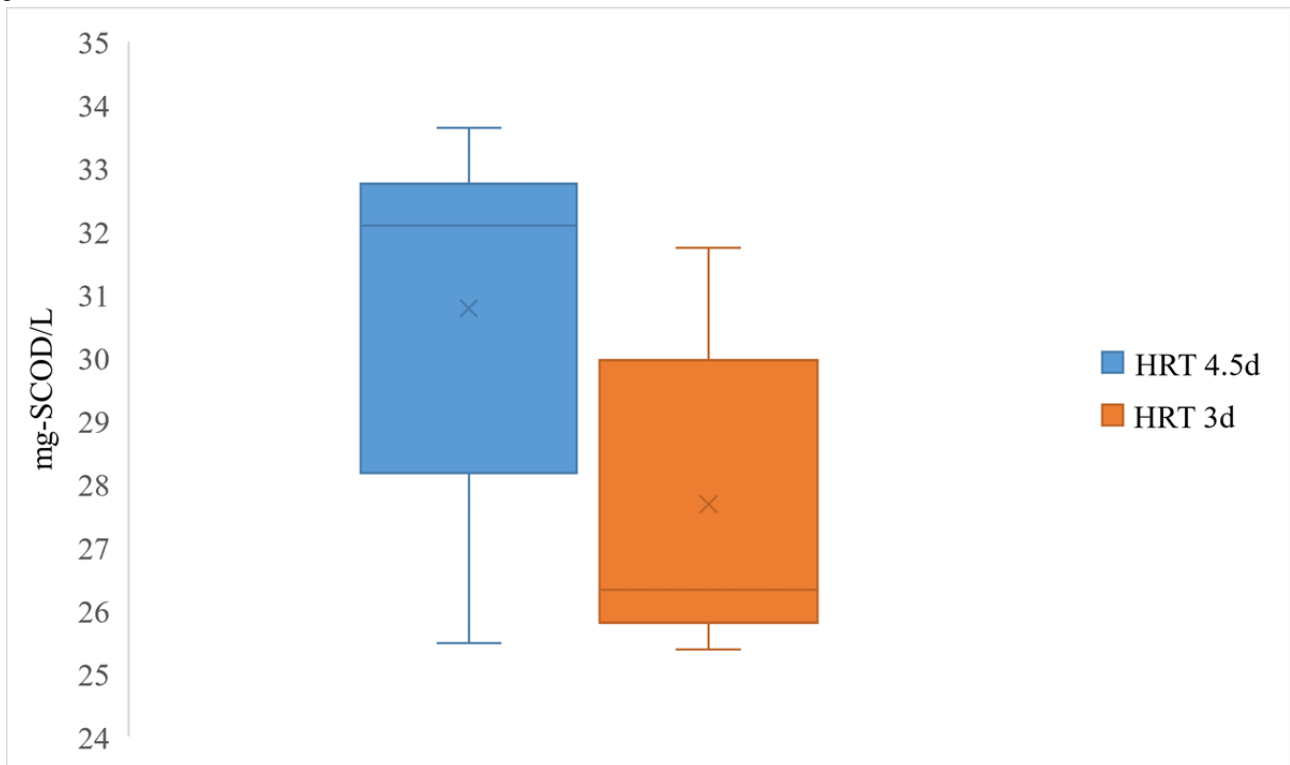
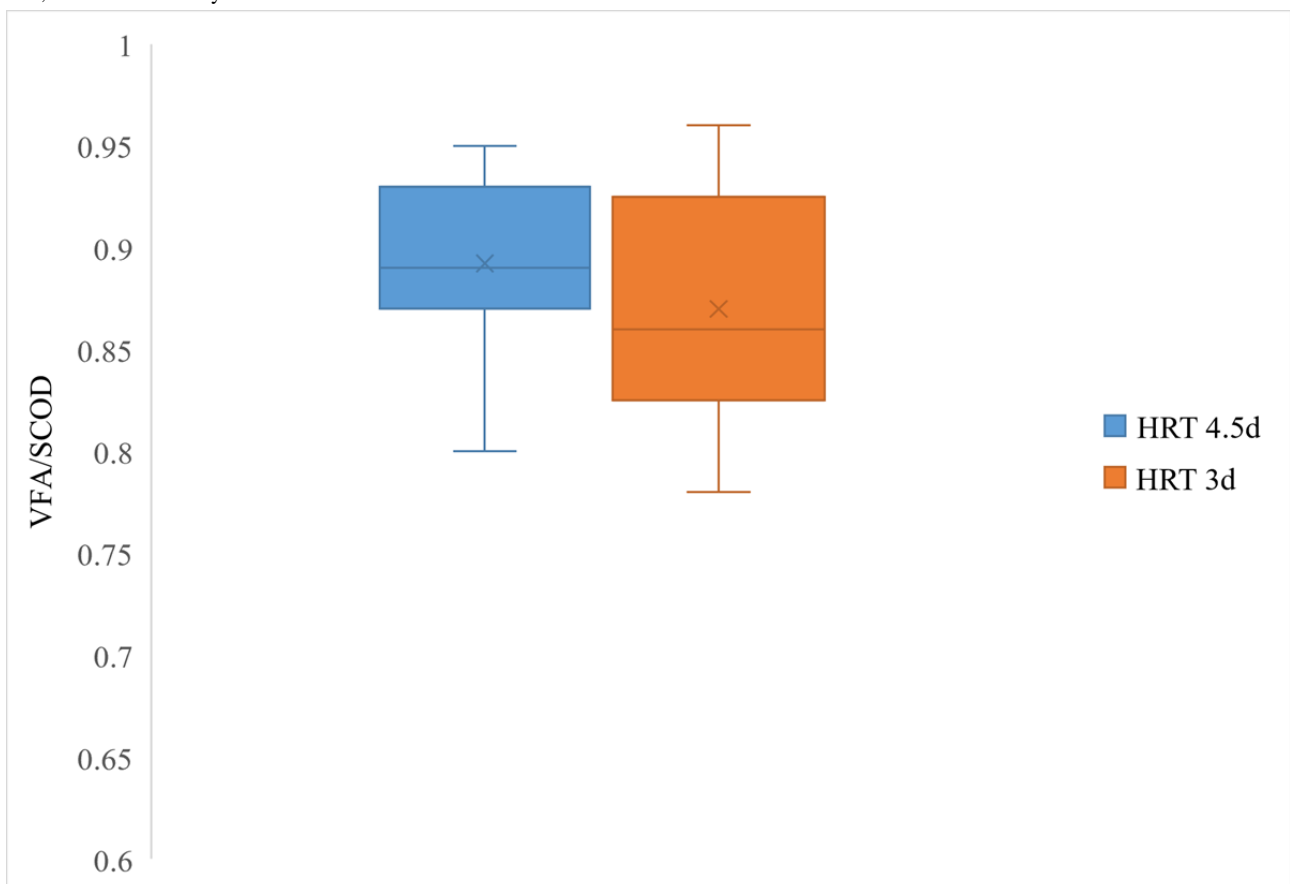


Figure 4. Box plot of VFA/SCOD ratios for mesophilic acidogenic fermentation. HRT: hydraulic retention time; SCOD: soluble chemical oxygen demand; VFA: volatile fatty acid.



Performance parameters for the 2 HRTs are given in Table 5. As can be seen, the HRT of 4.5 days gave higher COD

solubilization and released more ammonia and phosphate than the HRT of 3 days. Moreover, the 0.57 VFA yield per gram of

VS for the HRT of 4.5 days was significantly higher than 0.5 for the HRT of 3 days ($t_g = -2.94$; $P = .02$; $CI = 95\%$).

In the biopolymer-synthesizing process, the aim was to generate a stable VFA weight ratio distribution with a high VFA/SCOD ratio for an efficient PHA synthesis during the whole process. Concisely, the VFA stream with a higher dominance of even numbers of carbon atom acids means a higher 3-hydroxybutyrate monomer synthesis compared to the 3-hydroxyvalerate, which is correlated with the net prevalence of odd numbers of carbon atom acids (propionic, valeric, and isovaleric acid) [33]. As can be inferred, the stability in the VFA spectrum means a

predictable and reproducible PHA monomer production. Accordingly, the physical and mechanical features of synthesized biopolymers are stable [34,35].

Figure 5 reports the weight ratio distribution of the VFAs for the 2 HRTs. The main fractions were acetic acid (38% - 42%), butyric acid (24%), caproic acid (16% - 18.5%), propionic acid (9% - 11%), and valeric acid (5%). This VFA distribution, with a major part of butyric and acetic acid, is in line with those reported in similar studies [29,31]. In this respect, the VFA weight ratio distribution is determined by the type of feedstock and food waste rather than the operational conditions.

Table . Performance parameters of two different operational conditions used in mesophilic acidogenic fermentation.

Hydraulic retention time (days)	Solubilization (Δg -soluble chemical oxygen demand/g-VS ^a ₀), mean (SD)	Y_{VFA}^b (Δg -VFA/g-VS ₀), mean (SD)	Ammonia release (%), mean (SD)	Phosphate release (%), mean (SD)
4.5 ^c	0.28 (0.06)	0.57 (0.06)	35 (10.74)	13.7 (8.77)
3 ^d	0.19 (0.05)	0.50 (0.06)	29 (0.11)	11 (0.06)

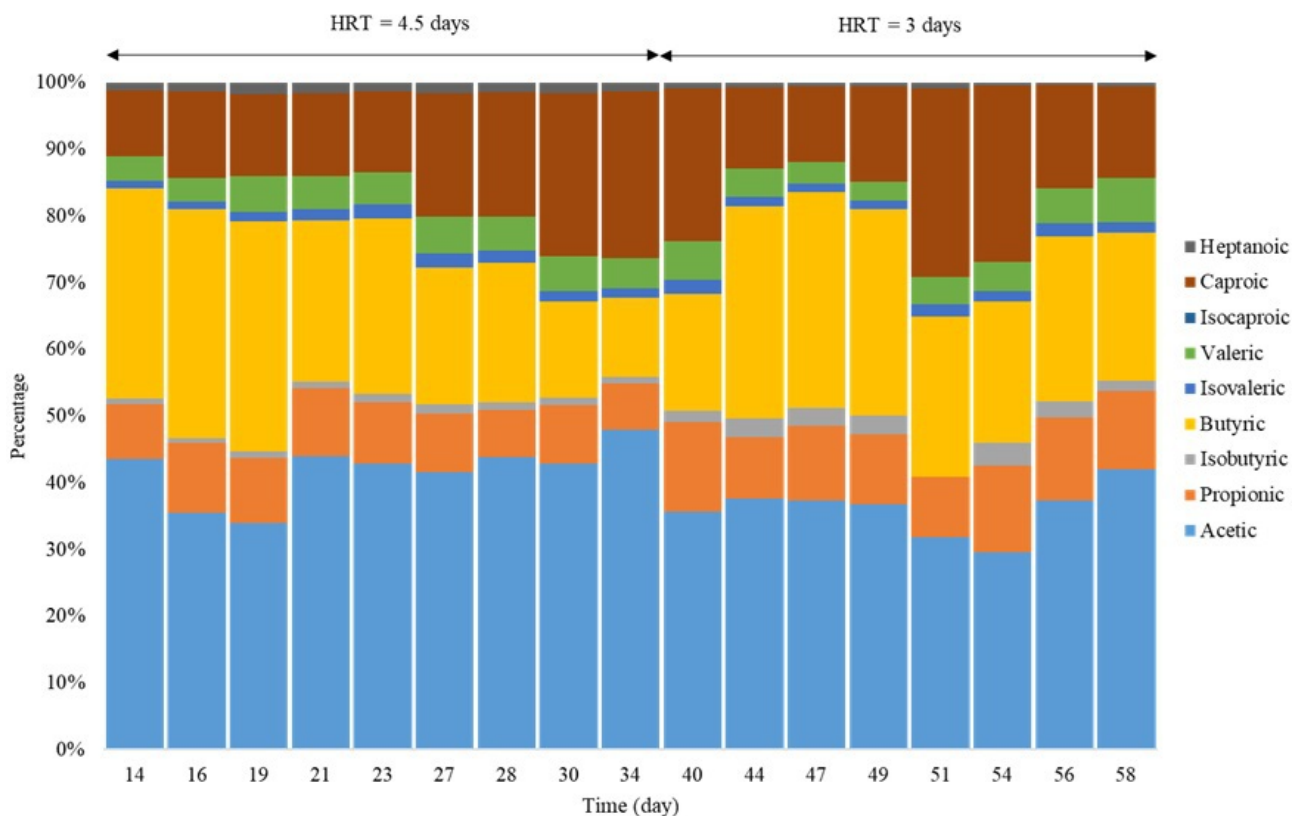
^aVS: volatile solids.

^bVFA: volatile fatty acid.

^c9 measurements for solubilization (Y_{VFA}); 8 measurements for ammonia and phosphate release.

^d9 measurements for solubilization (Y_{VFA}); 7 measurements for ammonia and phosphate release.

Figure 5. Volatile fatty acid weight ratio distribution for mesophilic acidogenic fermentation. HRT: hydraulic retention time.



Anaerobic Digestion

Table 6 summarizes the performance parameters and the results from the kinetics study for anaerobic digestion. This study obtained a remarkably high value for the hydrolysis rate (ie, 0.58, 1/d) with no lag phase. Besides, a biogas yield of

0.61 - 0.83 g-biogas/g-VS, SMP of 0.133 - 0.204 CH₄-Nm³/kg-VS, and an average composition of 45% - 58% methane (v-CH₄/v-biogas) were obtained. According to Figure 6, adding biochar provided the desirable conditions for the growth of hydrogen using methanogenesis manifested through

a higher maximum volumetric methane content (86% vs 66% volumetric basis [v/v]).

Table 6. The performance indicators for anaerobic digestion and results from the kinetics study for two models: (1) first-order rate and (2) modified Gompertz.

Experiments	Specific methane production (CH ₄ ^a -Nm ³ /kg-VS ^b)	Specific gas production (CH ₄ -Nm ³ /kg-VS)	K ^c (1/d)	R _m ^d (CH ₄ -mL/g-VS.d)	τ ^e (days)	RMSE ^f first-order (CH ₄ -Nm ³ /kg-VS)	RMSE modified Gompertz (CH ₄ -Nm ³ /kg-VS)	Max CH ₄ content (v/v) ^g , %
Without biochar	0.204	0.540	0.57	76.12	0	10.4	6.82	68.5
Biochar (0.12 g-biochar/g-)	0.133	0.567	0.69	62.42	0	5.74	5.59	86
Biochar (0.24 g-biochar/g-)	0.177	0.500	0.58	65.17	0	9.64	3.39	76.5

^aCH₄: methane.

^bVS: volatile solids.

^cHydrolysis rate.

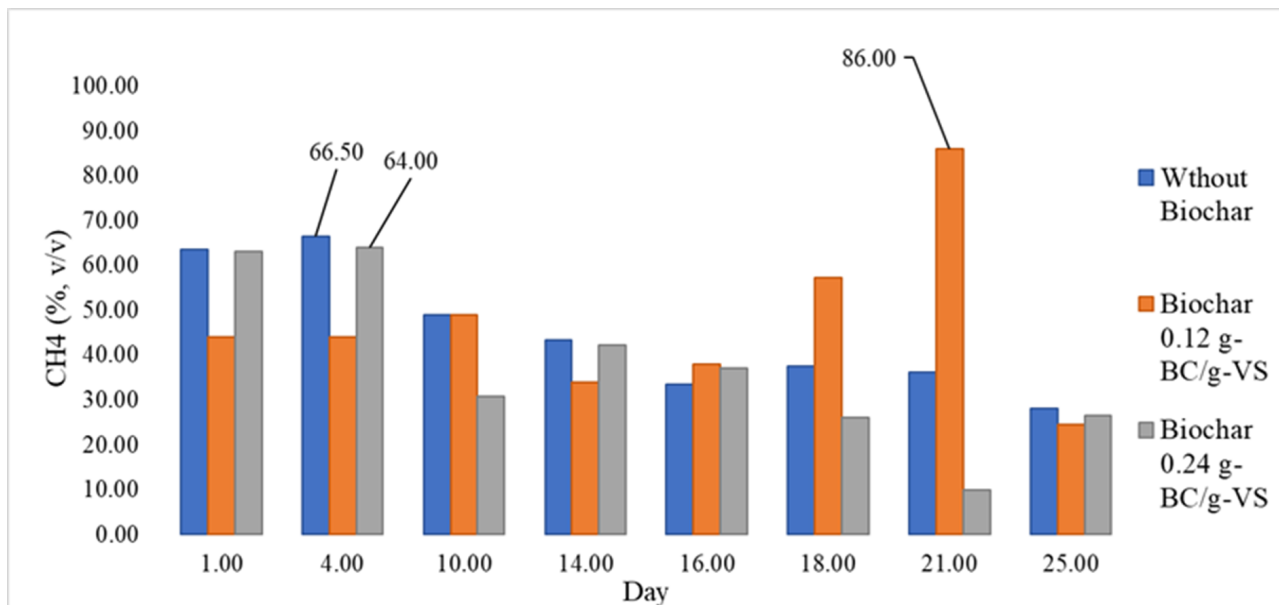
^dMaximum methane production rate.

^eLag phase.

^fRMSE: root mean squared error.

^gv/v: volumetric basis.

Figure 6. CH₄ content in v/v for 3 different biochar dosages in anaerobic digestion. BC: biochar; CH₄: methane; VS: volatile solids; v/v: volumetric basis.



The mass flow model was adopted for 0.12 g-biochar/g-VS as the only feasible solution. Unlike other dosages, it could satisfy the assumptions for an FS/IN ratio of 0.3 at an HRT of 20 days, which was adequately long enough to let the methanogens reproduce themselves. Detailed information is available in the Excel sheet named "DIGESTER DESIGN" [27]. Besides, the high alkalinity of the biochar as reported in Table S1 in Multimedia Appendix 1 signifies a benefit of the biochar addition in limiting the concern about decreases in pH for a high OLR in full-scale implementation. Accordingly, almost 4-fold of the ordinary OLR was obtained, that is, 6.25 kg-VS/m³.d, by minimum water dilution, knowing that the biochar could maintain the stability of the process. Therefore,

the digester volume will decline at the rate of 28 L/PE. Hence, the presented mass flow line model was implemented based on the results of 0.12 g-biochar/g-VS, the weighted average composition of biomethane as 35% v/v, and the SGP as 0.56 biogas-Nm³/kg-VS for an HRT of 20 days corresponding to an FS/IN ratio of 0.3.

Based on the root mean squared error reported in Table 6, both models were almost identical in describing biomethane production for a biochar dosage of 0.12 g-biochar/g-VS, and for simplicity, we used the first-order rate model in the feasibility study.

Technical and Economic Assessment

Assuming an imaginary municipality of 70,000 PEs and the amount of TS production per capita as 0.3 kg/PE per day [36], the inlet to the scale-up line would be 21,000 kg-TS per day.

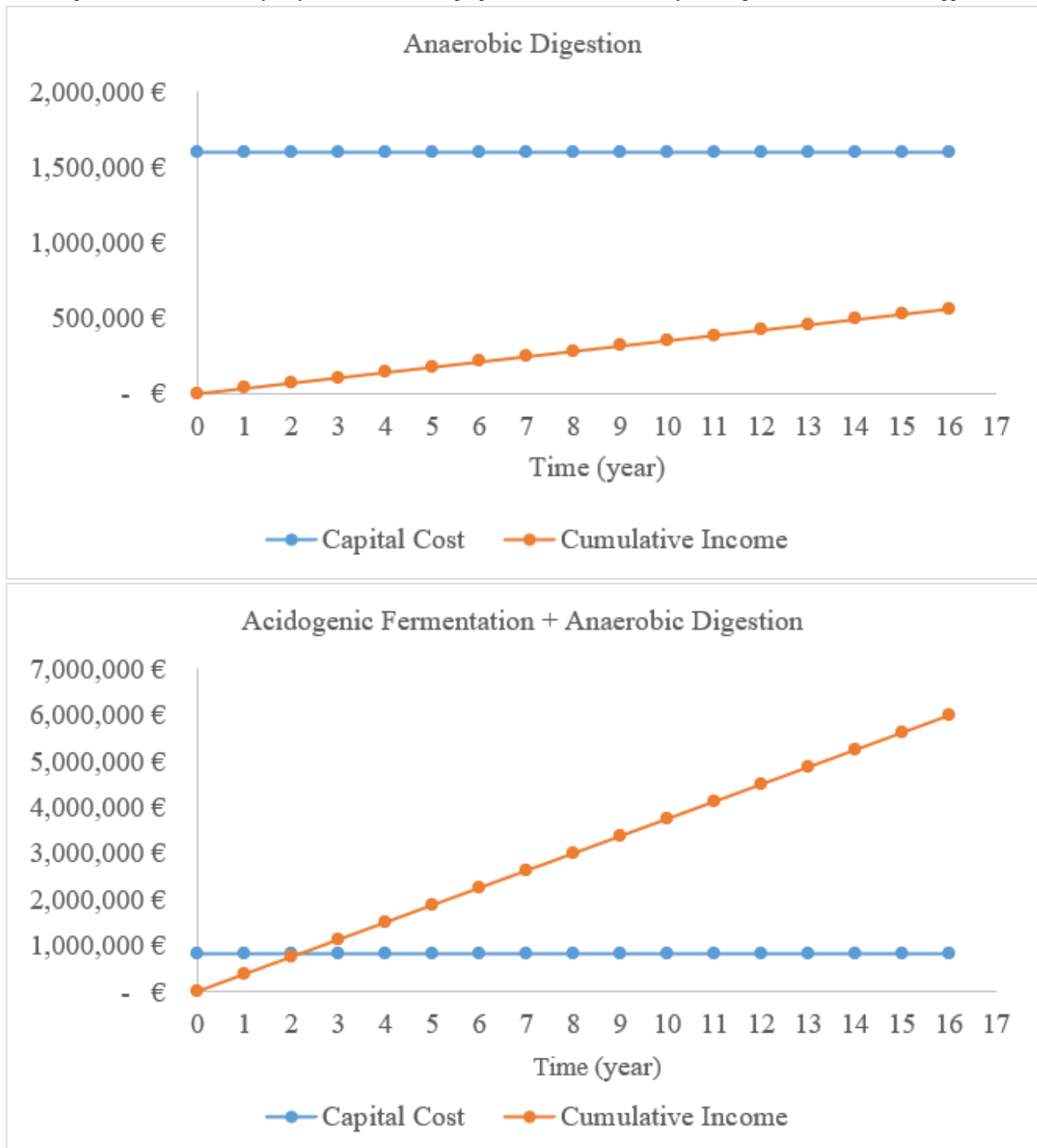
In the first scenario, the biowaste stream, after passing through the screw press and pretreatment unit, had a mass flow of 113,788 kg per day, TS of 4.1% kg/kg, and VS of 3.1% kg/kg. Then, the mixture was heated to 37 °C before and in the acidogenic fermenter, which was operated at an HRT of 4.5 days and OLR of 6.89 kg-VS/m³.d. This process was performed to convert biosolids into the VFAs and SCOD at concentration levels of 30.77 g-SCOD/L and 34 g-SCOD/L, respectively. At this step, the gaseous flow rate was assumed to be zero, as an HRT of 4.5 days is short for any adequate growth of methanogens in mesophilic conditions. The stream out of the acidogenic fermenter had a mass flow rate of 113,788 kg per day, with a VFA content of 3501 kg-SCOD per day, which could be used in the PHA-synthesizing step [37]. The outlet of this step was used in the separator to gain overflow and solid cake. Later, the solid cake was minimally diluted by water before being fed into a mesophilic anaerobic digester with a biochar addition of 0.12 g-biochar/g-VS. The anaerobic digester received a TS content of 18% kg/kg and a flow rate of 18,180 kg per day, corresponding to an HRT of 20 days and OLR of 6.25 kg-VS/m³.d. Overall, an SGP of 0.285 (Nm³-biogas/kg-VS)

was obtained assuming zero gas production in acidogenic fermentation.

In the second scenario, the fresh feedstock, after being screw-pressed, had a mass flow rate of 4678 kg-TS per day and 28% kg/kg dry matter. Then, it was diluted with water and heated before being fed into the anaerobic digester. At this step, the mass flow rate of 85,012 kg per day with a TS of 6% kg/kg entered the digester with a volume of 2125 m³, leading to an HRT of 25 days and OLR of 1.7 kg-VS/m³.d. The SMP of 0.311 Nm³-biogas/kg-VS was obtained by destroying 80% of the VS.

In this study, working volumes of 512 m³ and 364 m³ were adopted for the acidogenic fermenter and anaerobic digester in the first scenario, respectively, and 2125 m³ for the anaerobic digester in the second scenario. As a result, the capital cost for the presented line was almost €809,000, roughly half of the quantity for the single-step anaerobic digestion (Figure 7). Unlike the single-step anaerobic digestion that converts all VS to biogas, this novel line shared the recovery of VS between higher added-value VFAs and biogas production, and expectedly generated 10-fold higher benefits (€375,085). Consequently, the payback period was reduced by more than 20 times in 2 years (Figure 7). This period was achieved using less surplus energy (2251 megajoules [MJ]/d) for the 2-step fermentation (vs 21,567 MJ/d for the single-step anaerobic digestion).

Figure 7. Capital cost and cumulative yearly income for the two proposed scenarios. A currency exchange rate of €1=US \$1.05 is applicable.



Discussion

Principal Results

We showed that multistep fermentation followed by anaerobic digestion is both economically and technically feasible. The findings indicated that producing VFAs and biogas in separate stages can significantly reduce the payback period for upcoming investments in biorefinery projects and result in the creation of a highly desired stream that is rich in VFAs. Additionally, the process stability could be maintained even at a high OLR by adding biochar and converting the VS's easily biodegradable

COD content into VFAs in the first phase. This would preserve energy and water, and reduce the digester's volume.

Comparison With Previous Studies

Because of the extra pretreatment unit in this research, the VFA yield of 0.57 - 0.63 Δ g-VFA/g-COD_{IN} was roughly double the value reported by Valentino et al [31] for the same OMSW.

Our results also indicate a substantial improvement in the process kinetics, which was manifested through a more than 8-fold rise in the hydrolysis rate (0.58 vs 0.07, 1/d) and a full decrease in the lag phase (0 vs 2.69 days) as opposed to the previous study by Karki et al [38]. This improvement is

attributed to the destruction of the solids structure caused by bacterial enzymes and a hot alkaline solution. Additionally, a higher active biomass per feedstock was provided using a fine-tuned FS/IN ratio of 0.3 (VS basis), which was noticeably lower than the quantities (1 and 0.5) reported in similar studies [38,39].

The values for SMP and mean methane volumetric content presented in this study are lower than those reported by Valentino et al [29] (ie, 0.25 CH₄-Nm³/kg-VS and 63% - 64% v/v, respectively). This difference is explained by the added fresh WS, which has a higher digestible content and better nutrient balance than the fermented solids. Similarly, the SMP in this study was lower than the 0.384 CH₄-Nm³/kg-VS found in the study by Moreno et al [39]. This study investigated the anaerobic digestion of residual solids from two steps of bioethanol production and saccharification on OMSW. In this respect, bioethanol production can only convert part of the cellulose, starch, and some dissolved carbohydrates. Consequently, a great part of the biosolids' volatile content, nearly 70%, is still available to be harvested in different biorefinery schemes compared with the one proposed in this method with 55%. Besides, the fermented OMSW would have a completely incompatible composition since it did not only come from different geographical locations (Spain and the United Kingdom) with different food habits but also underwent different biological pretreatment. Further, the multistep recovery line proposed in our study is more practicable technically. As the method studied by Moreno et al [39] requires sterilization conditions, imposing an additional operational cost and bioethanol concentration should be high enough to lower the cost of the subsequent distillation step.

Furthermore, our method for VFA production distinctively from biogas was preferable to the study by Papa et al [9], wherein the operational alteration on a single anaerobic digester was performed to obtain VFAs and biogas. These researchers asserted that the single-step recovery of biogas and VFAs was feasible by increasing the OLR while keeping the SMP of the reactor almost unaffected. The main recovery path for the VS was still biogas production in their study, which accounted for more than 90% of the VS conversion. Meanwhile, our study obtained 36% and 64% of the biogas and VFA conversion share, respectively. Further, whereas the destruction of VS of around 70% was achieved in both studies, their proposal limited the VFA distribution to propionic and butyric acid. The explanation is that some of the VFAs were converted into biogas in the same unit, which could negatively affect the PHA synthesis step later.

Conclusion and Limitations

This paper demonstrated the technical and economic feasibility of a multistep recovery line for OMSW. The results of this study indicate that the production of VFAs and biogas in distinct steps can considerably shorten the payback period for future investments in biorefinery projects and produce a highly desirable VFA-rich stream. Further, adding biochar and converting easily degradable COD content in the VS into VFAs in the first step could maintain the process stability even with a high OLR in anaerobic digestion. As a result, it leads to energy and water preservation and a decrease in the digester volume. However, consideration should be paid to the full-scale implementation since the pilot studies cannot resemble the stability of the real process. For instance, operational alterations such as raising the OLR and the addition of biochar in the full-scale implementation might perturb the process pH or the synergetic balance between the bacterial communities and stop the process completely, which was never observed in our experimental study. Further, the superb profitability of the proposed line was highly variable because our cost analysis was too simplistic and did not elaborate on all the possible associated expenditures and incomes. Besides, since many of its components were from subject matter experts rather than the pilot studies' budget, they were prone to site variations and uncertainties. Addressing the systematic uncertainty in the labor and material costs due to the changes in the supply chain issues, inflation, and site variations is beyond our scope. Moreover, caution should also be considered regarding the significance of the BMP results with the marginal difference since the number of samples was not large enough for statistical analysis. Nevertheless, the results presented in this study were prepared cautiously both technically and financially to encourage the revolution in the current state of organic waste valorization in Italy and any similar location.

In conclusion, a robust framework was proposed to assess the valorization of organic waste through experimental tests, statistical analysis, process kinetics, and mass and energy flow analysis. The findings support considerably higher profitability and, thus, a shorter payback period for the multistep fermentation than the current single anaerobic digestion. Additionally, our results encourage the circular economy perspective on converting OMSW into biogas and VFAs with the benefit of fewer residual solids due to reusing them in a pyrolysis line.

Acknowledgments

The author gratefully acknowledges the Italian Ministry of University and Research and the University of Ca' Foscari for financially supporting and providing a study design for this research in the frame of Programma Operativo Nazionale Ricerca e Innovazione 2014-2020. In addition, the author appreciates Marco Gottardo for his helpful assistance with gas chromatography, and Francesco Valentino for his helpful comments and guidance during the study, as well as Alessio Dell'Olivo and Aditi Parmar Chitharanjan for their helpful advice with laboratory experiments and material flow analysis. The author also attests that they used generative artificial intelligence in checking the text regarding grammar and correct word usage.

Data Availability

The codes for statistical analysis as well as the datasets generated and analyzed during this study are available from a repository [27]. Research Data Policy Type 2 (for life sciences) by Springer Nature is distributed under the terms of the Creative Commons Attribution 4.0 International License.

Conflicts of Interest

The author declares his current expert witness position as a peer reviewer in the *Journal of Medical Internet Research*.

Multimedia Appendix 1

Supplementary table, equation, digester design, and code.

[DOCX File, 31 KB - [xmed_v6i1e50458_app1.docx](#)]

References

1. Preparatory study on food waste across EU 27: final report. Publications Office of the European Union. 2011. URL: <https://data.europa.eu/doi/10.2779/85947> [accessed 2024-01-29]
2. Pfaltzgraff LA, De bruyn M, Cooper EC, Budarin V, Clark JH. Food waste biomass: a resource for high-value chemicals. *Green Chem* 2013;15(2):307-314. [doi: [10.1039/c2gc36978h](https://doi.org/10.1039/c2gc36978h)]
3. Circular economy action plan. European Commission: Environment. 2022. URL: https://ec.europa.eu/environment/strategy/circular-economy-action-plan_en#:~:text= [accessed 2025-01-23]
4. Mazur-Wierzbicka E. Towards circular economy—a comparative analysis of the countries of the European Union. *Resources* 2021;10(5):49. [doi: [10.3390/resources10050049](https://doi.org/10.3390/resources10050049)]
5. Mattioli A, Gatti GB, Mattuzzi GP, Cecchi F, Bolzonella D. Co-digestion of the organic fraction of municipal solid waste and sludge improves the energy balance of wastewater treatment plants: Rovereto case study. *Renewable Energy* 2017 Dec;113:980-988. [doi: [10.1016/j.renene.2017.06.079](https://doi.org/10.1016/j.renene.2017.06.079)]
6. Cabbai V, De Bortoli N, Goi D. Pilot plant experience on anaerobic codigestion of source selected OFMSW and sewage sludge. *Waste Manag* 2016 Mar;49:47-54. [doi: [10.1016/j.wasman.2015.12.014](https://doi.org/10.1016/j.wasman.2015.12.014)] [Medline: [26739455](https://pubmed.ncbi.nlm.nih.gov/26739455/)]
7. Girotto F, Alibardi L, Cossu R. Food waste generation and industrial uses: a review. *Waste Manag* 2015 Nov;45:32-41. [doi: [10.1016/j.wasman.2015.06.008](https://doi.org/10.1016/j.wasman.2015.06.008)] [Medline: [26130171](https://pubmed.ncbi.nlm.nih.gov/26130171/)]
8. Tamisa J, Lužkov K, Jiang Y, van Loosdrecht MCM, Kleerebezem R. Enrichment of Plasticumulans acidivorans at pilot-scale for PHA production on industrial wastewater. *J Biotechnol* 2014 Dec 20;192 Pt A:161-169. [doi: [10.1016/j.jbiotec.2014.10.022](https://doi.org/10.1016/j.jbiotec.2014.10.022)] [Medline: [25456060](https://pubmed.ncbi.nlm.nih.gov/25456060/)]
9. Papa G, Pepè Sciarria T, Carrara A, Scaglia B, D'Imporzano G, Adani F. Implementing polyhydroxyalkanoates production to anaerobic digestion of organic fraction of municipal solid waste to diversify products and increase total energy recovery. *Bioresour Technol* 2020 Dec;318:124270. [doi: [10.1016/j.biortech.2020.124270](https://doi.org/10.1016/j.biortech.2020.124270)] [Medline: [33099102](https://pubmed.ncbi.nlm.nih.gov/33099102/)]
10. Jung S, Shetti NP, Reddy KR, et al. Synthesis of different biofuels from livestock waste materials and their potential as sustainable feedstocks – a review. *Energy Conversion Manage* 2021 May;236:114038. [doi: [10.1016/j.enconman.2021.114038](https://doi.org/10.1016/j.enconman.2021.114038)]
11. Pagliano G, Ventrino V, Panico A, Pepe O. Integrated systems for biopolymers and bioenergy production from organic waste and by-products: a review of microbial processes. *Biotechnol Biofuels* 2017 May 2;10:113. [doi: [10.1186/s13068-017-0802-4](https://doi.org/10.1186/s13068-017-0802-4)] [Medline: [28469708](https://pubmed.ncbi.nlm.nih.gov/28469708/)]
12. Sun J, Zhang L, Loh KC. Review and perspectives of enhanced volatile fatty acids production from acidogenic fermentation of lignocellulosic biomass wastes. *Bioresour Bioprocess* 2021 Aug 2;8(1):68. [doi: [10.1186/s40643-021-00420-3](https://doi.org/10.1186/s40643-021-00420-3)] [Medline: [38650255](https://pubmed.ncbi.nlm.nih.gov/38650255/)]
13. Sampath P, Brijesh, Reddy KR, et al. Biohydrogen production from organic waste – a review. *Chem Eng Technol* 2020 Jul;43(7):1240-1248. [doi: [10.1002/ceat.201900400](https://doi.org/10.1002/ceat.201900400)]
14. Gottardo M, Dosta J, Cavinato C, et al. Boosting butyrate and hydrogen production in acidogenic fermentation of food waste and sewage sludge mixture: a pilot scale demonstration. *J Cleaner Production* 2023 Jun 10;136919. [doi: [10.1016/j.jclepro.2023.136919](https://doi.org/10.1016/j.jclepro.2023.136919)]
15. Micolucci F, Gottardo M, Bolzonella D, Pavan P, Majone M, Valentino F. Pilot-scale multi-purposes approach for volatile fatty acid production, hydrogen and methane from an automatic controlled anaerobic process. *J Cleaner Production* 2020 Dec 20;277:124297. [doi: [10.1016/j.jclepro.2020.124297](https://doi.org/10.1016/j.jclepro.2020.124297)]
16. Anderottola G, Canziani R, Foladori P, Ragazzi M, Tatano F. Laboratory scale experimentation for RBCOD production from OFMSW for BNR systems: results and kinetics. *Environ Technol* 2000 Dec;21(12):1413-1419. [doi: [10.1080/09593332208618173](https://doi.org/10.1080/09593332208618173)]
17. Stoyanova E, Lundaa T, Bochmann G, Fuchs W. Overcoming the bottlenecks of anaerobic digestion of olive mill solid waste by two-stage fermentation. *Environ Technol* 2017 Feb;38(4):394-405. [doi: [10.1080/09593330.2016.1196736](https://doi.org/10.1080/09593330.2016.1196736)] [Medline: [27279450](https://pubmed.ncbi.nlm.nih.gov/27279450/)]

18. Sauer M, Porro D, Mattanovich D, Branduardi P. Microbial production of organic acids: expanding the markets. *Trends Biotechnol* 2008 Feb;26(2):100-108. [doi: [10.1016/j.tibtech.2007.11.006](https://doi.org/10.1016/j.tibtech.2007.11.006)] [Medline: [18191255](https://pubmed.ncbi.nlm.nih.gov/18191255/)]
19. Dahiya S, Sarkar O, Swamy YV, Venkata Mohan S. Acidogenic fermentation of food waste for volatile fatty acid production with co-generation of biohydrogen. *Bioresour Technol* 2015 Apr;182:103-113. [doi: [10.1016/j.biortech.2015.01.007](https://doi.org/10.1016/j.biortech.2015.01.007)] [Medline: [25682230](https://pubmed.ncbi.nlm.nih.gov/25682230/)]
20. Ma Y, Gu J, Liu Y. Evaluation of anaerobic digestion of food waste and waste activated sludge: soluble COD versus its chemical composition. *Sci Total Environ* 2018 Dec 1;643:21-27. [doi: [10.1016/j.scitotenv.2018.06.187](https://doi.org/10.1016/j.scitotenv.2018.06.187)] [Medline: [29935360](https://pubmed.ncbi.nlm.nih.gov/29935360/)]
21. Montalvo S, Vielma S, Borja R, Huilifñir C, Guerrero L. Increase in biogas production in anaerobic sludge digestion by combining aerobic hydrolysis and addition of metallic wastes. *Renewable Energy* 2018 Aug;123:541-548. [doi: [10.1016/j.renene.2018.02.004](https://doi.org/10.1016/j.renene.2018.02.004)]
22. Kumar M, Xiong X, Sun Y, et al. Critical review on biochar - supported catalysts for pollutant degradation and sustainable biorefinery. *Adv Sustainable Syst* 2020 Oct;4(10):1900149. [doi: [10.1002/adsu.201900149](https://doi.org/10.1002/adsu.201900149)]
23. Kumar M, Dutta S, You S, et al. A critical review on biochar for enhancing biogas production from anaerobic digestion of food waste and sludge. *J Clean Prod* 2021 Oct 10;305:127143. [doi: [10.1016/j.jclepro.2021.127143](https://doi.org/10.1016/j.jclepro.2021.127143)] [Medline: [36570877](https://pubmed.ncbi.nlm.nih.gov/36570877/)]
24. Inyang M, Gao B, Pullammanappallil P, Ding W, Zimmerman AR. Biochar from anaerobically digested sugarcane bagasse. *Bioresour Technol* 2010 Nov;101(22):8868-8872. [doi: [10.1016/j.biortech.2010.06.088](https://doi.org/10.1016/j.biortech.2010.06.088)] [Medline: [20634061](https://pubmed.ncbi.nlm.nih.gov/20634061/)]
25. Lipps WC, Braun-Howland EB, Baxter TE, editors. *Standard Methods for the Examination of Water and Wastewater*, 24th edition: American Public Health Association; 2022.
26. Cinar S, Cinar S, Kuchta K. Machine learning algorithms for temperature management in the anaerobic digestion process. *Fermentation* 2022 Jan 30;8(2):65. [doi: [10.3390/fermentation8020065](https://doi.org/10.3390/fermentation8020065)]
27. Borhany H. Supplementary documents for converting organic municipal solid waste into volatile fatty acids and biogas: experimental pilot and batch studies with statistical analysis. Zenodo. 2024 Jul 14. URL: <https://zenodo.org/records/12739504> [accessed 2025-01-23]
28. Mumme J, Srocke F, Heeg K, Werner M. Use of biochars in anaerobic digestion. *Bioresour Technol* 2014 Jul;164:189-197. [doi: [10.1016/j.biortech.2014.05.008](https://doi.org/10.1016/j.biortech.2014.05.008)] [Medline: [24859210](https://pubmed.ncbi.nlm.nih.gov/24859210/)]
29. Valentino F, Moretto G, Gottardo M, Pavan P, Bolzonella D, Majone M. Novel routes for urban bio-waste management: a combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production. *J Cleaner Production* 2019 May 20;220:368-375. [doi: [10.1016/j.jclepro.2019.02.102](https://doi.org/10.1016/j.jclepro.2019.02.102)]
30. Moretto G, Valentino F, Pavan P, Majone M, Bolzonella D. Optimization of urban waste fermentation for volatile fatty acids production. *Waste Manag* 2019 Jun 1;92:21-29. [doi: [10.1016/j.wasman.2019.05.010](https://doi.org/10.1016/j.wasman.2019.05.010)] [Medline: [31160023](https://pubmed.ncbi.nlm.nih.gov/31160023/)]
31. Valentino F, Gottardo M, Micolucci F, et al. Organic fraction of municipal solid waste recovery by conversion into added-value polyhydroxyalkanoates and biogas. *ACS Sustainable Chem Eng* 2018 Dec 3;6(12):16375-16385. [doi: [10.1021/acssuschemeng.8b03454](https://doi.org/10.1021/acssuschemeng.8b03454)]
32. Gottardo M, Micolucci F, Bolzonella D, Uellendahl H, Pavan P. Pilot scale fermentation coupled with anaerobic digestion of food waste - effect of dynamic digestate recirculation. *Renewable Energy* 2017 Dec;114:455-463. [doi: [10.1016/j.renene.2017.07.047](https://doi.org/10.1016/j.renene.2017.07.047)]
33. Estévez-Alonso Á, Pei R, van Loosdrecht MCM, Kleerebezem R, Werker A. Scaling-up microbial community-based polyhydroxyalkanoate production: status and challenges. *Bioresour Technol* 2021 May;327:124790. [doi: [10.1016/j.biortech.2021.124790](https://doi.org/10.1016/j.biortech.2021.124790)] [Medline: [33582521](https://pubmed.ncbi.nlm.nih.gov/33582521/)]
34. Morgan-Sagastume F, Hjort M, Cirne D, et al. Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. *Bioresour Technol* 2015 Apr;181:78-89. [doi: [10.1016/j.biortech.2015.01.046](https://doi.org/10.1016/j.biortech.2015.01.046)] [Medline: [25638407](https://pubmed.ncbi.nlm.nih.gov/25638407/)]
35. Valentino F, Morgan-Sagastume F, Campanari S, Villano M, Werker A, Majone M. Carbon recovery from wastewater through bioconversion into biodegradable polymers. *N Biotechnol* 2017 Jul 25;37(Pt A):9-23. [doi: [10.1016/j.nbt.2016.05.007](https://doi.org/10.1016/j.nbt.2016.05.007)] [Medline: [27288751](https://pubmed.ncbi.nlm.nih.gov/27288751/)]
36. Metcalf & Eddy, Inc, Tchobanoglous G, Stensel H, Tsuchihashi R, Burton F. *Wastewater Engineering: Treatment, Disposal, and Reuse*, 5th edition: McGraw-Hill Education; 2014.
37. Tamis J, Joosse BM, Loosdrecht MCMV, Kleerebezem R. High-rate volatile fatty acid (VFA) production by a granular sludge process at low pH. *Biotechnol Bioeng* 2015 Nov;112(11):2248-2255. [doi: [10.1002/bit.25640](https://doi.org/10.1002/bit.25640)] [Medline: [25950759](https://pubmed.ncbi.nlm.nih.gov/25950759/)]
38. Karki R, Chuenchart W, Surendra KC, Sung S, Raskin L, Khanal SK. Anaerobic co-digestion of various organic wastes: kinetic modeling and synergistic impact evaluation. *Bioresour Technol* 2022 Jan;343:126063. [doi: [10.1016/j.biortech.2021.126063](https://doi.org/10.1016/j.biortech.2021.126063)] [Medline: [34619321](https://pubmed.ncbi.nlm.nih.gov/34619321/)]
39. Moreno AD, Magdalena JA, Oliva JM, et al. Sequential bioethanol and methane production from municipal solid waste: an integrated biorefinery strategy towards cost-effectiveness. *Process Safety Environ Protection* 2021 Feb;146:424-431. [doi: [10.1016/j.psep.2020.09.022](https://doi.org/10.1016/j.psep.2020.09.022)]

Abbreviations

BMP: bio-methane potential

CH₄: methane
CO₂: carbon dioxide
COD: chemical oxygen demand
FS/IN: feedstock/inoculum
H₂: hydrogen molecule
HRT: hydraulic retention time
kPa: kilopascal
MJ: megajoules
MWh: megawatt-hour
N-NH₄⁺: ammonium
N₂: nitrogen molecule
NaOH: sodium hydroxide
O₂: oxygen molecule
OLR: organic loading rate
OMSW: organic municipal solid waste
P: phosphorous
P-PO₄³⁻: phosphate
PE: population equivalent
PHA: polyhydroxyalkanoate
rpm: rounds per minute
SCOD: soluble chemical oxygen demand
SGP: specific biogas production
SMP: specific methane production
TS: total solids
v/v: volumetric basis
v/v %: maximum volumetric methane content
VFA: volatile fatty acid
VS: volatile solids
WS: waste sludge
WWTP: wastewater treatment plant

Edited by T Leung; submitted 01.07.23; peer-reviewed by D Elsalamony, Anonymous; revised version received 08.07.24; accepted 12.07.24; published 04.02.25.

Please cite as:

Borhany H

Converting Organic Municipal Solid Waste Into Volatile Fatty Acids and Biogas: Experimental Pilot and Batch Studies With Statistical Analysis

JMIRx Med 2025;6:e50458

URL: <https://xmed.jmir.org/2025/1/e50458>

doi: [10.2196/50458](https://doi.org/10.2196/50458)

© Hojjat Borhany. Originally published in JMIRx Med (<https://med.jmirx.org>), 4.2.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Peer Review of “Safety and Efficacy of Chimeric Antigen Receptor T-Cell Therapy for Recurrent Glioblastoma: An Augmented Meta-Analysis of Phase 1 Clinical Trials (Preprint)”

Vanessa Fairhurst¹; Randa Salah Gomaa Mahmoud²; Toba Olatoye³; Sylvester Sakilay

¹PREreview, -, Portland, OR, United States

²Faculty of Human Medicine, Zagazig University, Zagazig, Egypt

³University of Ilorin, Ilorin, Nigeria

Related Article:

Companion article: <https://www.medrxiv.org/content/10.1101/2024.10.23.24316015v1>

(*JMIRx Med* 2025;6:e71293) doi:[10.2196/71293](https://doi.org/10.2196/71293)

KEYWORDS

CAR T-cell therapy; cancer; glioblastoma; brain tumor; meta-analysis; chimeric antigen receptor

This is a peer-review report for the preprint “Safety and Efficacy of Chimeric Antigen Receptor T-cell Therapy for Recurrent Glioblastoma: An Augmented Meta-Analysis of Phase 1 Clinical Trials.”

This review is the result of a virtual collaborative live review discussion organized and hosted by PREreview and JMIR Publications on Dec 12, 2024. The discussion was joined by 11 people: 3 facilitators, 1 member of the JMIR Publications team, and 7 live review participants including 3 who agreed to be named but did not assist in compiling the final review: Eudora Nwanaforo, Kelechi Elechi, and Murtala Haruna Bawa. The authors of this review have dedicated additional asynchronous time over the course of 2 weeks to help compose this final report using the notes from the live review. We thank all participants who contributed to the discussion and made it possible for us to provide feedback on this preprint.

Summary

The study [1] was designed to address the limitations of previous studies and evaluate the safety and efficacy of chimeric antigen receptor (CAR) T-cell therapy for recurrent glioblastoma. The results of this study are predictive rather than confirmatory. CAR T-cell therapy for glioblastoma was not predicted to significantly improve survival or achieve substantial complete responses. Stable disease rates were modest, while disease progression was notable. Adverse events, especially CAR T-cell therapy-related encephalopathy, raise safety concerns. Overall survival was 6.49 months in patients receiving CAR T-cell therapy after augmented analysis, and only 80% of patients exhibited this outcome. It was not statistically different from the median overall survival observed in patients with recurrent glioblastoma undergoing standard treatment, thereby indicating that CAR T-cell therapy, in its current form, does not offer substantially improved survival compared to standard treatments. Further trials and refinements are needed to enhance CAR T-cell therapy's effectiveness and safety in glioblastoma treatment.

An interesting fact is that a novel statistical technique (augmented meta-analyses) was used in this study. It was a combination of a cross-sectional (quantitative) and augmented meta-analysis (qualitative).

List of Major Concerns and Feedback

Methods

Augmented Meta-Analysis

- This section is limited in its description of the methodology used in the study. It would be helpful to include more information on the machine learning model or language model used to generate the extra cases.
- The title and aim specify that the study focuses on recurrent glioblastoma, but this specificity is not reflected in the inclusion criteria. It would be helpful to adjust the inclusion criteria to explicitly state that the study is targeting patients with recurrent glioblastoma. This will align the methodology with the aim as stated.
- The inclusion criteria do not specify that patients are in phase 1 clinical trials, where safety is a primary focus. Clearly state in the inclusion criteria that patients are part of phase 1 clinical trials. This will provide context for the study's focus on safety.
- There is no reference to the earlier use of augmented meta-analysis in cancer or medical research, nor is it explicitly stated if this is a new application. If augmented meta-analysis has been previously applied, cite relevant references. If this is its first application, explicitly state so and highlight its novelty.

Results

Literature Review and Risk of Bias Assessment Section

- It would be helpful to add the details of Figure 1 and Table 1 that explain the details of the cause of exclusion, the

results of the Newcastle Ottawa Scale, which study reached the high-quality level, etc.

Discussion

- It is important to add a comparison between the mean overall survival for patients with glioblastoma who underwent CAR T-cell therapy and the median overall survival observed in patients receiving the standard protocol for recurrent glioblastoma treatment to the Results section, as this comparison is mentioned in the first paragraph of the Discussion section.

Reproducibility of the Study

- The data presented in the study are beneficial for reproducibility except for the augmented meta-analysis, which is hindered by the lack of clear documentation on the large language model settings.
- The details of the augmented meta-analysis are not available. Provide access to the source code or methodological details for augmented meta-analysis, either as supplementary material or a public repository link. Transparency will strengthen the study's reproducibility.

List of Minor Concerns and Feedback

Concerns With Techniques/Analyses

- Abbreviations like “IL-13Ralpha-2,” “EGFRvIII,” “HER2,” and “HepHA2” are not identified in the Included Study Characteristics section. Expand the abbreviations and provide their full names (eg, “Interleukin-13 Receptor Subunit Alpha-2”) when first mentioned. This ensures clarity for readers not familiar with the terms.
- The last line of the large language model statement on page 16 does not explain how augmented meta-analysis was applied. Elaborate on how augmented meta-analysis was applied, especially in terms of methodology and integration with the study data.

Figures and Tables

- The screening section in Figure 1 is missing a rectangle to indicate the exclusion of 300 records. Update it using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart to include a rectangle that details the 300 excluded records and ensures the causes of exclusion are clearly stated.
- The reasons for exclusion are not detailed in the PRISMA flowchart. Follow PRISMA guidelines to specify the causes of exclusion, such as duplicates, irrelevance, or incomplete data, within the flowchart.
- Comments following Figure 1 are not in line with its instructions. Restructure the comments to follow the instructions and present the details of the research study accordingly.

Additional Comments

- No reference is provided for the trim-and-fill method mentioned in the augmented meta-analysis of overall survival (page 10). Cite a relevant source, such as [2] or another appropriate reference.
- The Cochrane Handbook (Part 2, Chapter 9) should be referenced in the Statistical Analysis section and its numbered reference cited in the text.
- References in the third paragraph of the Introduction mix meta-analyses and clinical trials without clear distinction. Rearrange and clarify the references while ensuring that references to meta-analyses and clinical trials are grouped and contextualized appropriately to avoid confusion.
- Repetition of the sentence “Egger’s test for publication bias could not be performed since the number of included studies in this outcome was less than ten” could be avoided by mentioning it once in the Methods section as the total number of the included studies is 8.
- In addition, the repetition of the sentence “The wide range of the 95% confidence interval was suggestive of data sparsity, so augmented meta-analysis was indicated before making conclusions” could be avoided by mentioning it once in the Augmented Meta-Analysis section of the Methods.

Acknowledgments

PREreview and JMIR Publications thank the authors of the preprint for posting their work openly for feedback. We also thank all participants of the live review call for their time and for engaging in the lively discussion that generated this review.

Conflicts of Interest

VF was a facilitator of this call and one of the organizers. No other competing interests were declared by the reviewers.

References

1. Azzam AY, Morsy MM, Azab MA, et al. Safety and efficacy of chimeric antigen receptor T-cell therapy for recurrent glioblastoma: an augmented meta-analysis of phase 1 clinical trials. medRxiv. Preprint posted online on Oct 24, 2024. [doi: [10.1101/2024.10.23.24316015](https://doi.org/10.1101/2024.10.23.24316015)]
2. Shi L, Lin L. The trim-and-fill method for publication bias: practical guidelines and recommendations based on a large database of meta-analyses. *Medicine (Baltimore)* 2019 Jun;98(23):e15987. [doi: [10.1097/MD.00000000000015987](https://doi.org/10.1097/MD.00000000000015987)] [Medline: [31169736](https://pubmed.ncbi.nlm.nih.gov/31169736/)]

Abbreviations

CAR: chimeric antigen receptor

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Edited by A Schwartz; submitted 14.01.25; this is a non-peer-reviewed article; accepted 14.01.25; published 24.01.25.

Please cite as:

Fairhurst V, Mahmoud RSG, Olatoye T, Sakilay S

Peer Review of "Safety and Efficacy of Chimeric Antigen Receptor T-Cell Therapy for Recurrent Glioblastoma: An Augmented Meta-Analysis of Phase 1 Clinical Trials (Preprint)"

JMIRx Med 2025;6:e71293

URL: <https://xmed.jmir.org/2025/1/e71293>

doi: [10.2196/71293](https://doi.org/10.2196/71293)

© Vanessa Fairhurst, Randa Salah Goma Mahmoud, Toba Olatoye, Sylvester Sakilay. Originally published in JMIRx Med (<https://med.jmirx.org>), 24.1.2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work, first published in JMIRx Med, is properly cited. The complete bibliographic information, a link to the original publication on <https://med.jmirx.org/>, as well as this copyright and license information must be included.

Publisher:
JMIR Publications
130 Queens Quay East.
Toronto, ON, M5A 3Y5
Phone: (+1) 416-583-2040
Email: support@jmir.org

<https://www.jmirpublications.com/>