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Peer Review of “Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study”

Anonymous

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Companion article: https://med.jmirx.org/2023/1/e50515

Companion article: https://med.jmirx.org/2023/1/e29587

(JMIRx Med 2023;4:e50501) doi:10.2196/50501

KEYWORDS

COVID-19; pandemic; SARS-CoV-2; seroprevalence; serology; epidemiology; Niger State; Nigeria; COVID-19 testing; social distancing

This is a peer-review report submitted for the paper “Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study.”

Review Round 1

General Comments

This paper, “Seroprevalence of COVID-19 in Niger State: A Pilot Cross-Sectional Study” by Majiya et al [1], is valuable and worthy of publication. The paper describes the seroprevalence of COVID-19 in Niger State. The COVID-19 asymptomatic rate in the state was 46.81%. The study also observed that the chances of infection are almost the same for both urban and rural dwellers. Of great interest is the finding that health care workers and those who had contact with persons who traveled out of Nigeria in the last 6 months are twice as likely to be at risk of being infected with the virus. The paper is relevant and contributes to the knowledge of the epidemiology of the virus. However, one primary concern is that the information about the virus from which inferences were made in this paper seems outdated. There is a need for an update. Also, the work appears to be underpowered in terms of sample size.

Specific Comments

1. The abstract is unusually extended; consider summarizing it, especially the results aspect.
2. There is a need for editing and restructuring some sentences.
3. Some long paragraphs have the same references; consider using other references as well.
4. Give a reference or definition for your sampling technique and probably describe how you achieved your sample size.
5. Avoid repeating the methodology in the Discussion session.
6. Add references to back up your inferences.
7. The authors should make inferences in light of observation and the literature; asymptomatic cases seem to foster community transmission. More so, isolation, quarantine, and lockdown, if need be, are some public health measures to halt transmission. I would instead advise that the authors make recommendations based on the data generated from the study.

Review Round 2

General Comments

This paper, “Seroprevalence of COVID-19 in Niger State: A Pilot Cross-Sectional Study,” is a credible addition to the body of knowledge about COVID-19 in Niger State and Nigeria as a whole. The Abstract has been refined, and the Discussion better articulated. The authors might want to consider reducing the Introduction to about one and a half pages, making it more concise.

Specific Comments

1. The authors should read through the paper to adjust for typographical errors.

Conflicts of Interest

None declared.

Reference

1. Majiya H, Aliyu-Paiko M, Balogu VT, Musa DA, Salihu IM, Kawu AA, et al. Seroprevalence of SARS-CoV-2 in Niger State: pilot cross-sectional study. JMIRx Med 2023;4:e29587. [doi: 10.2196/29587]

Edited by E Meinert; submitted 03.07.23; this is a non–peer-reviewed article;accepted 03.07.23; published 17.10.23.

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URL: https://xmed.jmir.org/2023/1/e50501
doi:10.2196/50501

Peer Review of "Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study"

Zied Hadrich

Department of Surgery, Faculty of Medicine of Tunis, Tunis, Tunisia

Related Articles:

Companion article: https://preprints.jmir.org/preprint/38852

Companion article: https://med.jmirx.org/2023/1/e53817

Companion article: https://med.jmirx.org/2023/1/e38852

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KEYWORDS

waist circumference; computed tomography; abdominal CT; mobile health; health apps; CT; CT scan; CT image; mobile app; app; application; waist; body; body mass; BMI; morbidity; mortality; clinical; tool; prototype; design; obesity; abdominal; usability; validity; medical

This is a peer-review report submitted for the paper "Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study."

Round 1 Review

This manuscript [1] is well written.

This paper presents an original idea to simplify patient care. It can be generalized to other specialties.

No specific comments.

Major Comment

This mobile app could be used for other measurements.

Conflicts of Interest

None declared.

Reference

1. Masmoudi A, Zouari A, Bouzid A, et al. Predicting waist circumference from a single computed tomography image using a mobile app (Measure It): development and evaluation study. JMIRx Med 2023;4:e38852. [doi: 10.2196/38852]

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Peer Review of "Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study"

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Related Articles:

Companion article: https://preprints.jmir.org/preprint/38852

Companion article: https://med.jmirx.org/2023/1/e53817

Companion article: https://med.jmirx.org/2023/1/e38852

(JMIRx Med 2023;4:e54012) doi:10.2196/54012

KEYWORDS

waist circumference; computed tomography; abdominal CT; mobile health; health apps; CT; CT scan; CT image; mobile app; app; application; waist; body; body mass; BMI; morbidity; mortality; clinical; tool; prototype; design; obesity; abdominal; usability; validity; medical

This is a peer-review report submitted for the paper "Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study."

Round 1 Review

General Comments

The authors created a mobile app that predicts waist circumference (WC) from computed tomography (CT) images [1]. After creating the app, the authors conducted a preliminary study involving 20 patients. The results showed that the developed app can predict WC from CT images with high accuracy. Though the paper showed some promising results, the authors still need to clarify a few important points. I hope the authors would be happy to clarify those points.

Specific Comments

Major Comments

- 1. What was the primary reason for selecting equation 1 as a reference method for WC calculation? Isn't it possible to calculate the exact circumference from the CT images using image-processing algorithms? Wouldn't it be more representative compared to the manual WC detection procedure?
- 2. Keeping the mobile app aside, how much different is this study compared to Ciudin et al [2]?
- 3. On page 3, please expand the discussion on "App Requirements." It is not evident what was meant by "app requirements" in this section.
- 4. How many images were taken from each CT slice? As the measurements for the waist parameters (a and b) were taken using a manual process, what kind of procedure was followed to ensure that the person-to-person variability remains low?
- 5. In Figures 3 and 4, there is a small dot around the top of the figures. Is this a data point? Additionally, proper x- and y-axis labels are missing. Please add appropriate units on the x- and y-axis.
- 6. In the Discussion section, it was claimed that "this is the first of a kind mobile app helping physicians to estimate WC." Do the authors think the physicians would be able to use apps such as [3] to assess WC?
- 7. In the Discussion section, it was stated that "Moreover, the simplicity of the app may reduce the time required for physicians to assess WC." How fast is the app compared to the manual approach?

Minor Comments

- 8. The authors stated that "WC cannot be physically assessed in patients with intellectual or motor disabilities" but did not provide any other details as to why it can't be assessed. The authors should discuss this in detail in the Introduction.
- 9. The sentence "However, for a radiologist, this method requires training and can be more or less time consuming" seems confusing. If possible, please restructure this sentence.
- 10. In equation 1, what is denoted by "p"?
- 11. Although the authors discussed in the Methods section how the measurements were taken just above the iliac crest and the CT images were taken from the last slice to ensure that those are not taken from different places, do the authors think that there could be some positional errors being introduced based on your approach?

- 12. On page 3, it was stated that there were further modifications to the app design. What kind of modifications were carried out? Did the authors discard the prior mobile app–based WC measurements (mWCs) after modifying the app?
- 13. Please try to make sure that periods and commas are being used appropriately. On page 4, one of the sentences was "The mean BMI was 26±4; 27,8±2,7 for women and 24.2±4,4 for men." For women, a comma was used as a decimal point. On the other hand, for men, a period was used as a decimal point.
- 14. In Table 1, what is the unit for "Confidence Interval"?
- 15. What kind of procedure was used to perform the diagnostic test to detect abdominal obesity? Please discuss this in the Methods section.

Round 2 Review

Thanks to the authors for providing a detailed revised version and comments.

If the authors can clear up a few more confusions, then it would be great.

Minor Comments

- 1. The authors stated that the app has an accuracy of 83% when using the mWC to detect abdominal obesity. Is it sufficient compared to the conventional approaches? Just a simple comparison/comment would suffice.
- 2. Related to comment 11 of the round 1 review, how much impact can positional errors have in abdominal obesity classification? This can be explained or discussed in the Discussion.
- 3. The Figure 3 regression shows that one of the app measurements was (WC_App=120) when the true value should have been around ~65 (standing app difference=55). But in Figure 4, that point seems to be missing (mean of standing + app ~92, so the difference ~55 should be around ~92 in the Bland-Altman plot). Can you please clarify this? If my calculations are wrong, I am extremely sorry about that.

Overall, the authors discussed all of the comments raised by the reviewer.

Conflicts of Interest

None declared.

References

1. Masmoudi A, Zouari A, Bouzid A, et al. Predicting waist circumference from a single computed tomography image using a mobile app (Measure It): development and evaluation study. JMIRx Med 2023;4:e38852. [doi: 10.2196/38852]
2. Ciudin A, Salvador R, Budoy A, et al. Measurement of waist circumference for retrospective studies - prospective validation of use of CT images to assess abdominal circumference. Endocrinol Nutr 2014 Mar;61(3):147-152. [doi: 10.1016/j.endonu.2013.10.004] [Medline: 24342428]
3. 3DLOOK. URL: https://3dlook.ai/ [accessed 2023-11-16]

Abbreviations

CT: computed tomography
mWC: mobile app–based waist circumference measurement
WC: waist circumference

Edited by E Meinert; submitted 26.10.23; this is a non–peer-reviewed article;accepted 26.10.23; published 12.12.23.

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Peer Review of “Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study”

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Related Articles:

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Companion article: https://med.jmirx.org/2023/1/e38852

(JMIRx Med 2023;4:e54045) doi:10.2196/54045

KEYWORDS

waist circumference; computed tomography; abdominal CT; mobile health; health apps; CT; CT scan; CT image; mobile app; app; application; waist; body; body mass; BMI; morbidity; mortality; clinical; tool; prototype; design; obesity; abdominal; usability; validity; medical

T*his is a peer-review report submitted for the paper “Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study.”*

Round 1 Review

General Comments

This paper [1] describes the results of using an author-created app to determine the waist circumference (WC) of patients in both retrospective and anticipatory circumstances. Although the manuscript makes a sound plausibility argument for the use of a smartphone app to determine WC from an existing computed tomography (CT) scan, it offers little rationale for using a pretreatment CT scan in preference to a conventional measurement with a tape measure or equivalent, especially as that measurement modality is taken as the comparison standard.

Specific Comments

Major Comments

- 1. The authors admit that their conclusion is based on a very small sample of patients. In recommending further studies, the authors should offer specific guidelines, especially with respect to establishing the precision of each measurement modality. The material speaks only to the accuracy, but the plots in Figures 4 and 5 display some significant outliers.
- 2. The manuscript should present quantitative evidence of the degree to which an ellipse is an accurate representation of the body shape at the waist.
- 3. The comment that this technique is important to less developed countries is puzzling considering the simplicity and extremely low cost of obtaining tape measure data prior to treatment.
- 4. The authors claim that the WC cannot be assessed in patients with intellectual or motor disabilities. Why? That hardly seems like a satisfactory reason to subject the patient to the radiation dose of a CT scan.
- 5. Were the statistics presented controlled for variations in BMI and the effect of BMI on the body shape at the waist?

Minor Comments

- 6. The WC is a characteristic of the patient. It is not a parameter. The text needs careful proofreading.
- 7. Unless needed for other clinical reasons, CT scans are not of such limited cost.
- 8. In the discussion of statistics, use consistent numbers for significant figures.
- 9. In Figures 3 and 4, add the dimensions in the captions.
- 10. In the Discussion, why aren’t tape measurements of WC routinely made if this characteristic is so important in treatment planning as the authors claim?
- 11. The comment “Also, for a radiologist, conventional CT scan method requires training and can be more or less time consuming” is puzzling in light of the ease of using a tape measure in pretreatment planning.
- 12. “Since smartphones are commonly available even in low- and middle-income countries”—CT scanners are not so prevalent. This is a pointless polemic.
- 13. In the references, please give PubMed numbers whenever they are available. For websites, give the last date accessed.
- 14. The suggestion of using AI in an upgraded app is hardly compelling without a clear explanation of why the ellipse fitting is of questionable validity.

Conflicts of Interest

None declared.

Reference

1. Masmoudi A, Zouari A, Bouzid A, et al. Predicting waist circumference from a single computed tomography image using a mobile app (Measure It): development and evaluation study. JMIRx Med 2023;4:e38852. [doi: 10.2196/38852]

Abbreviations

CT: computed tomography
WC: waist circumference

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Please cite as:
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Peer Review of “Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study”

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Biostatistics Centre, University of Otago, Dunedin, New Zealand

Related Articles:

Companion article: https://www.medrxiv.org/content/10.1101/2020.08.04.20168112v1

Companion article: https://med.jmirx.org/2023/1/e50515

Companion article: https://med.jmirx.org/2023/1/e29587

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KEYWORDS

COVID-19; pandemic; SARS-CoV-2; seroprevalence; serology; epidemiology; Niger State; Nigeria; COVID-19 testing; social distancing

This is a peer-review report submitted for the paper “Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study.”

Round 1 Review

This is a pilot study [1] to determine the COVID-19 seroprevalence, patterns, dynamics, and risk factors in Niger State, Nigeria. The study design is a cross-sectional survey using clustered, stratified random sampling over 5 days; the prevalence was measured by detecting antibodies.

Major point: the study design uses clustered, stratified random sampling. The authors haven’t described the clusters or stratification. However, I understand this as study participants were allowed to have different, but known, probabilities of being selected for the sample. This is different to study designs where participants are selected with equal probability. However, none of the analyses presented in the manuscript accounted for this different probability of selection; all the analyses have assumed an equal probability of selection. This is a fundamental mistake of the analysis. This invalidates all the results presented in the manuscript. The term “sampling weights” is not used at all.

The aims include determining the risk factors and dynamics of COVID-19. Not sure if the authors measured the dynamic of COVID-19 at all. Also, they need to say what is meant by risk factors because they haven’t measured it if a risk factor means a causative risk factor.

For the above reasons, it is unnecessary to review this manuscript further. However, some of the points I have already noticed are listed below if the authors would like to consider them.

- The justification for this pilot study is unclear. Specifically, what will be the full study that corresponds to this pilot? Since the COVID-19 situation changes rapidly, can the lessons from this study be used for designing a full study at a later stage?
- Some of the people sampled have not consented. How do they fill those gaps? Did they sample someone else in those places? What was the response rate as a measure of sampling bias in estimating prevalence?
- The inclusion and exclusion criteria are not given. The presented results are simple percentages from participants.
- The stratification is by place of residence (2 groups), gender (2 groups), occupation (unknown number of groups), and age (unknown number of groups). Therefore the number of strata should be large, although unknown to me. I wonder what could be the justification of these strata that must have resulted in a very small number of people per strata given the total sample size of 185.
- There are multiple places that require references (eg, second paragraph under section 2.4).
- Not sure what the value is of lots of bar graphs. Almost all of the information in those graphs is already in the text.
- The text needs revising in some places. For example, the first 1.5 paragraphs under section 3.2 do not belong in the Results section. Two of the subfigures in Figure 3 have been cited but mixed up in the second paragraph of that section.
- Have they considered the incubation period needed to develop antibodies when interpreting the calculated percentages as prevalence?
- Authors have determined the sensitivity and specificity as 100% for test kits; this was using the results from 15 individuals. I am skeptical to accept that in the absence of CIs.

Conflicts of Interest

None declared.

Reference

1. Majiya H, Aliyu-Paiko M, Balogu VT, Musa DA, Salihu IM, Kawu AA, et al. Seroprevalence of SARS-CoV-2 in Niger State: pilot cross-sectional study. JMIRx Med 2023;4:e29587. [doi: 10.2196/29587]

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Peer Review of “Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study”

Nadège Bourgeois-Nicolaos[1,2], PhD, PharmD

1

2

Related Articles:

Companion article: https://www.medrxiv.org/content/10.1101/2020.08.04.20168112v1

Companion article: https://med.jmirx.org/2023/1/e50515

Companion article: https://med.jmirx.org/2023/1/e29587

(JMIRx Med 2023;4:e50391) doi:10.2196/50391

KEYWORDS

COVID-19; pandemic; SARS-CoV-2; seroprevalence; serology; epidemiology; Niger State; Nigeria; COVID-19 testing; social distancing

This is a peer-review report submitted for the paper “Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study.”

Review Round 1

General Comments

This article is a pilot study [1] that was conducted to determine the prevalence, patterns, and dynamics of COVID-19 and the risk factors for contracting the disease in Niger State from June 26 to 30, 2020.

This study is a cross-sectional study and uses a clustered, stratified random sampling method. Only 185 participants were included in the study. The sample size is small.

The seroprevalence of COVID-19 was found to be 25.4% and 2.16% for the positive IgG and IgM, respectively. These seroprevalence results mean that herd immunity to COVID-19 has yet to be achieved, and the population is still susceptible to more infection and transmission of the virus.

Specific Comments

Major Comments

1. Samples were taken randomly from 185 participants for COVID-19 IgG and IgM rapid tests and questionnaires. Information on the number of patients included in the different sampling points is missing. Have serology results been confirmed by other techniques?
2. The results are expressed as a percentage; it would be interesting to have the data on the number of samples or the number of patients.How many participants tested positive for only IgG and for both IgG and IgM?

1. Bibliographic references are not formatted in the correct format.

Minor Comments

1. Page 1: explain “NCDC”
2. Page 5: italicize *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Treponema pallidum*
3. Page 9: add percent majority (61.62%)
4. Page 11: explain “ATM”
5. Page 14: replace igM with IgG, “while the Kit detecting only IgM means that...”
6. Page 19: explain “PPE”

Review Round 2

General Comments

This paper describes the seroprevalence of COVID-19 in Niger State. This is a pilot study.

Despite the authors’ efforts to respond specifically to comments, some points are still missing.

Specific Comments

Major Comments

1. Please include more quantitative results in the abstract (odds ratio with CIs).
2. The relative results (percentage) are not well presented. Mostly, if n is less than 100, do not use decimal points in your percentages. We need to review the data in Table 2.
3. The 95% CIs for the odds ratios are missing in Table 2.
4. The SARS-CoV-2 script must be homogenized throughout the manuscript.
5. The meaning of the a is missing in Table 2.
6. Almost all the information in Table 2 is already in the text.

Minor Comments

1. Page 3: replace “COVI-19” with “COVID-19” and remove “Coronavirus disease 2019”
2. The SARS-CoV-2 script must be homogenized throughout the manuscript
3. How were the kits validated by polymerase chain reaction?
4. Page 17: explain “ATMs” in the paper

Conflicts of Interest

None declared.

Reference

1. Majiya H, Aliyu-Paiko M, Balogu VT, Musa DA, Salihu IM, Kawu AA, et al. Seroprevalence of SARS-CoV-2 in Niger State: pilot cross-sectional study. JMIRx Med 2023;4:e29587. [doi: 10.2196/29587]

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Peer-Review Report

Peer Review of "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years"

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Related Articles:

Companion article: http://preprints.jmir.org/preprint/45220

Companion article: https://med.jmirx.org/2023/1/e45280/

Companion article: https://med.jmirx.org/2023/1/e45220/

(JMIRx Med 2023;4:e45278) doi:10.2196/45278

KEYWORDS

aging; angiotensin converting enzyme; lymphocytes; immunosenescence; inflammaging

This is a peer-review report submitted for the paper "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years." [1]

Round 1 Review

General Comments

The study is interesting, and the title promises for me more than the manuscript finally contains. The background, question, and the aim are relevant as explained in the Introduction.

The major concerns the small size of the material (6 subjects), the small age difference (64-67 years), and the lack of younger controls.

Specific Comments

Title: ACE seems better than ACE1; or, does the sophisticated, elegant method include both ACEs? The same should be explained and taken into consideration throughout the text.

Introduction: in the last chapter, the author should explain in more detail how Pawelec et al [2], Alves et al [3], Alves and Bueno [4], and Bueno et al [5] suggest that "ACE1 plays an important role in the aging process." Does "ACE1 plays" mean, that ACE1 is somehow regulating the aging process or are ACE1 levels changed with age?

Methods: The N value of the subjects should be mentioned here, as well the relation of females and males. Do the authors really regard 64-67 years "older age" nowadays? The study lacks younger controls. Why were the initial assays done many years after the collection of blood samples? Are the samples still useable and not destroyed? Did the subjects have some diseases or were taking drugs because they possibly were from a hospital sample bank? Provide the companies' details.

Results: "Table 1 shows that older adults….." The comparison between the present data and historical studies belongs to the Discussion. Also, provide individual ages and genders of the subjects in Table 1. What do *P* values mean here—what is being compared, or are interindividual differences being highlighted in the particular variables? This should be explained. The numbering of tables and the text seems confusing to me. Only 3 tables, but in the text, 4 are mentioned. Table 4 does not exist. It would be good to have a list of abbreviations used in the description of the cell types for an unfamiliar reader.

Discussion: A major part of the discussion deals with previous publications and not meaning or clinical significance of the present findings and comparison between the present and earlier studies. In those previous studies, ACE2 has also has been reported; why is it not studied here? In the limitations paragraph, the authors fairly mention the real problem—the small sample size, and I would like to add a lack of younger subjects. The point regarding the COVID-19 pandemic, seemingly worth mentioning, is too far from this study and unnecessary. Linguistic checking would improve the manuscript.

In summary, I recommend the acceptance of the manuscript for publication after the authors carefully rethink the message of the Results and correct per the minor comments. I hope that in the future, possible age -related correlations to old age of up to >80 years would be possible.

Decision

Verified with reservations: The content is scientifically sound but has shortcomings that could be improved by further studies and minor revisions.

Decision Changed

Verified manuscript: The content is scientifically sound, and only minor amendments (if any) are suggested.

Round 2 Review

I read with pleasure the very detailed answers to my comments. I very warmly recommend the acceptance of this manuscript for publication without any further notes.

Decision Changed

Verified manuscript: the content is scientifically sound, and only minor amendments (if any) are suggested.

Conflicts of Interest

None declared.

Editorial Notice

This paper was peer-reviewed by the Plan P Hashtag Community partner #PeerRef.

References

1. Bueno V, Destro PH, Teixeira D, Frasca D. Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years. JMIRx Med 2023 Jan;4:e45220 [FREE Full text]
2. Pawelec G, Picard E, Bueno V, Verschoor CP, Ostrand-Rosenberg S. MDSCs, ageing and inflammageing. Cell Immunol 2021 Apr;362:104297. [doi: 10.1016/j.cellimm.2021.104297] [Medline: 33550187]
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Peer-Review Report

Peer Review of "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years"

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Related Articles:

Companion article: http://preprints.jmir.org/preprint/45220

Companion article: https://med.jmirx.org/2023/1/e45280/

Companion article: https://med.jmirx.org/2023/1/e45220/

(JMIRx Med 2023;4:e45279) doi:10.2196/45279

KEYWORDS

aging; angiotensin converting enzyme; lymphocytes; immunosenescence; inflammaging

This is a peer-review report submitted for the paper "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years." [1]

Round 1 Review

General Comments

The paper is essentially anecdotal because it studies the cells of 6 subjects without any comparison with other age groups. There is also a serious limitation because beyond the age and sex, there is no information on the donors (how and why they were recruited, what drugs they took, etc). To infer that chronological and biological ages do not match is inappropriate in the absence of the above information.

However, the paper is of some interest because there are few studies on the topic.

Specific Comments

Essential revisions that are required to verify the manuscript

Although we do not have data on donors, placing an age and gender column in all tables adds a minimum of useful information for the reader.

Inflammaging means low grade of inflammation. The CRP value of 23.1 suggests acute inflammation (also because albumin has high values, while in chronic inflammation its values decrease). Therefore the averages do not have to take this subject into account.

Other suggestions to improve the manuscript

The authors write that their findings suggest that ACE1 could play a role in several processes linked to aging including the generation and activation of autoimmune cells, due to the experimental evidence that inhibitors of ACE suppress the autoimmune process in a number of autoimmune diseases such as EAE, arthritis, autoimmune myocarditis [2]. They do not appear to have these findings in their paper. So, they need to change the sentence.

Decision

Requires revisions: The manuscript contains objective errors or fundamental flaws that must be addressed and major revisions are suggested.

Decision Changed

Verified manuscript: The content is scientifically sound, only minor amendments (if any) are suggested.

Round 2 Review

Decision Changed

Verified manuscript: The content is scientifically sound, and only minor amendments (if any) are suggested.

Conflicts of Interest

None declared.

Editorial Notice

This paper was peer-reviewed by the Plan P Hashtag Community partner #PeerRef.

References

1. Bueno V, Destro PH, Teixeira D, Frasca D. Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years. JMIRx Med 2023 Jan;4:e45220 [FREE Full text]
2. Platten M, Youssef S, Hur EM, Ho PP, Han MH, Lanz TV, et al. Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1- and TH17-mediated autoimmunity. Proc Natl Acad Sci U S A 2009 Sep 01;106(35):14948-14953 [FREE Full text] [doi: 10.1073/pnas.0903958106] [Medline: 19706421]

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Caruso C
Peer Review of "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years"
JMIRx Med 2023;4:e45279
URL: https://med.jmirx.org/2023/1/e45279
doi:10.2196/45279
PMID:

Peer-Review Report

Peer Review of "Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review"

Dacre Knight[1], MD

Mayo Clinic, Jacksonville, FL, United States

Related Articles:

Companion article: https://preprints.jmir.org/preprint/43880

Companion article: https://med.jmirx.org/2023/1/e43880/

(JMIRx Med 2023;4:e45304) doi:10.2196/45304

KEYWORDS

COVID-19; long COVID; post–COVID-19 condition; postacute sequelae of COVID-19; PASC; psychiatry; epidemiology; evidence-based medicine

This is a peer-review report submitted for the paper "Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review."

Round 1 Review

Serious Concerns

Do you have any serious concerns about the manuscript such as fraud, plagiarism, unethical or unsafe practices?

No.

Have authors' provided the necessary ethics approval (from authors' institution or an ethics committee)?

Not applicable.

Language Quality

How would you rate the English language quality?

High quality.

Validity and Reproducibility

Is the reasons for conducting the study and its objectives clearly explained?

Yes.

Is the study design appropriate?

Yes.

Are sufficient details provided so that the method can be replicated?

Yes.

Are datasets available so that others could use them?

Not applicable.

Suggestions

Based on your answers in section 3 how could the author improve the protocol?

There is a more specific definition of PASC that should be included (with a reference). There is a need to list specific medical databases to search and not just mention "various" [1]. PECO criteria need to be listed and not only implied that they will be used.

Do you have any other suggestions, feedback, or comments for the Author?

The GRADE approach will be useful, as is mentioned along with a narrative synthesis if needed. Strengths and limitations seem accurate and are good to list.

Decision

Verified with reservations: The content is scientifically sound but has shortcomings that could be improved by further studies and minor revisions.

Conflicts of Interest

None declared.

Editorial Notice

This paper was peer-reviewed by the Plan P Hashtag Community partner #PeerRef.

Reference

1. Effiong A. Postacute sequelae of COVID-19 and adverse psychiatric outcomes: protocol for an etiology and risk systematic review. JMIRx Med 2023;4(1):e43880 [FREE Full text]

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Knight D
Peer Review of "Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review"
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Peer-Review Report

Peer Review of "Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review"

Yin Qianlan[1], MD

Navy Medical University, Shanghai, China

Related Articles:

Companion article: https://preprints.jmir.org/preprint/43880

Companion article: https://med.jmirx.org/2023/1/e43880/

(JMIRx Med 2023;4:e45306) doi:10.2196/45306

KEYWORDS

COVID-19; long COVID; post–COVID-19 condition; postacute sequelae of COVID-19; PASC; psychiatry; epidemiology; evidence-based medicine

This is a peer-review report submitted for the paper "Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review."

Round 1 Review

Serious Concerns

Do you have any serious concerns about the manuscript such as fraud, plagiarism, unethical or unsafe practices?

No.

Have authors' provided the necessary ethics approval (from authors' institution or an ethics committee)?

Yes.

Language Quality

How would you rate the English language quality?

High quality.

Validity and Reproducibility

Is the reasons for conducting the study and its objectives clearly explained?

No.

Is the study design appropriate?

Yes.

Are sufficient details provided so that the method can be replicated?

Yes.

Are datasets available so that others could use them?

Not applicable.

Suggestions

Based on your answers in section 3 how could the author improve the protocol?

As an important part of a review is the declaration of the purpose of the protocol [1], the Introduction section should be the core of the article. However, after reading the beginning of the paper, I realized the seriousness of COVID-19, but I could not see the key point of the research. There are a lot of data to emphasize the worse outcomes, but I do not know how these data contributed to the relationship between the major topic of postacute sequelae of COVID-19 and adverse psychiatric outcomes; for example, the narrative on the effect of therapies. Hence, a more organized structure for the Introduction section with more conciseness would be easier for the readers.

Decision

Requires revisions: The manuscript contains objective errors or fundamental flaws that must be addressed and major revisions are suggested.

Conflicts of Interest

None declared.

Editorial Notice

This paper was peer-reviewed by the Plan P Hashtag Community partner #PeerRef.

Reference

1. Effiong A. Postacute sequelae of COVID-19 and adverse psychiatric outcomes: protocol for an etiology and risk systematic review. JMIRx Med 2023;4(1):e43880 [FREE Full text]

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Peer-Review Report

Peer Review for " Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review"

Daniel Griffin[1], MD, PhD

Columbia University, New York City, NY, United States

Related Articles:

Companion article: https://preprints.jmir.org/preprint/43880

Companion article: https://med.jmirx.org/2023/1/e43880/

(JMIRx Med 2023;4:e45308) doi:10.2196/45308

KEYWORDS

COVID-19; long COVID; post–COVID-19 condition; postacute sequelae of COVID-19; PASC; psychiatry; epidemiology; evidence-based medicine

This is a peer-review report submitted for the paper "Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review."

Round 2 Review

General Comments

The authors lay out a reasonable protocol for this type of investigation [1].

Serious Concerns

Do you have any serious concerns about the manuscript such as fraud, plagiarism, unethical or unsafe practices?

No.

Have authors' provided the necessary ethics approval (from authors' institution or an ethics committee)?

Yes.

Language Quality

How would you rate the English language quality?

High quality.

Validity and Reproducibility

Is the reasons for conducting the study and its objectives clearly explained?

Yes.

Is the study design appropriate?

Yes.

Are sufficient details provided so that the method can be replicated?

Yes.

Are datasets available so that others could use them?

Not applicable.

Suggestions

Based on your answers in section 3 how could the author improve the protocol?

It is appropriate as it is.

Do you have any other feedback or comments for the Author?

The authors lay out a reasonable protocol for this type of investigation that is based on a fairly standard approach with the standard GRADE grading approach.

Decision

Verified manuscript: The content is scientifically sound, and only minor amendments (if any) are suggested.

Conflicts of Interest

None declared.

Editorial Notice

This paper was peer-reviewed by the Plan P Hashtag Community partner #PeerRef.

Reference

1. Effiong A. Postacute sequelae of COVID-19 and adverse psychiatric outcomes: protocol for an etiology and risk systematic review. JMIRx Med 2023;4(1):e43880 [FREE Full text]

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Griffin D
Peer Review for " Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review"
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Peer-Review Report

Peer Review of “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”

Ramesh Poluru[1], PhD

The INCLEN Trust International, New Delhi, India

Related Articles:

Companion article: https://preprints.jmir.org/preprint/34598

Companion article: https://med.jmirx.org/2023/1/e46944/

Companion article: https://med.jmirx.org/2023/1/e34598/

(JMIRx Med 2023;4:e46906) doi:10.2196/46906

KEYWORDS

COVID-19; infection; pandemic; vaccine; vaccination; epidemiology; transmissibility; health care; hospital admission; COVID-19 variants; SARS-CoV-2

This is a peer-review report submitted for the paper “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study.”

Round 1 Review

The manuscript [1] attempts to investigate the impact of SARS-CoV-2 lineages in South African COVID-19 epidemiology. I would like to congratulate the authors on this useful attempt. The manuscript is well written, and the subject addressed in this manuscript is worth investigating; however, the manuscript partly failed to present a clear picture of its analytical methodology and presentation of results. The following are some minor concerns for consideration. I suggest that the authors (a) extend the study to include the recent Omicron variant, (b) present results with complete models, (c) avoid excessive references (~71).

In conclusion, the subject addressed in this manuscript is worth investigating and acceptable after taking into account the abovementioned minor issues.

Conflicts of Interest

None declared.

Reference

1. Mabuka T, Naidoo N, Ncube N, Yiga T, Ross M, Kurehwa K, et al. The impact of SARS-CoV-2 lineages (variants) and COVID-19 vaccination on the COVID-19 epidemic in South Africa: regression study. JMIRx Med 2023;4:e34598 [FREE Full text]

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Poluru R
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Peer-Review Report

Peer Review of “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”

Anonymous

Related Articles:

Companion article: https://preprints.jmir.org/preprint/34598

Companion article: https://med.jmirx.org/2023/1/e46944/

Companion article: https://med.jmirx.org/2023/1/e34598/

(JMIRx Med 2023;4:e46908) doi:10.2196/46908

KEYWORDS

COVID-19; infection; pandemic; vaccine; vaccination; epidemiology; transmissibility; health care; hospital admission; COVID-19 variants; SARS-CoV-2

This is a peer-review report submitted for the paper “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study.”

Round 1 Review

General Comments

This paper [1] discusses the impact of the SARS-CoV-2 lineage in the South African COVID-19 epidemiology because it is important to investigate the evolution of distinct SARS-CoV-2 lineage that dominates among three epidemic waves in South Africa. The authors begin by recalling the background of the COVID-19 global pandemic and introducing the SARS-CoV-2 lineage and its variants. In section 2, their methodology is introduced. The data were obtained from public sources. Descriptive statistics, paired sample *t* test, and regression analysis with new variables such as active cases, deaths, and daily patient discharge are provided. The authors interpret the results of statistical analyses and discuss their findings from the data in section 3. However, the manuscript should be polished. Here are some comments.

Specific Comments

Major Comments

1. Throughout the manuscript, the notation of numbers is not consistent. For example, in the middle of the second paragraph in section 1, Introduction, “The genome of SARS-CoV-2 is a single positive-stranded RNA approximately 29 903 bases (nucleotides) pairs in length 9 [2-5].” It looks like a space between numbers indicates a digit of a thousand, and a comma is omitted. However, in the middle of the paragraph in section 2.2.1., “Table 2 shows that the mean COVID-19 daily tests in the first, second and third South African COVID-19 epidemic wave period were 20 575±14 062, 31 046±14 115 and 46 822±18 460 respectively.” A space between numbers indicates a decimal point, not a comma.

2. Sections 2 and 3 are extremely difficult to read because they are too lengthy, although subsections indicate each statistical analysis that was performed. I believe that the authors do not need to provide outputs copied from SPSS directly. Are all columns in each table meaningful? Should readers know both standard deviation and variance for each statistic, for example? I strongly suggest that the authors get rid of unnecessary columns in each table and move unnecessary tables from sections 2 and 3 to the appendix.

3. I believe that the *P* values in the manuscript do not need to be specific. For example, Table 3 displays Pearson and Spearman correlation coefficients and *P* values. Many people may not understand what 9.94E-79 means. It can be simplified to “<0.001” or 0.

Minor Comments

4. The font style and size are not consistent throughout the manuscript.

Round 2 Review

General Comments

The authors have tried to improve the quality of the manuscript. However, the manuscript still needs substantial improvement. Please see my comments.

Specific Comments

Major Comments

1. This issue has not been resolved. The authors said that the space between numbers indicates a digit of a thousand. However, according to JMIR house style and editorial guidelines, numbers greater than 999 have a comma to separate thousands, millions, etc. Please see [6] and update the style of numbers throughout the manuscript.

2. The authors have reduced unnecessary columns. However, the JMIR production team suggests no more than 5 tables per manuscript. There are still unnecessary tables in the manuscript, that do not provide meaningful information and are just the same outputs of SPSS. What is the purpose of including so many tables without interpretation? Should Table 1 really be placed in the main manuscript? Why? Please see [7].

3. The authors have updated the representation of *P* values according to the suggestion of the editorial director [8].

4. The font style is still not consistent throughout the manuscript. Please revise the font style.

5. The Introduction in the manuscript is too long. I would suggest reducing the Introduction in the manuscript.

6. There are 13 equations in the manuscript. I believe that the authors can reduce the number of equations in the manuscript by combining similar equations. Listing all equations is unnecessary. Also, reference numbers for equations could be a number in the parenthesis such as (1) instead of Equation 1.

7. Detailed information about the paired test (what pairs to what) will be placed in the footnote in the corresponding table or figure.

8. Why do the authors think that the following text or Table 3 is needed in the manuscript?

"Table 3 shows that the Pearson (Spearman) Correlation Coefficients between COVID-19 daily tests (Independent Variable) and cases (Dependent Variable) in the first, second, third and fourth COVID-19 epidemic wave in South Africa were 0.910 (0.955), 0.877 (0.751), 0.893 (0.847) and 0.854 (0.812) respectively."

This text and Table 3 are the same information.

9. What is the reason to provide Pearson correlation and Spearman rho together? Do the authors want to show a linear relationship or an ordinal relationship?

Minor Comments

10. The footnotes in Tables 3 and 4 are redundant. Where are the superscripts a, b, or c in the tables?

11. There is an inconsistent number of digits in all tables in the manuscript.

12. From Tables 1 to 16, why do the authors think that the minimum and maximum provide meaningful information in 2?

13. Please use "95% confidence interval" instead of "95 % confidence interval."

Round 3 Review

General Comments

The authors have improved the manuscript's quality compared to the previous version. However, I would assume that the quality could be improved more if the authors addressed the following comments.

Major Comments

1. In "Covariance and Regression of South African Epidemiological Data," the authors stated that the 2-tailed Pearson correlation above 0.850 with $P<.001$ was considered as having a high degree of linearity. Pearson correlation coefficient has a value between –1 and 1. A negative value (eg, –0.850) could also be considered as a strong negative relationship between two variables. Was a negative relationship included in the determination of linearity?

2. In "Normalisation and Paired T-tests on South African Epidemiological Data," the authors considered only 7 pairs among 5 periods. Normalized parameter 2 and 4, normalized parameter 2 and 5, and normalized parameter 3 and 5 were not included in pairing. Was there a specific reason to exclude these three pairs in the paired *t* test?

3. In the Discussion, the authors stated that the Pfizer-BioNTech (Comirnaty) and the Johnson & Johnson/Janssen COVID-19 vaccines have shown high efficacy against severe COVID-19 at 85% and 88.9%, respectively. However, two terms, vaccine efficacy and effectiveness, are used in different settings. According to [9], Pfizer demonstrated their COVID-19 vaccine efficacy based on randomized controlled trials. However, Johnson & Johnson did not show their COVID-19 vaccine efficacy according to [10]. Instead, Johnson & Johnson demonstrated their COVID-19 vaccine effectiveness based on observational studies, which is in a real-world setting. Could you please clarify this? (Please see [11].)

Minor Comments

4. The authors did not explain what the special characters after SARS-CoV-2 variants mean (eg, BA.4# or BA.2.75***). Could you please provide details on what the special characters after SARS-CoV-2 variants indicate?

5. The authors used unnecessary abbreviations throughout the manuscript. Could you please review the manuscript and remove some unnecessary abbreviations that are not used in a section of the manuscript?

Round 4 Review

Specific Comments

Major Comments

1. It is difficult to understand what Tables 2 and 3 show. Table 3 provides the mean difference between two daily positive COVID-19 tests in a percentage. If we look at the paired differences mean of pair 5 (daily positive COVID-19 test 2 – daily positive COVID-19 test 3), the difference is –1.20. However, the mean of the daily positive COVID-19 test 2 is

11.5 and the mean of the daily positive COVID-19 test 3 is 13.3 in Table 2. Could you please clarify what you compare between the two groups? How do we understand Tables 2 and 3 together? The same comment will be applied to Tables 4 and 5.

Minor Comments

2. The notation of *P* values throughout the manuscript is inconsistent.

On page 5, "with Pearson correlations above 0.850 or below -0.850 with *P*<.001 considered as having a high degree of linearity." On page 8, "The Spearman's correlation coefficients and P-values between the daily cumulative COVID-19 vaccinated people and the daily COVID-19 cases in the first half period of the third, fourth and fifth COVID-19 epidemic wave in South Africa were 0.930 (95% CI 0.890-0.956), 0.842 (95% CI 0.713-0.916) and 0.811 (95% CI 0.673-0.895) respectively with *P*-values<.001. While the Spearman's correlation coefficients and P-values between the daily cumulative COVID-19 vaccinated people and the change in daily COVID-19 cases were 0.031 (*P*=.79 95% CI -0.207-0.266), -0.014 (*P*=.93 95% CI -0.341-0.316) and -0.077 (*P*=.62 95% CI -0.374-0.233) respectively." Could you please make an update on the notation?

Round 5 Review

General Comments

The authors' responses are clear. However, this paper still needs cosmetic improvement. I have some minor comments to improve the quality of this manuscript.

Specific Comments

Minor Comments

1. In Tables 1 and 2, some minimum values are "-." Does this mean zero or unknown? Could you please specify what "-" is?

2. The format of *P* values in Table 3 and the tables in the appendix is incorrect. Please edit based on [8].

3. Tables 6, 7, and 8 show both standard deviation and variance. Are there any specific reasons that the authors display both? If there is no reason, it is sufficient to show the standard deviation only.

Conflicts of Interest

None declared.

References

1. Mabuka T, Naidoo N, Ncube N, Yiga T, Ross M, Kurehwa K, et al. The impact of SARS-CoV-2 lineages (variants) and COVID-19 vaccination on the COVID-19 epidemic in South Africa: regression study. JMIRx Med 2023;4:e34598 [FREE Full text]
2. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020 Mar;579(7798):265-269 [FREE Full text] [doi: 10.1038/s41586-020-2008-3] [Medline: 32015508]
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6. JMIR House Style and Editorial Guidelines. JMIR Publications Knowledge Base and Help Center. URL: https://support.jmir.org/hc/en-us/articles/360019504191 [accessed 2023-03-24]
7. How many tables and figures can I include in my article? JMIR Publications Knowledge Base and Help Center. URL: https://support.jmir.org/hc/en-us/articles/360021623072 [accessed 2023-03-24]
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9. SAGE Working Group on COVID-19. mRNA vaccines against COVID-19: Pfizer-BioNTech COVID-19 vaccine BNT162b2. World Health Organization. 2020. URL: https://apps.who.int/iris/bitstream/handle/10665/338096/WHO-2019-nCoV-vaccines-SAGE_evaluation-BNT162b2-2020.1-eng.pdf?sequence=1&isAllowed=y [accessed 2023-03-24]
10. Johnson and Johnson COVID-19 vaccine demonstrates 85 percent effectiveness against hospitalization in South Africa when Omicron was dominant. Johnson & Johnson. 2022. URL: https://tinyurl.com/yuptnvv7 [accessed 2023-03-24]
11. Vaccine efficacy, effectiveness and protection. World Health Organization. 2021. URL: https://www.who.int/news-room/feature-stories/detail/vaccine-efficacy-effectiveness-and-protection [accessed 2023-03-24]

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Anonymous
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Peer-Review Report

Peer Review of “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”

Anonymous

Related Articles:

Companion article: https://preprints.jmir.org/preprint/34598

Companion article: https://med.jmirx.org/2023/1/e46944/

Companion article: https://med.jmirx.org/2023/1/e34598/

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KEYWORDS

COVID-19; infection; pandemic; vaccine; vaccination; epidemiology; transmissibility; health care; hospital admission; COVID-19 variants; SARS-CoV-2

This is a peer-review report submitted for the paper “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study.”

Round 1 Review

General Comments

In this article [1], the authors study the emerging variants of SARS-CoV-2 at the immune and epidemiological levels. The authors conclude that the Delta, Beta I VOC SARS-CoV-2, and lineage cluster, predominantly B.1.1.54, B.1.1.56 C.1 SA SARS-CoV-2 were observed to cause similar cases of COVID-19 hospital mortality and discharge rates in South African hospitals.

Specific Comments

The article seems good to me but too complex and difficult to follow, it should be “lightened.”

Major Comments

When talking about COVID-19 and its variants, some important points should be clarified that inform and prepare the reader well to deal with the specifics. Therefore, to make this paper more complete and interesting for the readers of this important journal, the authors should expand a bit of the discussion on cytokines. On this subject, three important articles have recently been reported. Below I list these interesting articles that should be studied, incorporated into the meaning, and reported briefly in the discussion and in the list of references.

- Conti P, Caraffa A, Tetè G, Gallenga CE, Ross R, Kritas SK, et al. Mast cells activated by SARS-CoV-2 release histamine which increases IL-1 levels causing cytokine storm and inflammatory reaction in COVID-19. *J Biol Regul Homeost Agents*. 2020;34(5):1629-1632. PMID:32945158 doi:10.23812/20-2EDIT
- Ronconi G, Teté G, Kritas SK, Gallenga CE, Caraffa A, Ross R, et al. SARS-CoV-2, which induces COVID-19, causes kawasaki-like disease in children: role of pro-inflammatory and anti-inflammatory cytokines. *J Biol Regul Homeost Agents*. 2020;34(3):767-773. PMID:32476380 doi:10.23812/EDITORIAL-RONCONI-E-59
- Conti P, Caraffa A, Gallenga CE, Ross R, Kritas SK, Frydas I, et al. Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: a promising inhibitory strategy.*J Biol Regul Homeost Agents*. 2020 Nov-Dec;34(6):1971-1975. PMID:33016027 doi:10.23812/20-1-E

Minor Comments

Some legends should be expanded.

I believe these suggestions are important for improving this paper. Without these corrections, the paper cannot be published. So I recommend minor revision.

Conflicts of Interest

None declared.

Reference

1. Mabuka T, Naidoo N, Ncube N, Yiga T, Ross M, Kurehwa K, et al. The impact of SARS-CoV-2 lineages (variants) and COVID-19 vaccination on the COVID-19 epidemic in South Africa: regression study. JMIRx Med 2023;4:e34598 [FREE Full text]

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Peer-Review Report

Peer Review "The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study"

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Related Articles:

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Companion article: https://med.jmirx.org/2023/1/e46944/

Companion article: https://med.jmirx.org/2023/1/e34598/

(JMIRx Med 2023;4:e47143) doi:10.2196/47143

KEYWORDS

COVID-19; infection; pandemic; vaccine; vaccination; epidemiology; transmissibility; health care; hospital admission; COVID-19 variants; SARS-CoV-2

This is a peer-review report submitted for the paper "The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study."

Round 1 Review

General Comments

This paper [1] provides an epidemiological analysis and report on the COVID-19 pandemic in South Africa and provides insight into the potential impact that various SARS-CoV-2 lineages may have had on the epidemic. Overall, this paper notes that the nonpharmaceutical interventions such as movement restrictions through lockdown measures and the evolution of the COVID-19 virus had significant impacts on the disease burden and epidemiology of disease observed in South Africa through the 3 waves that have occurred.

This manuscript is well written, comprehensive, and filled with detail. This is both a strength and a possible weakness. The strength is that the data included have been analyzed in depth, and one can be fairly certain that the results obtained are likely to be accurate. On the other hand, depending on the audience, some readers may struggle to engage with the data appropriately; the dissemination of data and reporting has not been formatted and simplified in a manner that improves readability without compromising on accuracy. The use of scientific notation for P values to the 11th power, use of 3 or 4 decimal places for proportions, etc, and extensive reporting of findings instead of picking a few of the most relevant findings with reference to the table for other findings are a few examples of this. However, this does not detract from the large amount of work that has gone into this manuscript, and the author team should be commended for it. Please find specific comments below.

Specific Comments

Major Comments

1. I have not seen whether time was included as a potential confounder/covariate in any of the regression models that were conducted. Increasing immunity, the initiation of vaccination campaigns halfway through the third wave, and movement restrictions have not been discussed adequately.

2. Please provide brief details on how data used to assess movement restriction were obtained and analyzed.

3. Please comment on the appropriateness of using means and standard deviations for the description of the majority of some of these data, which may or may not have been normally distributed.

4. Please provide ethical considerations in the manuscript for the data and analysis, whether approval was required or not, and justify.

Minor Comments

1. "While, there is global consensus on the health risk posed by COVID-19, ground-breaking vaccine developments, and a great drive towards the vaccination of the world population against COVID-19."

This sentence is fragmented. Please revise.

2. "emergent." Possible typo error, consider using "emergence."

3. National Coronavirus Command Council: A one-liner describing the National Coronavirus Command Council would be beneficial to the reader.

4. “Beta SARS-CoV-2 lineage required a half Maximal inhibitory concentration (IC50) 6 to 200 fold higher than the lineages identified in the first wave.” What reagent/antibody/method is used to test the IC 50 cited here?

5. “estimated that it was 1.29 (95%CI: 1.9601.58)).” Unsure what the confidence interval is there. Please review.

6. “period) showed significant difference at 95 % confidence interval between the respective COVID-19 epidemic periods with P values of 1.82×10-11 and 5.87×10-05 respectively.”

The author team can check submission guidelines, and the editor can confirm, but I believe that P values <.001 should be stated as such.

7. Table entries with variable names that have underscores and labeling could be cleaned up to improve readability.

8. As noted above, the use of 3 or 4 decimal places and exponential notation of extremely small P values reduces the clarity and readability. Consider reviewing.

Round 2 Review

The manuscript has been improved based on previous reviewer comments but is still unnecessarily too long, dense, and bloated. I believe that the adage “simpler is better” would have suited the objectives of this paper well. The average reader may find it difficult to read to the end, and some readers may have difficulty fully engaging with the content as a result. Five pages on the virology of SARS-CoV-2 as an introduction is likely unnecessary for a manuscript whose data focus on the epidemiology and statistics of COVID-19 rather than its virology.

There are many statistical tests conducted here; however, the authors do not appear to have performed any adjustments for the multiple tests conducted. The familywise error rate is bound to be higher than 0.05, so some of your conclusions based on the statistical probability may be inaccurate.

Finally, there are some statements that have been made based on the Discussion and Conclusion sections that I do not believe are adequately supported by the data presented, and these may need to be reconsidered/softened. Please see specific comments below.

1. Methods: Many hypothesis tests are conducted in this paper. Was adjustment for multiple testing performed? Otherwise, the possibility of making type 1 errors is quite high. This should either be reviewed or listed as a key limitation.

2. South Africa community mobility data: How is movement in these data measured? Kilometers? Significant movement out of the house? The number of people in an area? Please describe.

3. “The mean daily positive COVID-19 tests in South Africa’s first and second COVID-19 epidemic wave had no statistically significant difference.”

Please report the data and P values or reference the table where these data can be found.

4. Please insert a legend for the figures (eg, Figures 7 and 8).

5. Table 1: The maximum COVID-19 hospitalized intensive care unit percentage of 7 and 814.1 is unclear.

6. Discussion: “The values of the Pearson and Spearman Correlation Coefficients obtained between the daily COVID-19 tests and cases in this study indicated a strong positive correlation between daily COVID-19 tests and cases with more than 95 % confidence in the four COVID-19 epidemic waves in South Africa.”

Please review this interpretation of your correlation significance and 95% confidence intervals. It is technically incorrect to say that “there is more than 95% confidence.”

As a suggestion, you may leave the 95% confidence part out altogether and just say that testing was significantly related to case incidence in the 4 COVID-19 waves.

Consider also reviewing the American Statistical Association papers on P values and moving toward more conservative reliance on statistical significance overall (Wasserstein RL, Schirm AL, Lazar NA. Moving to a world beyond “*P*<0.05”. *Am Statistician*. 2019;73(sup1):1-19. doi:10.1080/00031305.2019.1583913).

7. These data, as presented, do not allow you to make this conclusion as you have not made a relationship of causality, but rather have demonstrated an association, as you rightly say in the following lines. Please revise to describe this as a significant association rather than a causal relationship.

8. “To understand the causality of relationships between two or more variables, statistical theory must be applied.” Text like this is unnecessary and contributes to the bloating of your manuscript. Consider removing.

9. “Daily COVID-19 tests in South Africa were observed to be normally distributed while the daily COVID-19 cases were positively skewed with a lognormal distribution (Galton distribution).”

I do not recall the data distributions being assessed or described in the Results, so it is surprising that they are now included in the Discussion. Consider including or revising the need to discuss the data distributions (a similar comment applies to the following paragraph).

10. I have reservations about the use of the word “confounder” in this discussion. While the movement is most likely a potential contributing factor in the detection rate of COVID-19, this was not analyzed or demonstrated using appropriate statistical methods such as multiple regression or interaction tests.

Showing that there was a correlation between population movement and COVID-19 detection does not automatically demonstrate that movement is a significant confounder. The messaging may have to be altered to suggest a possible confounding effect, or alternatively, this would need to be demonstrated by conducting appropriate data analysis.

11. “The values of the Spearman Correlation Coefficients obtained between the daily cumulative COVID-19 vaccinated people and change in daily COVID-19 cases in the half period of the third and fourth COVID-19 epidemic wave in this study indicated a low correlation between the daily cumulative COVID-19 vaccinated people and change in daily COVID-19 cases with this correlation statistically insignificant.”

This statement should be reconsidered. If vaccination does indeed have a significant effect on daily infection rates, there is bound to be a lag between exposure and effect, and this would need to be demonstrated in a robust time series analysis. Correlating the vaccination rate with the COVID-19 case rate without adjustment for time periods would not adequately demonstrate the effect of vaccination if such an effect existed. This is particularly important because the statement “These results suggest that COVID-19 vaccines administered in South Africa had no significant effect on the transmission of COVID-19” would be a controversial conclusion to come to without solid evidence to support this statement that may be seen as inflammatory in the politically charged topic of vaccines and vaccine hesitancy in South Africa.

12. “This result can be explained by the percentage of the population per age group who had received at least one dose of the COVID-19 vaccine by the end of the fourth COVID-19 epidemic wave.”

This statement appears to contradict your earlier statement that vaccines did not appear to have an impact on COVID-19 transmission in South Africa. Please review and reconcile. Also, natural immunity and potentially reduced virulence of the Omicron variant are important factors to consider in the reduced mortality in the fourth wave.

13. “showed statistical significant indifferences at 95 % confidence.” Unusual wording and terminology such as indifference at 95% confidence. Please revise.

14. “While COVID-19 vaccines administered in South Africa had no significant effect on the transmission of COVID-19 within the South African population.”

Again, this statement is not supported by the data provided and should be reviewed and reconsidered.

15. Table A. 1: Consider formatting these large sums of square and mean square values including thousand separators for readability.

Round 3 Review

Thank you for the review comments and revisions.

Comments

1. Table 8: Consider having the cumulative COVID-19 death risk ratio value for the reference group as “Ref” for reference. It may be confusing to have a risk ratio for the reference category.

2. Table 9: Case-fatality rate is abbreviated as “CRF” at times (and in subsequent text) and as “CFR” at times.

Conflicts of Interest

None declared.

Reference

1. Mabuka T, Naidoo N, Ncube N, Yiga T, Ross M, Kurehwa K, et al. The impact of SARS-CoV-2 lineages (variants) and COVID-19 vaccination on the COVID-19 epidemic in South Africa: regression study. JMIRx Med 2023;4:e34598 [FREE Full text]

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Peer Review “The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study”
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Authors' Response to Peer Reviews of "Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study"

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Companion article: https://med.jmirx.org/2023/1/e50501

Companion article: https://med.jmirx.org/2023/1/e29587

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KEYWORDS

COVID-19; pandemic; SARS-CoV-2; seroprevalence; serology; epidemiology; Niger State; Nigeria; COVID-19 testing; social distancing

This is the authors' response to peer-review reports for "Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study."

Round 1 Review

Thank you for going through and reviewing our manuscript. We have corrected, changed, and included most of the things you suggested. Please find as follows the specific responses to each of the comments made by the reviewers. Responses are placed immediately after each comment made by the reviewers.

Reviewer S [1]

This is a pilot study [2] to determine the COVID-19 seroprevalence, patterns, dynamics, and risk factors in Niger State, Nigeria. The study design is a cross-sectional survey using clustered, stratified random sampling over 5 days; the prevalence was measured by detecting antibodies.

Major point: the study design uses clustered, stratified random sampling. The authors haven't described the clusters or stratification. However, I understand this as study participants were allowed to have different, but known, probabilities of being selected for the sample. This is different to study designs where participants are selected with equal probability. However, none of the analyses presented in the manuscript accounted for this different probability of selection; all the analyses have assumed an equal probability of selection. This is a fundamental mistake of the analysis. This invalidates all the results presented in the manuscript. The term "sampling weights" is not used at all.

Response: Details were added to the sampling strategies in the Methods and Results sections as requested.

Other considerations:

- The justification for this pilot study is unclear. Specifically, what will be the full study that corresponds to this pilot? Since the COVID-19 situation changes rapidly, can the lessons from this study be used for designing a full study at a later stage?
- Response: This is a pilot study and was aimed at giving a quick feel of the COVID-19 situation at the time before the follow-up study, which can give the status and pattern of COVID-19 in the state.
- Some of the people sampled have not consented. How do they fill those gaps? Did they sample someone else in those places? What was the response rate as a measure of sampling bias in estimating prevalence?
- Response: All people that participated consented. More details were added to the sampling strategies in the Methods to give more clarifications.
- The inclusion and exclusion criteria are not given. The presented results are simple percentages from participants.
- Response: The age range covered all people and other stratifications, and therefore, unless they did not consent to the participation, all residents could be approached to participate and sampled. The exclusion criterion was not consenting to participate; the inclusion criterion was consenting to participate.
- The stratification is by place of residence (2 groups), gender (2 groups), occupation (unknown number of groups), and age (unknown number of groups). Therefore the number of strata should be large, although unknown to me. I wonder what could be the justification of these strata that must have resulted in a very small number of people per strata given the total sample size of 185.
- Response: More details were added to the sampling strategies in the Methods to give more clarifications.
- There are multiple places that require references (eg, second paragraph under section 2.4).
- Response: References were added as requested.
- Not sure what the value is of lots of bar graphs. Almost all of the information in those graphs is already in the text.
- Response: The Results are now summarized in tables in the revised manuscript. The results in the bar graphs were removed.
- The text needs revising in some places. For example, the first 1.5 paragraphs under section 3.2 do not belong in the Results section. Two of the subfigures in Figure 3 have been cited but mixed up in the second paragraph of that section.
- Response: The manuscript was revised as suggested and errors were corrected.
- Have they considered the incubation period needed to develop antibodies when interpreting the calculated percentages as prevalence?
- Response: Yes, we considered the incubation period needed to develop antibodies when doing the calculations; this is clear in the revised manuscript.
- Authors have determined the sensitivity and specificity as 100% for test kits; this was using the results from 15 individuals. I am skeptical to accept that in the absence of CIs.
- Response: The sample size of the participants and the small number of kits for validation were some of the limitations of the study; these were stated in the revised manuscript.

Reviewer AV [3]

General Comments

This article is a pilot study that was conducted to determine the prevalence, patterns, and dynamics of COVID-19 and the risk factors for contracting the disease in Niger State from June 26 to 30, 2020.

This study is a cross-sectional study and uses a clustered, stratified random sampling method. Only 185 participants were included in the study. The sample size is small.

Response: The sample size of the participants and the small number of kits for validation were some of the limitations of the study; these were stated in the revised manuscript.

The seroprevalence of COVID-19 was found to be 25.4% and 2.16% for the positive IgG and IgM, respectively. These seroprevalence results mean that herd immunity to COVID-19 has yet to be achieved, and the population is still susceptible to more infection and transmission of the virus.

Specific Comments

Major Comments

1. Samples were taken randomly from 185 participants for COVID-19 IgG and IgM rapid tests and questionnaires. Information on the number of patients included in the different sampling points is missing. Have serology results been confirmed by other techniques?

Response: More information was added in the Methods section to clarify more. The serology results were not confirmed by other methods, but the kits were validated by polymerase chain reaction.

2. The results are expressed as a percentage; it would be interesting to have the data on the number of samples or the number of patients.How many participants tested positive for only IgG and for both IgG and IgM?

Response: Absolute numbers were provided for the relative results (percentages) in the tables.

3. Bibliographic references are not formatted in the correct format.

Response: References were done as requested.

Minor Comments

4. Page 1: explain “NCDC”

Response: It is now explained in the revised manuscript. It means Nigeria Center for Disease Control.

5. Page 5: italicize *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Treponema pallidum*

Response: Noted, but that part was removed from the manuscript.

6. Page 9: add percent majority (61.62%)

Response: Done as requested

7. Page 11: explain “ATM”

Response: automated teller machine

8. Page 14: replace igM with IgG, “while the Kit detecting only IgM means that...”

Response: Done as requested

9. Page 19: explain “PPE”

Response: personal protective equipment

Anonymous [4]

General Comments

This paper, “Seroprevalence of COVID-19 in Niger State: A Pilot Cross-Sectional Study” by Majiya et al, is valuable and worthy of publication. The paper describes the seroprevalence of COVID-19 in Niger State. The COVID-19 asymptomatic rate in the state was 46.81%. The study also observed that the chances of infection are almost the same for both urban and rural dwellers. Of great interest is the finding that health care workers and those who had contact with persons who traveled out of Nigeria in the last 6 months are twice as likely to be at risk of being infected with the virus. The paper is relevant and contributes to the knowledge of the epidemiology of the virus. However, one primary concern is that the information about the virus from which inferences were made in this paper seems outdated. There is a need for an update. Also, the work appears to be underpowered in terms of sample size.

Response: The manuscript was revised and updated with regard to the recent COVID-19 situation in Nigeria. The sample size of the participants and the small number of kits for validation were some of the limitations of the study; these were stated in the revised manuscript.

Specific Comments

1. The abstract is unusually extended; consider summarizing it, especially the results aspect.

Response: The abstract was summarized and shortened as suggested.

2. There is a need for editing and restructuring some sentences.

Response: The manuscript was revised and grammar checked.

3. Some long paragraphs have the same references; consider using other references as well.

Response: More references were added as suggested.

4. Give a reference or definition for your sampling technique and probably describe how you achieved your sample size.

Response: More sampling information including the sample size calculation was added in the Methods as requested.

5. Avoid repeating the methodology in the Discussion session.

Response: Repetitions were removed as requested.

6. Add references to back up your inferences.

Response: More references were added where necessary for the key findings and interpretations.

7. The authors should make inferences in light of observation and the literature; asymptomatic cases seem to foster community transmission. More so, isolation, quarantine, and lockdown, if need be, are some public health measures to halt transmission. I would instead advise that the authors make recommendations based on the data generated from the study.

Response: More references were added where necessary for the key findings and interpretations. Those earlier interpretations that contradict public health measures were all removed in the revised manuscript.

References

1. Samaranayaka A. Peer review of "Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study". JMIRx Med 2023;4:e49866. [doi: 10.2196/49866]
2. Majiya H, Aliyu-Paiko M, Balogu VT, Musa DA, Salihu IM, Kawu AA, et al. Seroprevalence of SARS-CoV-2 in Niger State: pilot cross-sectional study. JMIRx Med 2023;4:e29587. [doi: 10.2196/29587]
3. Bourgeois-Nicolaos N. Peer review of "Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study". JMIRx Med 2023;4:e50391. [doi: 10.2196/50391]
4. Anonymous. Peer review of "Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study". JMIRx Med 2023;4:e50501. [doi: 10.2196/50501]

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Authors' Response to Peer Reviews of "Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study"
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Authors' Response to Peer Reviews of "Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study"

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KEYWORDS

waist circumference; computed tomography; abdominal CT; mobile health; health apps; CT; CT scan; CT image; mobile app; app; application; waist; body; body mass; body mass index; morbidity; mortality; clinical; tool; prototype; design; obesity; abdominal usability; validity; medical; BMI

This is the authors' response to peer-review reports for "Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study."

Round 1 Review

I would like to thank you for your important comments and questions. Please accept my finest greetings and my humble responses.

I will be answering each comment separately.

Reviewer K [1]

1. *What was the primary reason for selecting equation 1 as a reference method for waist circumference (WC) calculation? Isn't it possible to calculate the exact circumference from the computed tomography (CT) images using image-processing algorithms? Wouldn't it be more representative compared to the manual WC detection procedure?*

Response: Yes, indeed calculating the circumference using CT scan images has already been validated through many papers; however, what our study [2] is trying to do is create a simple and easy tool to retrospectively evaluate the WC using images from CTs, even real images from existing CT radio film papers (with a scale on it).

This method is very simple and easier for nonradiologists (taking a photo with the app and doing an estimation immediately, not waiting for a radiologist with experience in measuring WC with CT software).

The math formula used is a formula of an ellipse; the abdominal perimeter was estimated using the formula as validated by Ciudin et al [3] in their paper.

2. *Keeping the mobile app aside, how much different is this study compared to Ciudin et al [3]?*

Response: The study by Ciudin et al [3] compared a regular measurement of WC using the usual method with CT (drawing the perimeter of the abdomen manually with the CT scan software) and the estimation with the formula of an ellipse. We only used this result to estimate the WC in the mobile app, which is very different from using CT scan software. We also performed WC measurement on 10 healthy candidates using both the conventional tape method and the ellipse formula (with the mobile app). We then used a simple linear regression analysis to adjust the final WC formula to the gender of the

patient. So our results are adjusted to gender and are established with the mobile app, not the CT scan software—very different.

3. *On page 3, please expand the discussion on "App Requirements." It is not evident what was meant by "app requirements" in this section.*

Response: The app requirements are the ellipse formula, required measurements (a and b), final formula applied to gender, and the needed parameters and organization of the steps required by the physician to ameliorate the user experience. The text has been modified to further clarify this point.

4. *How many images were taken from each CT slice? As the measurements for the waist parameters (a and b) were taken using a manual process, what kind of procedure was followed to ensure that the person-to-person variability remains low?*

Response: Thank you for your comment and question.

Using the camera of the phone, the app used the CT scan image on the last slice, from cranial to caudal, not showing the iliac bone. The final goal was always to minimize time and make the method as rapid and simple as possible, so each time the first picture was satisfying and clear enough to be used, it was used. To minimize the variability in measurements (wideness of the screen of the phone, wideness of the finger of the user, and personal variability), we specifically used only two variable parameters (a and b). We also only used the CT scan image on the last slice, from cranial to caudal, not showing the iliac bone.

I want to remind you that, even if the precision of the measurement is very important, classifying the patient (with or without abdominal obesity) is the more important result to get from our app, and the small variability in the measurements does not affect it.

5. *In Figures 3 and 4, there is a small dot around the top of the figures. Is this a data point? Additionally, proper x- and y-axis labels are missing. Please add appropriate units on the x- and y-axis.*

Response: Yes, that is a data point; it does not represent a patient, but a difference of means of the measurements. The x-axis of the plot displays the average measurement of the two methods, and the y-axis displays the difference in measurements between the two methods.

The three lines also shown in the plot represent:

1. The average difference in measurements between the two methods,
2. The upper limit of the 95% CI for the average difference, and
3. The lower limit of the 95% CI for the average difference.

The horizontal line drawn in the middle of the chart shows the average difference in measurements between the two methods. This value is often referred to as the "bias" between the instruments.

The further this value is from zero, the larger the average difference in measurements between the methods.

In our case the Bland-Altman analysis showed a mean difference of 0.03 cm between the two measurements, which is very close to zero, indicating that our method using the mobile app is probably reliable.

The units are in centimeters, but with the Bland-Altman test, statistics specialists do not show the unit because it is a representation of how much the two methods of measurement are in accordance.

6. *In the Discussion section, it was claimed that "this is the first of a kind mobile app helping physicians to estimate WC." Do the authors think the physicians would be able to use apps such as [4] to assess WC?*

Response: There are many mobile apps to do measurements; we are not reinventing it, but our app is specifically designed to do measurements and apply a unique formula (applied to gender).

I would like to remind you that our app indicates a WC estimation, but the most important parameter is abdominal obesity, so even if the estimated WC does not match the real WC (conventional tape method) in extreme cases, we have an accuracy of 83% when using the mobile app–based WC measurement (mWC) to detect abdominal obesity.

7. *In the Discussion section, it was stated that "Moreover, the simplicity of the app may reduce the time required for physicians to assess WC." How fast is the app compared to the manual approach?*

Response: Conventional measuring of WC does not require too much time, but it requires the presence of a patient with the app; for any patient who has had a CT scan, the evaluation becomes feasible and easy (retrospectively).

Assessing WC using the conventional methods takes time and expertise for a radiologist; with the mobile app, even a CT image from the patient folder (even on paper or in old CT films) can make the measurement very easy, feasible, and reliable.

Minor Comments

8. *The authors stated that "WC cannot be physically assessed in patients with intellectual or motor disabilities" but did not provide any other details as to why it can't be assessed. The authors should discuss this in detail in the Introduction.*

Response: Taking a conventional tape WC measurement in patients with intellectual or motor disabilities can be challenging. Conventional measurement with tape requires a standing up position and a cooperating understanding patient.

The sentence was modified in the Introduction.

9. *The sentence "However, for a radiologist, this method requires training and can be more or less time consuming" seems confusing. If possible, please restructure this sentence.*

Response: Modified to "However, for a radiologist, this method may require time and training."

10. *In equation 1, what is denoted by "p"?*

Response: P (perimeter)=WC; this was modified in the text.

11. *Although the authors discussed in the Methods section how the measurements were taken just above the iliac crest and the*

CT images were taken from the last slice to ensure that those are not taken from different places, do the authors think that there could be some positional errors being introduced based on your approach?

Response: Maybe yes, but even with the positional errors, the goal of the measurement is not only to have an estimation of the WC but also more importantly to assess abdominal obesity (more important than the exact WC).

12. *On page 3, it was stated that there were further modifications to the app design. What kind of modifications were carried out? Did the authors discard the prior mWCs after modifying the app?*

Response: No, only the design and organization of the steps required by the physician to ameliorate the user experience were modified.

13. *Please try to make sure that periods and commas are being used appropriately. On page 4, one of the sentences was "The mean BMI was 26±4; 27,8±2,7 for women and 24.2±4,4 for men." For women, a comma was used as a decimal point. On the other hand, for men, a period was used as a decimal point.*

Response: Thank you for your comment. Corrected.

14. *In Table 1, what is the unit for "Confidence Interval"?*

Response: We do not usually express the units; it refers to the mean difference, which is in centimeters.

15. *What kind of procedure was used to perform the diagnostic test to detect abdominal obesity? Please discuss this in the Methods section.*

Response: Abdominal obesity is a simple parameter. Abdominal obesity was defined by WC measurements of >102 cm (~40 in) and >88 cm (~35 in) for men and women, respectively.

This is written in the Methods section.

Reviewer L [5]

Major Comments

1. *The authors admit that their conclusion is based on a very small sample of patients. In recommending further studies, the authors should offer specific guidelines, especially with respect to establishing the precision of each measurement modality. The material speaks only to the accuracy, but the plots in Figures 4 and 5 display some significant outliers.*

Response: Thank you for your valuable comment. True, our study is based on a very small sample of patients and that is why we did not write this paper as a validation of the method (mobile app method) but as an introduction to it. We will need a much bigger sample size and specific guidelines indeed, which will be detailed in the next paper (the validation of the method paper).

Even with the outliers, we succeeded in creating this useful tool that may be used as an easier method for physicians. Additionally, the goal of the mobile app was not only to have an estimation of the WC but also, more importantly, to assess abdominal obesity (we have good accuracy in doing that), so in retrospective studies, assessing this parameter may be very useful and important; we can do that using old CT scan images.

2. *The manuscript should present quantitative evidence of the degree to which an ellipse is an accurate representation of the body shape at the waist.*

Response: Thank you for your comment. In the study of Ciudin et al [3], the Pearson test was 0.987 with a mean error of 0.4 cm and the Bland-Altman analysis showed a mean difference of 1.4 cm between the standing and ellipse formula CT evaluation measurements.

I will add the details to our text to assure scientific honesty.

3. *The comment that this technique is important to less developed countries is puzzling considering the simplicity and extremely low cost of obtaining tape measure data prior to treatment.*

Response: This meant that in less developed countries, CT scan electronic archives are not often available or may not exist. So patient folders (like in low-income countries) are still physical (on paper) and contain images of CT scans (radio films) or CDs. So this method becomes very valuable since it gives the physician the opportunity to extract such a valuable parameter (abdominal obesity) retrospectively and from old paper folders and CDs.

4. *The authors claim that the WC cannot be assessed in patients with intellectual or motor disabilities. Why? That hardly seems like a satisfactory reason to subject the patient to the radiation dose of a CT scan.*

Response: The idea is to assess WC in patients who already have abdominal CT scans and certainly not to order a new one to only assess WC.

5. *Were the statistics presented controlled for variations in BMI and the effect of BMI on the body shape at the waist?*

Response: No, there might be positional errors with the effect of BMI on the shape of the waist; the goal of the measurement is not only to have an estimation of the WC but more importantly to assess abdominal obesity (more important than the exact WC).

Minor Comments

6. *The WC is a characteristic of the patient. It is not a parameter. The text needs careful proofreading.*

Response: Thank you for your comment. I agree; the valuable parameter is abdominal obesity.

10. *In the Discussion, why aren't tape measurements of WC routinely made if this characteristic is so important in treatment planning as the authors claim?*

Response: I agree that they should be. Abdominal obesity is an important morbidity risk factor in many medical and surgical specialties.

11. *The comment "Also, for a radiologist, conventional CT scan method requires training and can be more or less time consuming" is puzzling in light of the ease of using a tape measure in pretreatment planning.*

Response: I agree, and I modified it.

12. *"Since smartphones are commonly available even in low- and middle-income countries"—CT scanners are not so prevalent. This is a pointless polemic.*

Response: I agree—removed.

14. *The suggestion of using artificial intelligence (AI) in an upgraded app is hardly compelling without a clear explanation of why the ellipse fitting is of questionable validity.*

Response: I agree that when using an AI-upgraded app, the ellipse formula may not be needed. The AI will assess the WC directly using image analysis technology.

Reviewer R [6]

This manuscript is well written. This paper presents an original idea to simplify patient care. It can be generalized to other specialties.

No specific comments.

Major Comment

This mobile app could be used for other measurements.

Round 2 Review

I would like to thank you for your comments and questions. Please accept my finest greetings and my humble response.

Reviewer K

1. *The authors stated that the app has an accuracy of 83% when using the mWC to detect abdominal obesity. Is it sufficient compared to the conventional approaches? Just a simple comparison/comment would suffice.*

Response: Our estimation based on the app is quite accurate. The percentage of 83% is interesting. As said before, in most cases, it is sufficient, but the more we are talking about extreme numbers (WCs), the less accuracy we get. This comparison was added to the paper.

2. *Related to comment 11 of the round 1 review, how much impact can positional errors have in abdominal obesity classification? This can be explained or discussed in the Discussion.*

Response: In the same spirit as the last comment, the accuracy of WC measurement may be altered in some cases. This may be due to the measurement error in the conventional method or to particular body shapes and extreme values of WC. This comment was already added to the paper.

3. *The Figure 3 regression shows that one of the app measurements was (WC_App=120) when the true value should have been around ~65 (standing app difference=55). However, in Figure 4, that point seems to be missing (mean of standing + app ~92, so the difference ~55 should be around ~92 in the Bland-Altman plot). Can you please clarify this? If my calculations are wrong, I am extremely sorry about that.*

Response: Thank you for your comment. Figure 3 shows the Q-Q plot figure that shows the mean of differences between the two measurements. The Q-Q plot showed good overlapping with some dispersion of extreme values, but the difference between both never exceeds +20 or –10.

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Abbreviations

AI: artificial intelligence
CT: computed tomography
mWC: mobile app–based waist circumference measurement
WC: waist circumference

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Authors' Response to Peer Reviews

Authors' Responses to Peer Review Reports for "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years"

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KEYWORDS

aging; angiotensin converting enzyme; lymphocytes; immunosenescence; inflammaging

This is the authors' response to peer-review reports for "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years" [1].

Round 1 Review

Reviewer Heikki Vapaatalo [2]

Dear reviewer, thank you very much for the insightful suggestions; the manuscript improved a lot with the suggested changes. Please find our point-by-point answers to the raised questions. In the main text, all changes are highlighted in yellow. I hope that with the changes made, the new version is suitable for publication.

General Comments

1. The study is interesting, and the title promises for me more than the manuscript finally contains.

Answer: The manuscript is part of a project aiming to study ACE1 and ACE2 expression in cells from the immune system of aging and young adults. These initial results suggest that ACE1 (and probably ACE2) somehow plays a role in the process of aging.

2. The background, question, and the aim are relevant as explained in the Introduction.

Answer: We included some information in the Introduction, trying to link ACE1 expression in tissue cells and age-related diseases, as follows:

"ACE1 has been suggested to influence age-related diseases (ie, Alzheimer disease, sarcopenia, and cancer) but the associated mechanisms are still under investigation. *ACE1* polymorphisms have been correlated with susceptibility to Alzheimer disease [15,16]. In addition, it was shown recently that in normal aging, ACE1 expression is increased in brain

homogenates, and this expression is unchanged in early stages of Alzheimer disease [17]. Regarding sarcopenia, Yoshihara et al [18] found a weak correlation between *ACE1* polymorphism and physical function. In cancer (gastric or colorectal), patients presented higher expression of ACE1 in tumors than in healthy tissues [19,20]."

3. The major concerns the small size of the material (6 subjects), the small age difference (64-67 years), and the lack of younger controls.

Answer: We agree that the small number of studied subjects is a limitation of this study. In spite of the interesting results suggesting that ACE1 expression could be linked to the health status, it was not possible to perform correlation analysis due to the small sample size. Even though there is a small chronological difference among the subjects, the biological aging is very different among them and reflects the genetics, lifestyle, nutrition, and comorbidities. Another limitation is the lack of younger controls to compare with the subjects studied. Our next steps are to include younger controls, to increase the number of studied subjects, and, if possible, to obtain samples from older subjects (ie, aged 70-80, 80, and >80 years).

Specific Comments

1. Title: ACE seems better than ACE1; or, does the sophisticated, elegant method include both ACEs?

Answer: We evaluated only ACE1 expression, and thus, the title, abstract, and main text were changed to indicate ACE1 instead of ACE. We decided to change the title to "Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years."

2. Introduction: in the last chapter, the author should explain in more detail how Pawelec et al [3], Alves et al [4], Alves and Bueno [5], and Bueno et al [6] suggest that "ACE1 plays an important role in the aging process." Does "ACE1 plays" mean, that ACE1 is somehow regulating the aging process or are ACE1 levels changed with age?

Answer: These cited studies show that age-related diseases occurring in older adults are associated with changes in the immune system. To complete the text, we added the following:

"ACE1 has been suggested to influence age-related diseases (ie, Alzheimer disease, sarcopenia, and cancer) but the associated mechanisms are still under investigation. *ACE1* polymorphisms have been correlated with susceptibility to Alzheimer disease [15,16]. In addition, it was shown recently that in normal aging, ACE1 expression is increased in brain homogenates, and this expression is unchanged in early stages of Alzheimer disease [17]. Regarding sarcopenia, Yoshihara et al [18] found a weak correlation between *ACE1* polymorphism and physical function. In cancer (gastric or colorectal), patients presented higher expression of ACE1 in tumors than in healthy tissues [19,20]."

Methods:

1. The N value of the subjects should be mentioned here, as well the relation of females and males.

Answer: Text was corrected as suggested: "Blood was collected from adults (n=6, four females and 2 males) aged 64-67 years in 2015."

2. Do the authors really regard 64-67 years "older age" nowadays?

Answer: Nowadays, the most common term used for individuals older than 65 years is "older adults."

3. Why were the initial assays done many years after the collection of blood samples? Are the samples still useable and not destroyed?

Answer: Samples are part of UNIFESP Biobank and have been maintained in adequate conditions. We wanted to test cells from a period before the COVID-19 pandemic and those samples were the only ones that served our purpose. We compared samples used in this study with fresh blood samples (cell viability and percentage of $CD4^+$, $CD8^+$, and $CD19^+$ cells) and the results showed good preservation of the cells.

4. Did the subjects have some diseases or were taking drugs because they possibly were from a hospital sample bank?

Answer: The samples are part of UNIFESP Biobank, but unfortunately, we do not have information about diseases and medicaments.

5.Provide the companies' details.

Answer: Changes were made as required: "ACE CD143 fluorescein isothiocyanate (R&D Systems)."

Results:

1. "Table 1 shows that older adults….." The comparison between the present data and historical studies belongs to the Discussion.

Answer: Changes were made as required.

2. Also, provide individual ages and genders of the subjects in Table 1.

Answer: The manuscript version sent to medrxiv@medrxiv.org had age and gender on tables, but due to their request, any possible variable that could identify the study participant had to be removed. Hence, the present version these variables are not shown.

3.What do *P* values mean here—what is being compared, or are interindividual differences being highlighted in the particular variables? This should be explained.

Answer: We used *P* values for interindividual differences in each variable, since individuals age differently (biological aging); thus, physiological parameters could be affected by genetics, lifestyle, nutrition, and comorbidities. It is now explained in the Methods section.

4. The numbering of tables and the text seems confusing to me. Only 3 tables, but in the text, 4 are mentioned. Table 4 does not exist.

Answer: For some reason, Table 2 is missing in the main text. Please find the new version with Table 2 included.

5. It would be good to have a list of abbreviations used in the description of the cell types for an unfamiliar reader.

Answer: In each figure and table, we are now providing a description of cells evaluated.

Discussion:

1. A major part of the discussion deals with previous publications and not meaning or clinical significance of the present findings and comparison between the present and earlier studies.

Answer: The discussion was changed as suggested:

"Our results show that for the studied population, chronological aging and biological aging are asynchronous. Even among individuals with a small chronological difference (64 to 67 years), there is heterogeneity in physiological parameters such as glucose, urea, glycated hemoglobin, and CRP. Changes in the same functional parameters have been reported by Carlsson et al [22] and Helmerson-Karlqvist [23] in healthy older adults. Carlsson's [22] study found that the CRP level was 2.6% with a coefficient variation of 1.4%, whereas in our study, we observed higher levels of CRP in 5 out of 6 individuals. Increased CRP levels have been associated with inflammaging, and our findings show that the study population has changes in functional parameters, which are likely associated with an inflammatory profile [24].

The link between the RAS and inflammation has been suggested but its role is not completely clear under physiological and pathological conditions [25,26]. In addition, the association between altered ACE1 expression in tissues (brain, muscle, heart, and vessels) and the development and progression of age-related conditions such as Alzheimer disease, sarcopenia, and cardiovascular disease has been suggested, but results are controversial [17,27-30].

There are few studies showing the association between ACE1 expression in cells from the immune system (monocytes and T cells) and the progression of kidney and cardiovascular disease [8,9,31,32]. Therefore, considering the lack of information on this issue, we questioned whether ACE1 (CD143) was highly expressed in cells from the immune system during the aging process. We found that ACE1 was expressed in almost 100% of T (CD4+ and CD8+) and B lymphocytes and in all phenotypes of these cells. In nonlymphoid cells, mean ACE1 expression was 56.9% (SD 20.6%). In agreement with our findings, independent studies showed that T cells from healthy donors and monocytes from patients with congestive heart failure expressed ACE1, but there has been no investigation on cell phenotypes [25,26]. Our study is the first to show that either inexperienced (naïve) or fully activated (memory) cells expresses ACE1. Our findings suggest that the expression of ACE1 in lymphoid and nonlymphoid cells reflects health status, since our studied population presented changes in physiological parameters and high levels of ACE1 expression in immune cells. Previous independent studies showed that patients with unstable angina [32] or acute myocardial infarction [33] presented higher expression of ACE1 in T cells and dendritic cells than control subjects. In addition, markers of cell (lymphoid and nonlymphoid) functional status, such as inflammatory or growth factor production, could be modulated by ACE inhibitors (ACEi). Accordingly, mononuclear leukocytes from healthy subjects incubated with an endotoxin exhibited high levels of tissue factor activity, which was reduced in the presence of captopril in a dose-dependent pattern. This result could be related to the antithrombotic effect of ACEi [34]. In patients with congestive heart failure, immune cells cultured with lipopolysaccharide secreted high levels of the proinflammatory tumor necrosis factor α, and these levels were significantly reduced in the presence of captopril [35]."

2. In those previous studies, ACE2 has also has been reported; why is it not studied here?

Answer: Our subsequent studies will be focused on ACE1 and ACE2 expression in cells from the immune system in both younger and older adults.

3. In the limitations paragraph, the authors fairly mention the real problem—the small sample size, and I would like to add a lack of younger subjects.

Answer: We agree with the limitations pointed, and the text was changed as required:

"This study has limitations such as the small sample size and the lack of young adults for comparison. As an example, the subject presenting the highest CRP and albumin levels also exhibited a high percentage of ACE1 expression in T cells (CD4+ and CD8+), B cells, and nonlymphoid cells, in addition to the lowest percentage of CD4+ naïve cells, and the highest percentage of CD8+ terminally differentiated (EMRA) and DN B cells. However, due to the small sample size, it was not possible to associate the high expression of ACE1 in immune cells with inflammaging and immunosenescence. Correlation of physiological parameters and health status with ACE1 expression and investigating whether age and associated chronic diseases could lead to increased ACE1 expression would yield important information."

4. The point regarding the COVID-19 pandemic, seemingly worth mentioning, is too far from this study and unnecessary.

Answer: Our point was to emphasize the negative impact of chronic diseases for the outcome of the aging population during a viral infection and how ACE1/ACE2 expression could provide information regarding diagnosis and treatment. Therefore, we would like to maintain this information.

5. Linguistic checking would improve the manuscript.

Answer: We checked for possible language errors.

Reviewer Calogero Caruso [7]

Dear reviewer, thank you very much for suggesting revisions to our manuscript. It is a privilege to have a manuscript reviewed by a researcher with high expertise on the field of ageing. Please find our responses to your questions and corresponding revisions made to the main text.

General Comments

1. The paper is essentially anecdotal because it studies the cells of 6 subjects without any comparison with other age groups. There is also a serious limitation because beyond the age and

sex, there is no information on the donors (how and why they were recruited, what drugs they took, etc).

Answer: It is really a limitation to have only 6 individuals for the study, but they were the only ones meeting the criteria of the proposed study. The samples were from a central bank of cells at the UNIFESP, and participants were considered "healthy" but there was no further information in addition to what we displayed in the tables in the manuscript. They were not living on homecare or hospitalized.

Our aim was to evaluate samples from individuals aged 60-69 years before the COVID-19 pandemic or vaccination. In addition, there were no samples maintained under the same conditions (PBMCs at –80 °C), obtained from young individuals, and using fresh blood could yield a result that could not be compared mainly for myeloid cells and B cells as shown in Braudeau et al [8]. Our goal from now on is to expand this study with young and older adults' samples, since it is important to understand whether ageing is associated with an increase in ACE expression in immune cells.

2. To infer that chronological and biological ages do not match is inappropriate in the absence of the above information.

Answer: This information regarding chronological and biological age was required by another reviewer. I agree that the concept does not match without more information on the donors. However, the information is now provided in Vasto et al [9] and should be considered when older adults are studied.

3. However, the paper is of some interest because there are few studies on the topic.

Answer: Thank you for this positive comment. Few studies on the topic are the reason why we decided to send the manuscript for publication, even though there some important information on the donors is missing and a limited number of individuals was included.

Specific Comments

Essential revisions that are required to verify the manuscript

1. Although we do not have data on donors, placing an age and gender column in all tables adds a minimum of useful information for the reader.

Answer: The first table was submitted with age, but per requirement of MedRxiv, gender and age could not be linked to the metabolic results to preserve the anonymity of the donors.

2. Inflammaging means low grade of inflammation. The CR*P* value of 23.1 suggests acute inflammation (also because albumin has high values, while in chronic inflammation its values decrease). Therefore the averages do not have to take this subject into account.

Answer: Thank you for this comment. In a review of the literature, Heumann et al [10] found a CRP variation from 0.1 to 19.8. There is also an article from your group [11] showing that a CRP level of <5 g/dL and >5 g/dL will be considered to investigate how ageing impacts CRP levels. Considering the already small number of donors, data were maintained and statistics (mean and SD) with and without 23.1 mg/dL are now shown.

This will be the new version (Discussion) with respect to CRP: "Carlsson's [22] study found that the CRP level was 2.6% with a coefficient variation of 1.4%, whereas in our study, we observed higher levels of CRP in 5 out of 6 individuals. Increased CRP levels have been associated with inflammaging, and our findings show that the study population has changes in functional parameters, which are likely associated with an inflammatory profile [24]."

However, an individual presented CPR 23.1 mg/dL, suggesting acute inflammation instead, but as all donors were not hospitalized or living on homecare, this sample was considered a part of the study. Another study [12] evaluating gait speed found CRP levels varying from 0.1 to 19.8 mg/dL. Our study has an important limitation, that is, the lack of data on donors such as the use of continuous medicaments or sarcopenia, hypertension, and cognition, among others, and thus it was not possible to correlate CRP with age-related conditions.

Table 1. Updated

Other suggestions to improve the manuscript

1. The authors write that their findings suggest that ACE1 could play a role in several processes linked to aging including the generation and activation of autoimmune cells, due to the experimental evidence that inhibitors of ACE suppress the autoimmune process in a number of autoimmune diseases such as EAE, arthritis, autoimmune myocarditis [13]. They do not appear to have these findings in their paper. So, they need to change the sentence.

Answer: The sentence has been changed as follows: "Our findings suggest that ACE1 could play a role in several processes linked to aging, including the generation and activation of autoimmune cells, due to the experimental evidence that inhibitors of ACE1 suppress the autoimmune process in a number of autoimmune diseases such as experimental autoimmune encephalomyelitis, arthritis, autoimmune myocarditis [49]."

Editorial Notice
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Authors' Response to Peer Reviews

Authors' Response to Peer Reviews of "The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study"

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COVID-19; infection; pandemic; vaccine; vaccination; epidemiology; transmissibility; health care; hospital admission; COVID-19 variants; SARS-CoV-2

This is authors' response to peer-review reports for "The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study."

Round 1 Review

Reviewer AA [1]

The manuscript [2] is well written, and the subject addressed in this manuscript is worth investigating; however, the manuscript partly failed to present a clear picture of its analytical methodology and presentation of results.

Response: The authors have revised the methodology and presentation of the results.

The following are some minor concerns for consideration. I suggest that the authors (a) extend the study to include the recent Omicron variant.

Response: The authors have extended the study to include the Omicron variant.

The following are some minor concerns for consideration. I suggest that the authors (b) present results with complete models.

Response: The authors have presented results with complete model methodologies.

The following are some minor concerns for consideration. I suggest that the authors (c) avoid excessive references (~71).

Response: The authors have tried to reduce the references in the manuscript to critical references.

Reviewer BQ [3]

General Comments

This manuscript is well written, comprehensive, and filled with detail. This is both a strength and a possible weakness. The strength is that the data included have been analyzed in depth, and one can be fairly certain that the results obtained are likely to be accurate. On the other hand, depending on the audience, some readers may struggle to engage with the data appropriately; the dissemination of data and reporting has not been formatted and simplified in a manner that improves readability without compromising on accuracy.

Response: The authors have reworded most of the sections, particularly the Results and Discussion to make the manuscript more reader friendly.

The use of scientific notation for P values to the 11th power, use of 3 or 4 decimal places for proportions, etc, and extensive reporting of findings instead of picking a few of the most relevant findings with reference to the table for other findings are a few examples of this.

Response: The presentation of P values in the manuscript has been reformatted as required.

Specific Comments

Major Comments

1. I have not seen whether time was included as a potential confounder/covariate in any of the regression models that were conducted. Increasing immunity, the initiation of vaccination campaigns halfway through the third wave, and movement restrictions have not been discussed adequately.

Response: The authors have included nonpharmaceutical interventions and COVID-19 vaccination as cofounders in the study analysis.

2. Please provide brief details on how data used to assess movement restriction were obtained and analyzed.

Response: Data on community movement was obtained from the Google Community Mobility reports. The regression of movement and the daily COVID-19 effective contact rate was then conducted through a literature review of earlier work done by the authors on this.

3. Please comment on the appropriateness of using means and standard deviations for the description of the majority of some of these data, which may or may not have been normally distributed.

Response: The authors have addressed this key question in the manuscript. For comparative inferential statistical analysis of continuous variables using the magnitude of the mean and variance, the distribution of the continuous variable must be the same in the periods being compared.

4. Please provide ethical considerations in the manuscript for the data and analysis, whether approval was required or not, and justify.

Response: Information used in this study was from public sources with creative commons licenses. The authors ensured reference data sources and acknowledged relevant institutions. The data used was blinded regarding patient personal information.

Minor Comments

1. “While, there is global consensus on the health risk posed by COVID-19, ground-breaking vaccine developments, and a great drive towards the vaccination of the world population against COVID-19.”

This sentence is fragmented. Please revise.

Response: The sentence was revised to “There is global consensus on the health risk posed by COVID-19, ground-breaking vaccine developments, and a great drive towards the vaccination of the world population against COVID-19. However challenges still persist in controlling the Global COVID-19 transmission and severity.”

2. “emergent.” Possible typo error, consider using “emergence.”

Response: Typo corrected.

3. National Coronavirus Command Council: A one-liner describing the National Coronavirus Command Council would be beneficial to the reader.

Response: The authors felt this would be unnecessary considering the word limit. The relevant reference has been added for the reader interested in looking for more information. The background has relatively low relevance to the study.

4. “Beta SARS-CoV-2 lineage required a half Maximal inhibitory concentration (IC50) 6 to 200 fold higher than the lineages identified in the first wave.” What reagent/antibody/method is used to test the IC 50 cited here?

Response: The authors wish to guide you to the following paper for more information. This background has relatively low relevance to the study, particularly the information on the reagent used. Cele S, Gazy I, Jackson L, Hwa SH, Tegally H, Lustig G, et al. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. *Nature*. 2021;593(7857):142-146. doi:10.1038/s41586-021-03471-w.

5. “estimated that it was 1.29 (95%CI: 1.9601.58)).” Unsure what the confidence interval is there. Please review.

Response: This error has been corrected.

6. “period) showed significant difference at 95 % confidence interval between the respective COVID-19 epidemic periods with p-values of 1.82×10-11 and 5.87×10-05 respectively.”

The author team can check submission guidelines, and the editor can confirm, but I believe that P values <.001 should be stated as such.

Response: The presentation of P values in the manuscript has been reformatted as required.

7. Table entries with variable names that have underscores and labeling could be cleaned up to improve readability.

Response: The use of the underscore was left unchanged as the authors feel this is the best method of referencing the epidemic waves in multiple variables of the study. This is also described in the methodology for the reader to understand their meaning (underscore and number).

8. As noted above, the use of 3 or 4 decimal places and exponential notation of extremely small P values reduces the clarity and readability. Consider reviewing.

Response: The presentation of P values in the manuscript has been reformatted as required.

Reviewer Anonymous [4]

Specific Comments

The article seems good to me but too complex and difficult to follow, it should be "lightened."

Response: The authors have restructured the paper for easier readability.

Major Comments

When talking about COVID-19 and its variants, some important points should be clarified that inform and prepare the reader well to deal with the specifics. Therefore, to make this paper more complete and interesting for the readers of this important journal, the authors should expand a bit of the discussion on cytokines. On this subject, three important articles have recently been reported. Below I list these interesting articles that should be studied, incorporated into the meaning, and reported briefly in the discussion and in the list of references.

- Conti P, Caraffa A, Tetè G, Gallenga CE, Ross R, Kritas SK, et al. Mast cells activated by SARS-CoV-2 release histamine which increases IL-1 levels causing cytokine storm and inflammatory reaction in COVID-19. *J Biol Regul Homeost Agents*. 2020;34(5):1629-1632. PMID:32945158 doi:10.23812/20-2EDIT
- Ronconi G, Teté G, Kritas SK, Gallenga CE, Caraffa A, Ross R, et al. SARS-CoV-2, which induces COVID-19, causes kawasaki-like disease in children: role of pro-inflammatory and anti-inflammatory cytokines. *J Biol Regul Homeost Agents*. 2020;34(3):767-773. PMID:32476380 doi:10.23812/EDITORIAL-RONCONI-E-59
- Conti P, Caraffa A, Gallenga CE, Ross R, Kritas SK, Frydas I, et al. Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: a promising inhibitory strategy. *J Biol Regul Homeost Agents*. 2020 Nov-Dec;34(6):1971-1975. PMID:33016027 doi:10.23812/20-1-E

Response: The authors found the suggested papers interesting. The following paper "Mast Cells Activated by SARS-CoV-2 Release Histamine Which Increases IL-1 Levels Causing Cytokine Storm and Inflammatory Reaction in COVID-19" was included in the paper as a reference; however, the authors, due to the word limit, did not expand on this topic. Though interesting, it has low relevance to the study.

Minor Comments

Some legends should be expanded.

I believe these suggestions are important for improving this paper. Without these corrections, the paper cannot be published. So I recommend minor revision.

Response: Legends in the paper were expanded.

Reviewer Anonymous [5]

Specific Comments

Major Comments

1. Throughout the manuscript, the notation of numbers is not consistent. For example, in the middle of the second paragraph in section 1, Introduction, "The genome of SARS-CoV-2 is a single positive-stranded RNA approximately 29 903 bases (nucleotides) pairs in length 9 [6-9]." It looks like a space between numbers indicates a digit of a thousand, and a comma is omitted. However, in the middle of the paragraph in section 2.2.1., "Table 2 shows that the mean COVID-19 daily tests in the first, second and third South African COVID-19 epidemic wave period were 20 575±14 062, 31 046±14 115 and 46 822±18 460 respectively." A space between numbers indicates a decimal point, not a comma.

Response: The authors have corrected this error. A space between numbers in the manuscript represents a digit of a thousand.

2. Sections 2 and 3 are extremely difficult to read because they are too lengthy, although subsections indicate each statistical analysis that was performed. I believe that the authors do not need to provide outputs copied from SPSS directly. Are all columns in each table meaningful? Should readers know both standard deviation and variance for each statistic, for example? I strongly suggest that the authors get rid of unnecessary columns in each table and move unnecessary tables from sections 2 and 3 to the appendix.

Response: The authors have reduced the columns in the tables and moved some of the tables to the appendix. The authors have also rewritten these sections for easier readability.

3. I believe that the P values in the manuscript do not need to be specific. For example, Table 3 displays Pearson and Spearman correlation coefficients and P values. Many people may not understand what 9.94E-79 means. It can be simplified to "<0.001" or 0.

Response: The presentation of P values in the manuscript has been reformatted as required.

Minor Comments

4. The font style and size are not consistent throughout the manuscript.

Response: The font and style have been made consistent throughout the manuscript.

Round 2 Review

Reviewer BQ [3]

The manuscript has been improved based on previous reviewer comments but is still unnecessarily too long, dense, and bloated. I believe that the adage "simpler is better" would have suited

the objectives of this paper well. The average reader may find it difficult to read to the end, and some readers may have difficulty fully engaging with the content as a result. Five pages on the virology of SARS-CoV-2 as an introduction is likely unnecessary for a manuscript whose data focus on the epidemiology and statistics of COVID-19 rather than its virology.

Response: The authors agree with this review note and have cut down the Introduction (to 2.25 pages) to focus on the background of detected SARS-CoV-2 and COVID-19 vaccination in South Africa to prepare the reader for the study objectives.

There are many statistical tests conducted here; however, the authors do not appear to have performed any adjustments for the multiple tests conducted. The familywise error rate is bound to be higher than 0.05, so some of your conclusions based on the statistical probability may be inaccurate.

Response: Each descriptive and inferential statistical analysis conducted/applied on the analysis data sets and conclusions drawn from each inference were done independently as per the objective of the statistical analysis method. However, type 1 error are noted and covered in the limitations stated in the manuscript under Data Handling and Limitations.

Finally, there are some statements that have been made based on the Discussion and Conclusion sections that I do not believe are adequately supported by the data presented, and these may need to be reconsidered/softened. Please see specific comments below.

Response: Thank you for this review. The authors agree with your statements below.

1. Methods: Many hypothesis tests are conducted in this paper. Was adjustment for multiple testing performed? Otherwise, the possibility of making type 1 errors is quite high. This should either be reviewed or listed as a key limitation.

Response: The limitations of the manuscript have been listed under Data Handling and Limitations. Statistical tests were applied independently; however, the potential for type I or II errors has been noted.

2. South Africa community mobility data: How is movement in these data measured? Kilometers? Significant movement out of the house? The number of people in an area? Please describe.

Response: The Google Mobility reports are created with aggregated data from users who have turned on their Location History in their Google accounts. The baseline in these reports is the median values of movement in the respective locations from January 3 to February 6, 2020. This movement unit is the percentage from baseline (number of people in that location per time relative to the number observed at baseline).

3. “The mean daily positive COVID-19 tests in South Africa’s first and second COVID-19 epidemic wave had no statistically significant difference.”

Please report the data and P values or reference the table where these data can be found.

Response: P value added to this statement.

4. Please insert a legend for the figures (eg, Figures 7 and 8).

Response: Legends inserted for the figures.

5. Table 1: The maximum COVID-19 hospitalized intensive care unit percentage of 7 and 814.1 is unclear.

Response: This statement was removed in the writing of the Discussion section.

6. Discussion: “The values of the Pearson and Spearman Correlation Coefficients obtained between the daily COVID-19 tests and cases in this study indicated a strong positive correlation between daily COVID-19 tests and cases with more than 95 % confidence in the four COVID-19 epidemic waves in South Africa.”

Please review this interpretation of your correlation significance and 95% confidence intervals. It is technically incorrect to say that “there is more than 95% confidence.”

As a suggestion, you may leave the 95% confidence part out altogether and just say that testing was significantly related to case incidence in the 4 COVID-19 waves.

Consider also reviewing the American Statistical Association papers on P values and moving toward more conservative reliance on statistical significance overall (Wasserstein RL, Schirm AL, Lazar NA. Moving to a world beyond “*P*<0.05”. *Am Statistician*. 2019;73(sup1):1-19. doi:10.1080/00031305.2019.1583913).

Response: The statement was changed to “The values of the Pearson correlation coefficients obtained between the daily COVID-19 tests and cases in this study indicate a strong positive association between daily COVID-19 tests and cases in the five COVID-19 epidemic waves in South Africa,” and the “95% confidence” was removed.

7. These data, as presented, do not allow you to make this conclusion as you have not made a relationship of causality, but rather have demonstrated an association, as you rightly say in the following lines. Please revise to describe this as a significant association rather than a causal relationship.

Response: Instead of relationship, the word “association” was used to avoid an interpretation of causality instead of correlations.

8. “To understand the causality of relationships between two or more variables, statistical theory must be applied.” Text like this is unnecessary and contributes to the bloating of your manuscript. Consider removing.

Response: This statement was removed in the rewriting of the Discussion section.

9. “Daily COVID-19 tests in South Africa were observed to be normally distributed while the daily COVID-19 cases were positively skewed with a lognormal distribution (Galton distribution).”

I do not recall the data distributions being assessed or described in the Results, so it is surprising that they are now included in the Discussion. Consider including or revising the need to

discuss the data distributions (a similar comment applies to the following paragraph).

Response: The discussion of variable normal distributions was removed from the manuscript.

10. I have reservations about the use of the word "confounder" in this discussion. While the movement is most likely a potential contributing factor in the detection rate of COVID-19, this was not analyzed or demonstrated using appropriate statistical methods such as multiple regression or interaction tests.

Showing that there was a correlation between population movement and COVID-19 detection does not automatically demonstrate that movement is a significant confounder. The messaging may have to be altered to suggest a possible confounding effect, or alternatively, this would need to be demonstrated by conducting appropriate data analysis.

Response: The words "possible" and "association" were used since there were not enough multivariable statistical methods applied in the manuscript to avoid conclusive statements.

11. "The values of the Spearman Correlation Coefficients obtained between the daily cumulative COVID-19 vaccinated people and change in daily COVID-19 cases in the half period of the third and fourth COVID-19 epidemic wave in this study indicated a low correlation between the daily cumulative COVID-19 vaccinated people and change in daily COVID-19 cases with this correlation statistically insignificant."

This statement should be reconsidered. If vaccination does indeed have a significant effect on daily infection rates, there is bound to be a lag between exposure and effect, and this would need to be demonstrated in a robust time series analysis. Correlating the vaccination rate with the COVID-19 case rate without adjustment for time periods would not adequately demonstrate the effect of vaccination if such an effect existed. This is particularly important because the statement "These results suggest that COVID-19 vaccines administered in South Africa had no significant effect on the transmission of COVID-19" would be a controversial conclusion to come to without solid evidence to support this statement that may be seen as inflammatory in the politically charged topic of vaccines and vaccine hesitancy in South Africa.

Response: This statement was removed from the Discussion. The authors agree that there is not enough evidence generated in the results of the manuscript to make this conclusion.

12. "This result can be explained by the percentage of the population per age group who had received at least one dose of the COVID-19 vaccine by the end of the fourth COVID-19 epidemic wave."

This statement appears to contradict your earlier statement that vaccines did not appear to have an impact on COVID-19 transmission in South Africa. Please review and reconcile. Also, natural immunity and potentially reduced virulence of the Omicron variant are important factors to consider in the reduced mortality in the fourth wave.

Response: The statements on the impact of the COVID-19 vaccine on transmissibility were retracted in the manuscript due to insufficient evidence from the available data. Including this conclusive analysis will require data that captures COVID-19 daily cases and their vaccination status.

13. "showed statistical significant indifferences at 95 % confidence." Unusual wording and terminology such as indifference at 95% confidence. Please revise.

Response: The wording has been revised.

14. "While COVID-19 vaccines administered in South Africa had no significant effect on the transmission of COVID-19 within the South African population."

Again, this statement is not supported by the data provided and should be reviewed and reconsidered.

Response: The statements on the impact of the COVID-19 vaccine on transmissibility were retracted in the manuscript due to insufficient evidence from the available data. Including this conclusive analysis will require data that captures COVID-19 daily cases and their vaccination status.

15. Table A. 1: Consider formatting these large sums of square and mean square values including thousand separators for readability.

Response: Commas to separate thousands were included in the formatting of all numbers in the manuscript.

Reviewer Anonymous [5]

General Comments

The authors have tried to improve the quality of the manuscript. However, the manuscript still needs substantial improvement. Please see my comments.

Response: Thank you for this review. The authors agree with your statements below.

Specific Comments

Major Comments

1. This issue has not been resolved. The authors said that the space between numbers indicates a digit of a thousand. However, according to JMIR house style and editorial guidelines, numbers greater than 999 have a comma to separate thousands, millions, etc. Please see [10] and update the style of numbers throughout the manuscript.

Response: Commas to separate thousands were included in the formatting of all numbers in the manuscript.

2. The authors have reduced unnecessary columns. However, the JMIR production team suggests no more than 5 tables per manuscript. There are still unnecessary tables in the manuscript, that do not provide meaningful information and are just the same outputs of SPSS. What is the purpose of including so many tables without interpretation? Should Table 1 really be placed in the main manuscript? Why? Please see [11].

Response: The authors have moved unnecessary tables and figures to the appendix.

3. The authors have updated the representation of P values according to the suggestion of the editorial director [12].

Response: No updates required.

4. The font style is still not consistent throughout the manuscript. Please revise the font style.

Response: The font style has been revised and made consistent throughout the manuscript.

5. The Introduction in the manuscript is too long. I would suggest reducing the Introduction in the manuscript.

Response: The authors agree. The Introduction in the manuscript was reduced.

6. There are 13 equations in the manuscript. I believe that the authors can reduce the number of equations in the manuscript by combining similar equations. Listing all equations is unnecessary. Also, reference numbers for equations could be a number in the parenthesis such as (1) instead of Equation 1.

Response: The authors have removed unnecessary equations in the manuscript.

7. Detailed information about the paired test (what pairs to what) will be placed in the footnote in the corresponding table or figure.

Response: This was removed from the captions of the tables and described in the methodology.

8. Why do the authors think that the following text or Table 3 is needed in the manuscript?

"Table 3 shows that the Pearson (Spearman) Correlation Coefficients between COVID-19 daily tests (Independent Variable) and cases (Dependent Variable) in the first, second, third and fourth COVID-19 epidemic wave in South Africa were 0.910 (0.955), 0.877 (0.751), 0.893 (0.847) and 0.854 (0.812) respectively."

This text and Table 3 are the same information.

Response: Table 3 was moved to the appendix and the text was used instead for the Results section.

9. What is the reason to provide Pearson correlation and Spearman rho together? Do the authors want to show a linear relationship or an ordinal relationship?

Response: The authors used throughout the Spearman rho correlation coefficient and left the Pearson correlation for normally distributed variables.

Minor Comments

10. The footnotes in Tables 3 and 4 are redundant. Where are the superscripts a, b, or c in the tables?

Response: Footnotes in Tables 3 and 4 were removed, and the tables were moved to the appendix.

11. There is an inconsistent number of digits in all tables in the manuscript.

Response: The authors agree and have resolved all formatting of numbers according to JMIR guidelines.

12. From Tables 1 to 16, why do the authors think that the minimum and maximum provide meaningful information in Table 2?

Response: The minimum and maximum provide the lowest and highest values observed in the epidemic wave period, which corresponds to the start/end and the peak of the epidemic wave period.

13. Please use "95% confidence interval" instead of "95 % confidence interval."

Response: "95% confidence interval" was used instead, and all percentage values were formatted accordingly.

Round 3 Review

Reviewer BQ [3]

Comments

1. Table 8: Consider having the case-fatality age risk ratio value for the reference group as "Ref" for reference. It may be confusing to have a risk ratio for the reference category.

Response: Updated the caption of Table 8 and the values of the case-fatality age risk ratio reference group to make the case-fatality age risk ratio reference clearer.

2. Table 9: Case-fatality rate is abbreviated as "CRF" at times (and in subsequent text) and as "CFR" at times.

Response: The abbreviation of case-fatality rate in Table 9 and Table A12 was corrected to "CFR." The in-text reference to the case-fatality rate abbreviation was checked to ensure they are all abbreviated as "CFR."

Reviewer Anonymous [5]

Major Comments

1. In "Covariance and Regression of South African Epidemiological Data," the authors stated that the 2-tailed Pearson correlation above 0.850 with P<.001 was considered as having a high degree of linearity. Pearson correlation coefficient has a value between –1 and 1. A negative value (eg, –0.850) could also be considered as a strong negative relationship between two variables. Was a negative relationship included in the determination of linearity?

Response: Thank you for this comment. The authors agree with the reviewer. Indeed, a value of less than –0.850 implies a strong negative relationship/association between two variables. The authors did conduct their analysis in this manner; however, unfortunately, the wording was omitted in the methodology. The Methods section Covariance and Regression of South African Epidemiological Data has been updated to include "or below -0.850."

2. In "Normalisation and Paired T-tests on South African Epidemiological Data," the authors considered only 7 pairs among 5 periods. Normalized parameter 2 and 4, normalized parameter 2 and 5, and normalized parameter 3 and 5 were not included in pairing. Was there a specific reason to exclude these three pairs in the paired *t* test?

Response: The authors initially did consider having all possible test pairings; however, it would have complicated the analysis. We, therefore, chose two analysis groupings in terms of test pairing. The first one was comparing all COVID-19 epidemic waves to the first COVID-19 epidemic wave (pair 1 to pair 4). This would help us understand the impact of the evolution of SARS-CoV-2 (inclusive of other factors: nonpharmaceutical interventions, vaccination, etc) against the ancestral SARS-CoV-2 lineage (and initial conditions). The second analysis grouping was understanding the evolution per consecutive waves (pairs 5, 6, and 7). This would help us understand the impact of the evolution of SARS-CoV-2 (including changing conditions) between each consecutive wave. This simplified the analysis and allowed us valid inference between test pairings and an overview based on the two analysis test pairing groupings.

3. In the Discussion, the authors stated that the Pfizer-BioNTech (Comirnaty) and the Johnson & Johnson/Janssen COVID-19 vaccines have shown high efficacy against severe COVID-19 at 85% and 88.9%, respectively. However, two terms, vaccine efficacy and effectiveness, are used in different settings. According to [13], Pfizer demonstrated their COVID-19 vaccine efficacy based on randomized controlled trials. However, Johnson & Johnson did not show their COVID-19 vaccine efficacy according to [14]. Instead, Johnson & Johnson demonstrated their COVID-19 vaccine effectiveness based on observational studies, which is in a real-world setting. Could you please clarify this? (Please see [15].)

Response: Thank you for this comment, and it touches on an important discourse regarding the implications of using different methodologies to infer efficacy, with of course, randomized clinical trials being the standard. Certainly, the authors accept the reviewer's point; for the Discussion, the authors wanted to highlight these studies for reference in terms of the efficacy against severe COVID-19. Unfortunately, there are, of course, limitations in the inference of efficacy, as it does depend on the methodology of those studies. The authors in the manuscript used the reference to allow the reader to understand the current work regarding the association, which is highlighted by the manuscript (increasing vaccination, decreasing hospitalization). In light of the reviewer's point, we have updated reference [14] to Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against COVID-19. *N Engl J Med*. 2021;384:2187-2201. PMID:33882225 doi:10.1056/NEJMoa2101544.

Minor Comments

4. The authors did not explain what the special characters after SARS-CoV-2 variants mean (eg, BA.4# or BA.2.75***). Could you please provide details on what the special characters after SARS-CoV-2 variants indicate?

Response: The naming of these lineages with special characters "#" or "*" appeared due to an error in rendering our document. We have updated to remove these from the naming of the lineages.

5. The authors used unnecessary abbreviations throughout the manuscript. Could you please review the manuscript and remove some unnecessary abbreviations that are not used in a section of the manuscript?

Response: The authors reviewed the abbreviations used in the manuscript and removed unnecessary abbreviations.

Round 4 Review

Reviewer Anonymous [5]

Specific Comments

Major Comments

1. It is difficult to understand what Tables 2 and 3 show. Table 3 provides the mean difference between two daily positive COVID-19 tests in a percentage. If we look at the paired differences mean of pair 5 (daily positive COVID-19 test 2 – daily positive COVID-19 test 3), the difference is –1.20. However, the mean of the daily positive COVID-19 test 2 is 11.5 and the mean of the daily positive COVID-19 test 3 is 13.3 in Table 2. Could you please clarify what you compare between the two groups? How do we understand Tables 2 and 3 together? The same comment will be applied to Tables 4 and 5.

Response: Table 2 shows the descriptive statistics for the COVID-19 active cases and daily positive COVID-19 tests (%, ie, what percentage of the total COVID-19 tests were positive) for each epidemic wave. The descriptive statistics include the number of valid observations (n), minimum, maximum, mean, and standard deviation (std deviation).

While, Table 3 shows the paired sample *t* test results between test pairing (ie, between epidemic waves), showing the paired differences of the mean and standard deviation, the student *t* test value, degrees of freedom (*df*), and the P value.

Now discussing the pairings you are comparing, pair 5 in Table 3 is the comparison between the daily positive COVID-19 tests in the COVID-19 epidemics 2 and 3. The paired mean difference was –1.20; however, the actual mean difference (13.3 – 11.5) is 1.80 as you have stated. The discrepancy between Tables 2 and 3 is due to the degrees of freedom (*df*) in Table 3 and observations (n) in Table 2. Test pairing was done based on the epidemic day; therefore, the epidemic wave with the lowest observations will always be the *df* of the *t* test. We have to compare like with like; due to this, some of the observations in Table 2 are not included in the *t* test. This concept is the same for Tables 4 and 5.

Minor Comments

2. The notation of P values throughout the manuscript is inconsistent.

On page 5, "with Pearson correlations above 0.850 or below -0.850 with P<.001 considered as having a high degree of linearity." On page 8, "The Spearman's correlation coefficients and P-values between the daily cumulative COVID-19 vaccinated people and the daily COVID-19 cases in the first half period of the third, fourth and fifth COVID-19 epidemic wave in South Africa were 0.930 (95% CI 0.890-0.956), 0.842 (95% CI 0.713-0.916) and 0.811 (95% CI 0.673-0.895)

respectively with P-values<.001. While the Spearman's correlation coefficients and P-values between the daily cumulative COVID-19 vaccinated people and the change in daily COVID-19 cases were 0.031 (P=.79 95% CI -0.207-0.266), -0.014 (P=.93 95% CI -0.341-0.316) and -0.077 (P=.62 95% CI -0.374-0.233) respectively." Could you please make an update on the notation?

Response: The authors have updated the notation of P values in the manuscript. The authors have followed the recommendations in [12].

References

1. Poluru R. Peer review of "The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study". JMIRx Med 2023;4:e46906 [FREE Full text]
2. Mabuka T, Naidoo N, Ncube N, Yiga T, Ross M, Kurehwa K, et al. The impact of SARS-CoV-2 lineages (variants) and COVID-19 vaccination on the COVID-19 epidemic in South Africa: regression study. JMIRx Med 2023;4:e34598 [FREE Full text]
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Original Paper

The Impact of SARS-CoV-2 Lineages (Variants) and COVID-19 Vaccination on the COVID-19 Epidemic in South Africa: Regression Study

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Abstract

Background: Emerging SARS-CoV-2 variants have been attributed to the occurrence of secondary, tertiary, quaternary, and quinary COVID-19 epidemic waves threatening vaccine efforts owing to their immune invasiveness. Since the importation of SARS-CoV-2 in South Africa, with the first reported COVID-19 case on March 5, 2020, South Africa has observed 5 consecutive COVID-19 epidemic waves. The evolution of SARS-CoV-2 has played a major role in the resurgence of COVID-19 epidemic waves in South Africa and across the globe.

Objective: We aimed to conduct descriptive and inferential statistical analysis on South African COVID-19 epidemiological data to investigate the impact of SARS-CoV-2 lineages and COVID-19 vaccinations in South African COVID-19 epidemiology.

Methods: The general methodology involved the collation and stratification, covariance, regression analysis, normalization, and comparative inferential statistical analysis through null hypothesis testing (paired 2-tailed *t* tests) of South African COVID-19 epidemiological data.

Results: The mean daily positive COVID-19 tests in South Africa's first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 11.5% (SD 8.58%), 11.5% (SD 8.45%), 13.3% (SD 9.72%), 13.1% (SD 9.91%), and 14.3% (SD 8.49%), respectively. The COVID-19 transmission rate in the first and second COVID-19 epidemic waves in South Africa was similar, while the COVID-19 transmission rate was higher in the third, fourth, and fifth COVID-19 epidemic waves than in the aforementioned waves. Most COVID-19 hospitalized cases in South Africa were in the general ward (60%-79.1%). Patients with

COVID-19 on oxygen were the second-largest admission status (11.2%-16.8%), followed by patients with COVID-19 in the intensive care unit (8.07%-16.7%). Most patients hospitalized owing to COVID-19 in South Africa's first, second, third, and fourth COVID-19 epidemic waves were aged between 40 and 49 years (16.8%-20.4%) and 50 and 59 years (19.8%-25.3%). Patients admitted to the hospital owing to COVID-19 in the age groups of 0 to 19 years were relatively low (1.98%-4.59%). In general, COVID-19 hospital admissions in South Africa for the age groups between 0 and 29 years increased after each consecutive COVID-19 epidemic wave, while for age groups between 30 and 79 years, hospital admissions decreased. Most COVID-19 hospitalization deaths in South Africa in the first, second, third, fourth, and fifth COVID-19 epidemic waves were in the ages of 50 to 59 years (15.8%-24.8%), 60 to 69 years (15.9%-29.5%), and 70 to 79 years (16.6%-20.7%).

Conclusions: The relaxation of COVID-19 nonpharmaceutical intervention health policies in South Africa and the evolution of SARS-CoV-2 were associated with increased COVID-19 transmission and severity in the South African population. COVID-19 vaccination in South Africa was strongly associated with a decrease in COVID-19 hospitalization and severity in South Africa.

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KEYWORDS

COVID-19; SARS-CoV-2; vaccines; variants; lineages; South Africa; epidemiology; statistics

Introduction

Background

On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a global pandemic [1]. The COVID-19 pandemic has resulted in >6,490,817 deaths in the reporting period until August 30, 2022 [2]. Public health measures, such as nationwide lockdowns aimed at reducing the transmission of COVID-19, have come at a great cost to the global economy [3]. There is a global consensus on the health risk posed by COVID-19, groundbreaking vaccine developments, and a great drive toward vaccination of the world population against COVID-19. However, challenges persist in controlling the global COVID-19 transmission and severity. One challenge is the large disparity in access to vaccines between low-income and high-income countries [4]. Another challenge is the emergence of SARS-CoV-2 lineages and sublineages (variants) with increased transmissibility [5]. Lineages and sublineages are a series of entities (in this case, genetic) forming a single line of direct ancestry and descent [6]. Emerging SARS-CoV-2 variants have been attributed to the occurrence of secondary, tertiary, quaternary, and quinary COVID-19 epidemic waves and threatening vaccine efforts owing to their immune invasiveness [7].

SARS-CoV-2 Variants of Concern and Interest

SARS-CoV-2 is the virus that causes COVID-19 upon infecting a human host. Whole-genome sequencing of 104 strains of SARS-CoV-2 from patients with COVID-19 symptom onset from December 2019 to mid-February 2020 showed 99.9% homology, without major mutations [8]. However, the rapid spread of SARS-CoV-2 has allowed the virus opportune replications to evolve into lineages and sublineages. To prioritize global monitoring and research and to inform the ongoing response to the COVID-19 pandemic, SARS-CoV-2 variants have been characterized as either *variants of concern* (VOCs) or *variants of interest* (VOIs). The main characteristics of VOCs are that they have evidence of an increase in transmissibility and more severe disease that leads to increased hospitalization or deaths, thereby reducing the effectiveness of public health and social measures [9]. In addition, VOCs substantially reduce the neutralization of antibodies generated during previous infection or vaccination which ultimately reduces the effectiveness of treatments, vaccines, or diagnostic detection [10]. VOIs are lineages whose changes have predicted genetic markers that are known to affect virus characteristics, such as transmissibility, disease severity, immune escape, and diagnostic or therapeutic escape [9]. They are also identified to have a predictable increase in transmissibility or disease severity, thus having an apparent epidemiological impact to suggest an emerging risk to global public health [9,10]. The SARS-CoV-2 lineages that have been characterized as VOCs by the WHO are the alpha (B.1.1.7), beta (B.1.351, B1.351.2, and B.1.351.3), gamma (P.1, P.1.1, P.1.2, P.1.4, P1.6, and P.1.7), delta (B.1.617.2, AY.1, AY.2, AY.3, and AY.3.1), and omicron (B.1.1.529, BA.4, BA.5, BA.2.12.1, and BA.2.75) SARS-CoV-2 lineages [9,10]. The variants that have been characterized as VOIs are eta (B.1.525), iota (B.1.526), kappa (B.1.617.1), lambda (C.37), epsilon (B.1.427, B.1.429), zeta (P.2), theta (P.3), and mu (B.1.621) SARS-CoV-2 lineages [9].

SARS-CoV-2 Lineages in South Africa

Of interest in this study is the impact of the evolution of SARS-CoV-2 lineages and COVID-19 vaccination in the COVID-19 epidemiology in South Africa. Since the importation of SARS-CoV-2 in South Africa, with the first reported COVID-19 case on March 5, 2020, South Africa has observed 5 consecutive COVID-19 epidemic waves [2,11]. The response by the Government of South Africa toward the COVID-19 epidemic was the establishment of a National Coronavirus Command Council to oversee the epidemic; the use of health policy measures, including nonpharmaceutical interventions (NPIs) to try to mitigate the transmission of COVID-19; and the implementation of COVID-19 vaccination programs to try to vaccinate the South African population against COVID-19 [12-17].

"Globally, systems have been established and are being strengthened to detect 'signals' of potential VOIs or VOCs and assess these based on the risk posed to global public health" [9]. In South Africa, the Network for Genomics Surveillance in South Africa was formed to understand the spread of SARS-CoV-2 [18]. During the first COVID-19 epidemic wave in South Africa, 16 SARS-CoV-2 lineages specific to South

Africa were identified from 1365 high-quality whole genomes [18]. From these 16 lineages, 3 main clusters (B.1.1.54, B.1.1.56, and C.1 SARS-CoV-2 lineages) were identified to have caused approximately 42% of SARS-CoV-2 infections in South Africa [18]. Another sublineage specific to South Africa was the B.1.106 lineage that emerged in Kwa-Zulu Natal province in a nosocomial outbreak during the first COVID-19 epidemic wave [18]. The prevalence of this sublineage decreased as a result of control measures [18,19]. The C.1 lineage (first identified C lineage of SARS-CoV-2) was the most geographically spread lineage during the first COVID-19 epidemic wave in South Africa [18]. Before the resurgence of the second COVID-19 epidemic wave in South Africa, the beta (B.1.351, B1.351.2, and B.1.351.3 lineages) SARS-CoV-2 VOC (formerly GR/501Y.V2) was identified in an analysis of 2704 South African SARS-CoV-2 genotypes (samples collected till December 14, 2020) from the GISAID database. The beta (B.1.351 lineage) SARS-CoV-2 VOC was detected in samples collected in October 2020 [20]. The beta SARS-CoV-2 lineage became the dominant lineage in South Africa's second COVID-19 epidemic wave, rapidly replacing the 3 main clusters (B.1.1.54, B.1.1.56, and C.1 SARS-CoV-2 lineages) identified during the first COVID-19 epidemic wave [20]. During the resurgence of the third COVID-19 epidemic wave in South Africa, 4 SARS-CoV-2 variants were identified: alpha, beta, eta, and delta SARS-CoV-2 variants. Genomic data for South African samples identified 65% of 1147 whole genomes from May 2021 as the beta SARS-CoV-2 variant. The alpha, delta, and eta SARS-CoV-2 variants accounted for 6%, 16%, and 1% of those samples, respectively. In June 2021, with 2931 genetic sequences in that period, the delta SARS-CoV-2 variant had become the dominant variant in samples collected in South Africa at 66%, while the beta and alpha SARS-CoV-2 variants accounted for 16% and 4%, respectively [21]. By the end of South Africa's third COVID-19 epidemic wave in September 2021, the delta SARS-CoV-2 variant accounted for 96% of the 186 whole-genome sampled in that period, while the C1.2 SARS-CoV-2 lineage accounted for 1% of those samples [21]. The C1.2 SARS-CoV-2 lineage, a new South Africa-specific SARS-CoV-2 lineage (evolved from the C.1 SARS-CoV-2 lineage), was identified in South African samples in May 2021. The C.1.2 lineage was detected across the majority of South African provinces and in 7 other countries [22]. On November 25, 2021, the National Institute for Communicable Diseases (NICD) in South Africa confirmed the detection of the omicron SARS-CoV-2 VOC (B.1.1.529 lineage) in SARS-CoV-2 genomes of 22 laboratory-confirmed cases of COVID-19 [23]. The investigation into the initially identified cases of the B.1.1.529 SARS-CoV-2 lineage in South Africa was triggered by the absence of the S gene (S-gene dropout or-gene target failure) in a specific PCR assay because of the 69-70del deletion [24]. A similar observation was made during the early identification of the alpha SARS-CoV-2 VOC (B.1.1.7 lineage). The omicron SARS-CoV-2 VOC (B.1.1.529 lineage) was the dominant SARS-CoV-2 lineage in the fourth COVID-19 wave in South Africa, accounting for 82% of SARS-CoV-2 infections in November 2021 and 98.5% of SARS-CoV-2 infections in South Africa by January and February 2022 [25]. By the time of the resurgence of the fifth COVID-19 epidemic wave in South Africa in April 2022, the omicron SARS-CoV-2 VOC had evolved into sublineages. A total of 2459 whole genomes from South African samples with confirmed SARS-CoV-2 infection in April 2022 identified the omicron SARS-CoV-2 sublineages (BA.4, BA.2, and BA.5) in 54%, 25%, and 19% of the respective samples, respectively [26]. By the end of the fifth COVID-19 epidemic wave in South Africa in July 2022, 339 whole genomes from South African samples with confirmed SARS-CoV-2 infection in that period were identified to be largely omicron SARS-CoV-2 sublineages (BA.5 and BA.4), accounting for 96% of those samples [27].

COVID-19 Vaccination in South Africa

The immunity against COVID-19 in humans is thought to be both innate and adaptive. Most patients with COVID-19 who recovered developed antibodies against SARS-CoV-2 within 1 to 3 weeks [28]. The SARS-COV-2 seroprevalence in South Africa's first COVID-19 epidemic wave was estimated to be between 31% and 46% [29], while in the second COVID-19 epidemic wave, it was estimated to be 35.8% [30-32]. COVID-19 vaccination in South Africa commenced during South Africa's second COVID-19 epidemic wave and was limited to frontline workers, such as health care workers, owing to the limited access to vaccines at the time [33]. COVID-19 vaccines were then largely administered to the rest of the South African population during the third and fourth COVID-19 epidemic waves. According to the WHO, there are currently 52 COVID-19 candidate vaccines in the clinical evaluation stages, of which 13 are in phase 3. Some vaccines have been reported to have an efficacy greater than 90% (BioNTech or Fosun Pharma or Pfizer, AstraZeneca, Sinovac, and Sputnik V vaccines) [28]. South Africa's COVID-19 vaccination run through COVID-19 vaccination programs and clinical trials have largely administered the Pfizer-BioNTech (Comirnaty) and the Johnson & Johnson or Janssen doses to the South African population. By August 26, 2022, a total of 37.4 million people had been vaccinated against COVID-19 in South Africa, mainly with the Pfizer-BioNTech (Comirnaty) and the Johnson & Johnson or Janssen COVID-19 vaccines [34]. The Johnson & Johnson or Janssen COVID-19 vaccine is a viral vector vaccine, whereas the Pfizer-BioNTech (Comirnaty) COVID-19 vaccine is an mRNA vaccine. Both vaccines are dependent on the encoded SARS-CoV-2 spike (S) proteins to induce an immune response after vaccination. Thus, SARS-CoV-2 VOCs and VOIs with mutations on the spike (S) proteins emerging after the development of these vaccines have hampered their efficacy, requiring booster doses [35].

The evolution of SARS-CoV-2 has played a major role in the resurgence of the COVID-19 epidemic waves in South Africa and across the globe. South Africa has a unique observation of the evolution of SARS-CoV-2, with distinct SARS-CoV-2 lineages dominating certain epidemic periods. This unique observation allows for an investigation of the impact of the detected SARS-CoV-2 lineages on COVID-19 transmissibility and severity through the analysis of epidemiological data. In this study, a descriptive and inferential statistical analysis was conducted on South African COVID-19 epidemiological data to describe and investigate the impact of SARS-CoV-2 lineages

and COVID-19 vaccinations on COVID-19 transmission and severity in the South African population.

Methods

The general methodology in this study involved the collation and stratification of South African COVID-19 epidemiological data, covariance, regression analysis of epidemiological data, normalization, and comparative inferential statistical analysis through null hypothesis testing (paired 2-tailed *t* tests).

Collation of South African COVID-19 Epidemiological Data

South African COVID-19 reported case data (cumulative and daily COVID-19 cases, recovered, and deaths) for the reporting period from January 22, 2020, to August 18, 2022, were obtained from the Johns Hopkins University Center for Systems Science and Engineering COVID-19 Database [2]. The South African COVID-19 testing data (cumulative and daily COVID-19 tests) were obtained from the Our World In Data project [36] for the reporting period from February 14, 2020, to June 22, 2022. South African COVID-19 hospitalization data were obtained from the NICD DATCOV surveillance system [37] for the period May 24, 2020, to August 18, 2022. The NICD DATCOV surveillance system in South Africa only started publishing reports on the reporting date of May 24, 2020; thus, data from March 5, 2020, to May 23, 2020, in the first COVID-19 epidemic wave period are missing. Data from October 9, 2020, to October 26, 2020, in the second COVID-19 epidemic wave period were also missing. The number of hospitals reporting to the NICD DATCOV surveillance system during South Africa's first COVID-19 epidemic wave period was initially 204 facilities and the number of facilities increased to 666 by the end of the fifth COVID-19 epidemic wave period. The South African COVID-19 hospitalization data obtained in this study were composed of the number of facilities reporting, admission status data (daily COVID-19 hospital admission cases, hospitalized in high care, intensive care unit [ICU], isolation ward, on oxygen, and ventilator), cumulative COVID-19 admission age profile, cumulative COVID-19 hospital deaths age profile, and cumulative COVID-19 patients discharged alive. The COVID-19 hospital daily discharge rate (DR) and case fatality rate (CFR) were then calculated based on the methodology described in the study by Mabuka et al [38]. Weekly South African natural and excess (natural) deaths were obtained from the South African Medical Research Council [39] for the reporting period of December 29, 2019, to August 20, 2022. The weekly unreported excess deaths (natural) to COVID-19 death ratio (ECDR) was then calculated based on the methodology in the study by Mabuka et al [38].

South African COVID-19 vaccination data (cumulative number of people who received at least 1 dose of a COVID-19 vaccine per population age group) for the reporting period of February 17, 2021, to August 18, 2022, were obtained from the Department of Health Republic of South Africa COVID-19 Public Dashboard [34]. The data in the respective dashboards only contained COVID-19 vaccination records captured on the live Electronic Vaccination Data System and excluded vaccination records captured on paper [34]. South African community mobility data, which includes the change from baseline in movement in retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residential locations, were obtained from the Google Community Mobility Reports [40] with data from the period of February 15, 2020, to August 18, 2022. The reports are created using aggregated data from users who have turned on their location history in their Google accounts. The baseline in these reports was the median values of movement in the respective locations from January 3 to February 6, 2020.

Stratification of South African COVID-19 Epidemiological Data

Study analysis data sets from the collated South African COVID-19 epidemiological data were produced using SAS Base 9.4 software. To draw inferential comparisons regarding the impact of the evolution of SARS-CoV-2 and COVID-19 vaccinations in South African COVID-19 epidemiology, the collated South African COVID-19 epidemiological data were stratified based on the observed COVID-19 epidemic wave periods 1, 2, 3, 4, and 5 in South Africa. The South African COVID-19 epidemic wave periods 1, 2, 3, 4, and 5 were classified as collated data from March 5 to September 30, 2020; October 1, 2020, to April 26, 2021; April 27 to November 14, 2021; November 15, 2021, to April 1, 2022; and April 2 to July 31, 2022, respectively. The labels of stratified variables were given a suffix reference of "1," "2," "3," "4," and "5" for the 5 COVID-19 epidemic periods, respectively. For cumulative epidemiological data (South African cumulative COVID-19 admission age profile, cumulative COVIID-19 hospital deaths age profile, and cumulative COVID-19 patients discharged alive), the data were adjusted using equation 1 to remove the cumulative data from the previous COVID-19 epidemic period:

> Cumulative epidemic variable adj (n), i = cumulative epidemic variable (n), i − cumulative epidemic variable (n), j **(1)**

where n is the number of patients, i is the reported date, and j is the last reported date of the previous COVID-19 epidemic period. The stratification of data in this study was done by splitting the data using the epidemic period variable in SPSS (version 28; IBM Corp).

On the basis of a review of the literature [18,20,21,25-27,41], Table S1 in Multimedia Appendix 1 summarizes the South African SARS-CoV-2 lineage clusters observed in the South African genomic data during the respective observed COVID-19 epidemic wave periods. On the basis of this stratification, the cluster of lineages identified in Table S1 in Multimedia Appendix 1 was assumed to be the SARS-CoV-2 lineages resulting in the respective COVID-19 epidemic waves in South Africa.

Covariance and Regression of South African Epidemiological Data

In this study, covariance between the following epidemiological data was investigated: (1) COVID-19 daily tests and cases, (2) the number of reporting hospitals to NICD DATCOV and COVID-19 hospitalized cases, (3) COVID-19 active and hospitalized cases, (4) daily COVID-19 cases, change in daily

COVID-19 cases, hospital-to-active cases (HA), CFR, DR, admission status, and admitted death status with daily cumulative COVID-19 vaccinated people. The covariance was investigated by applying Bivariate Analysis using 2-tailed Pearson and Spearman tests in SPSS. The 2 respective parametric and nonparametric methods were applied, considering the distribution of data and linearity. The assumed linearity was based on 2-tailed Pearson correlations with Pearson correlations above 0.850 or below 0.850, with $P<.001$ considered as having a high degree of linearity. For data with assumed linearity, further analysis was conducted by applying an ANOVA using the Univariate General Linear Model.

Normalization and Paired *t* tests on South African Epidemiological Data

In this study, COVID-19 transmissibility was measured through the magnitude of mean and variance of the percentage of daily COVID-19 positive tests, considering the linear positive correlation between daily COVID-19 tests and cases. COVID-19 severity was measured through the magnitude of the mean and variance of the COVID-19 hospital admission cases, admission status, admission age profile, death age profile, CFR, DR, and ECDR. Considering the linear positive correlation between COVID-19 active and hospital admission cases, a normalized parameter (COVID-19 HA) was used to normalize the variance of active cases in hospital admission cases.

For the comparative inferential statistical analysis conducted to understand the impact of SARS-CoV-2 evolution on COVID-19 transmissibility and severity in South Africa, descriptive statistics and paired samples *t* test at 95% CIs were conducted on the normalized parameters in SPSS. The paired *t* test was conducted between the COVID-19 epidemic wave periods based on the following *t* test pairings: normalized parameter 1 with normalized parameter 2 (pair 1), normalized parameter 1 with normalized parameter 3 (pair 2), normalized parameter 1 with normalized parameter 4 (pair 3), normalized parameter 1 with normalized parameter 5 (pair 4), normalized parameter 2 with normalized parameter 3 (pair 5), normalized parameter 3 with normalized parameter 4 (pair 6), and normalized parameter 4 with normalized parameter 5 (pair 7) were the suffix number representing the COVID-19 epidemic wave period.

Data Handling and Limitations

The propagated error due to data capturing or data reliability from data sources were not accounted for. Each descriptive and inferential statistical analysis test was applied independently to the analysis data sets, and there was no codependent or propagated error in the results of the applied statistical tests. The time variance of data was accounted for by reporting values to 95% CIs. Missing data values were not included in the analysis. The Pearson and Spearman correlation coefficients are limited to determining the strength of the association between ≥2 or more variables; however, they do not determine the causality of this relationship. In this study, COVID-19 seroprevalence was not investigated as a confounder because of the limitations of these data.

Ethics Approval

An internal ethical assessment was conducted within the Afrikan Research Initiative at the start of the ARI COVID-19 Research Project, and no regional ethics approval was requested for this study. Data used in this study were obtained from public sources with an Open Data Licence. Patient data were obtained from the public source and anonymized following the local regulations of the Protection of Personal Information Act in South Africa.

Results

Covariance and Regression of South African COVID-19 Epidemiological Data

Table 1 shows that the mean COVID-19 daily tests in the first, second, third, fourth, and fifth South African COVID-19 epidemic wave periods were 20,575 (SD 14,062), 31,046 (SD 14,115), 41,315 (SD 16,108), 35,226 (SD 17,078), and 23,419 (SD 7229), respectively.

Table 1. Statistical sample number (n), range, mean (SD), and coefficient of skewness of daily COVID-19 tests and cases in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

Parameter and COVID-19 epidemic wave	Values, n[a]	Values, mean (SD; range)	Skewness
Daily COVID-19 cases			
1	237	2845 (3794; 0-13,944)	1.53
2	208	4336 (5034; 437-21,980)	1.79
3	197	6672 (6252; 0-26,485)	0.84
4	137	5705 (6816; 0-37,875)	1.95
5	82	3293 (2791; 0-13,613)	1.31
Daily COVID-19 tests			
1	203	20,575 (14,062; 4-56,663)	0.47
2	201	31,046 (14,115; 10,402-77,167)	1.16
3	191	41,315 (16,108; 13,507-96,896)	0.62
4	133	35,226 (17,078; 12,942-104,831)	1.40
5	61	23,419 (7299; 9149-39,613)	0.12

[a]Values, n represents the number of observations or records pooled for the mean sample size calculations. All other values represent absolute descriptive statistical values.

The Pearson (coefficient of determination) correlation coefficients between COVID-19 daily tests (independent variable) and cases (dependent variable) in the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa were determined to be 0.910 (0.828), 0.877 (0.769), 0.893 (0.797), 0.859 (0.737), and 0.749 (0.562), respectively (shown in Table S2 in Multimedia Appendix 1). The *F* test values between the mean square regression and residual for the daily COVID-19 tests and cases linear regression in the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa are shown in Table S3 in Multimedia Appendix 1, and the unstandardized and standardized coefficients are shown in Table S4 in Multimedia Appendix 1. The Pearson (coefficient of determination) correlation coefficients between facilities reporting to the NICD DATCoV (independent variable) and COVID-19 hospital admission cases (dependent variable) in the first, second, third, and fourth COVID-19 epidemic waves in South Africa were 0.336 (0.113), 0.212 (0.045), 0.385 (0.148), and −0.249 (−0.062), respectively (Table S5 in Multimedia Appendix 1). The Pearson (coefficient of determination) correlation coefficients between COVID-19 active (independent variable) and hospital admission cases (dependent variable) in the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa were 0.932 (0.869), 0.819 (0.671), 0.967 (0.936), 0.919 (0.845), and 0.863 (0.745), respectively (Table S5 in Multimedia Appendix 1). The unstandardized and standardized coefficients between the daily COVID-19 tests and cases in the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa are presented in Table S6 in Multimedia Appendix 1.

South Africa's COVID-19 NPI health policy response to the COVID-19 epidemic waves in South Africa was implemented in the form of National Lockdown Alert Level policies. The National Lockdown Alert Level policies were largely entry and exit screening at borders, limitations of movements and gatherings, closure and limitations of institutions and business activities, ban and limiting of alcohol and tobacco industries, isolation, quarantine of potentially infected persons, contact-tracing protocols, use of personal protective equipment, and hygienic protocols [38,42]. The adjustment in the alert levels resulted in eased movement restrictions compared with their predecessors. Table S7 in Multimedia Appendix 1 shows the summary of COVID-19 NPI policies implemented in South Africa during the first, second, third, fourth, and fifth COVID-19 epidemic waves.

The mean change in movement from baseline in the retail and recreation, grocery and pharmacy, parks, transit stations, workspaces, and residential locations in South Africa during the implementation of the no national lockdown; National Lockdown Level 5, 4, 3, and 2; and National Lockdown Adjusted Level 4, 3, 2, and 1 are shown in Multimedia Appendix 2. In general, COVID-19 NPI policies in South Africa resulted in a negative mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, and workplaces. Residential locations in South Africa had a positive mean change in movement from baseline. A decrease in the national lockdown alert levels resulted in a decrease in the modulus mean change in movement from the baseline in locations in South Africa. The national lockdown adjusted alert levels resulted in a lower modulus mean change in movement from the baseline in locations in South Africa than the lowest unadjusted Alert Level (National Lockdown Alert Level 2). Concerning movement, the most affected locations in South Africa by the COVID-19 NPI policies implemented were the retail and recreation, transit stations, workplaces, and residential locations. The National Lockdown Alert Level 5 had the largest impact on the movement in South African communities, whereas the National Lockdown Adjusted Alert Level 1 had the least impact on movement in South African communities. Figure 1 shows the mean change in movement from baseline in retail

and recreation, grocery and pharmacy, parks, transit stations, workspaces, and residential locations in South Africa during the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa. Figure 1 shows that the negative mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, and workspaces gradually decreased during each consecutive COVID-19 epidemic wave. The mean change in movement from the baseline in residential locations was positive for the 5 COVID-19 epidemic waves. Figure 1 also shows that the mean change in movement from baseline in South African locations was positive during the fourth and fifth COVID-19 epidemic waves.

The cumulative (maximum) number of people receiving at least 1 dose of the COVID-19 vaccine relative to the total population per age group in the first, second, third, fourth, and fifth COVID-19 epidemic waves is shown in Multimedia Appendix 3. By August 30, 2022, a total of 37,456,345 doses of COVID-19 vaccines had been administered in South Africa. Of the 37,456,345 nationally administered doses, 9,190,172 (24.5%) doses of the COVID-19 vaccine administered were the Johnson & Johnson COVID-19 vaccine, with 1,385,476 (3.7%) doses being the Johnson & Johnson COVID-19 vaccine booster dose; 28,266,173 (75.5%) of the national administered amount was the Pfizer COVID-19 vaccine, with 14,452,185 (38.6%), 11,515,875 (30.7%), and 2,298,113 (6.14%) being the Pfizer COVID-19 vaccine first, second, and third booster doses, respectively [34]. Figure 2 shows the mean daily number of COVID-19 vaccinated people per age group during the first, second, third, fourth, and fifth COVID-19 epidemic waves in South Africa. Figure 2 shows that the mean daily COVID-19 vaccination rate per age group was lowest in the second and fourth COVID-19 epidemic waves and highest in the third COVID-19 epidemic wave for all age groups. There were no reported administered COVID-19 vaccinations in South Africa during the first COVID-19 epidemic wave. Figure 2 shows that there was a decrease in the mean daily COVID-19 vaccination rate in all age groups after the third COVID-19 epidemic wave.

The Spearman correlation coefficients and *P* values among daily COVID-19 cases, change in daily COVID-19 cases, HA, CFR, DR, admission status, and admitted death status with daily cumulative COVID-19 vaccinated people are shown in Table S8 in Multimedia Appendix 1. The Spearman correlation coefficients and *P* values between the daily cumulative COVID-19 vaccinated people and the daily COVID-19 cases in the first half period of the third, fourth, and fifth COVID-19 epidemic waves in South Africa were 0.930 (95% CI 0.890-0.956), 0.842 (95% CI 0.713-0.916), and 0.811 (95% CI 0.673-0.895), respectively, with $P<.001$. The Spearman correlation coefficients and *P* values between the daily cumulative COVID-19 vaccinated people and the change in daily COVID-19 cases were 0.031 ($P=.79$; 95% CI −0.207 to 0.266), −0.014 ($P=.93$; 95% CI −0.341 to 0.316), and −0.077 ($P=.62$; 95% CI −0.374 to 0.233), respectively. These results show a significant strong positive monotonic correlation ($P<.001$) between daily COVID-19 cases and daily cumulative COVID-19 vaccinations and a weak monotonic correlation between the change in daily COVID-19 cases and daily cumulative COVID-19 vaccinations. The Spearman correlation coefficients and *P* values between the daily cumulative COVID-19 vaccinated people and the daily HA were −0.983 (95% CI −0.989 to −0.972), −0.852 ($P=.93$; 95% CI −0.921 to −0.731), and −0.917 ($P=.62$; 95% CI −0.955 to −0.850), respectively, with $P<.001$. These results show a significantly strong negative monotonic correlation ($P<.001$) between daily COVID-19 HA and daily cumulative COVID-19 vaccinated individuals. The Spearman correlation coefficients and *P* values between the daily cumulative COVID-19 vaccinated people and the CFR were 0.380 ($P<.001$; 95% CI 0.160-0.565), 0.192 ($P=.25$; 95% CI −0.150 to 0.494), and 0.264 ($P=.09$; 95% CI −0.049 to 0.529), respectively. These results show a moderate positive monotonic correlation between hospital CFR and daily cumulative COVID-19 vaccinated people. The Spearman correlation coefficients between daily cumulative COVID-19 vaccinated people and the percentage hospitalized age groups of 18 to 34 years showed a significantly strong positive monotonic correlation ($P<.001$), while the age groups of 50 to above 60 years showed a significantly strong negative monotonic correlation ($P<.001$).

Figure 1. Mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residences during the South African first, second, third, fourth, and fifth COVID-19 epidemic waves.

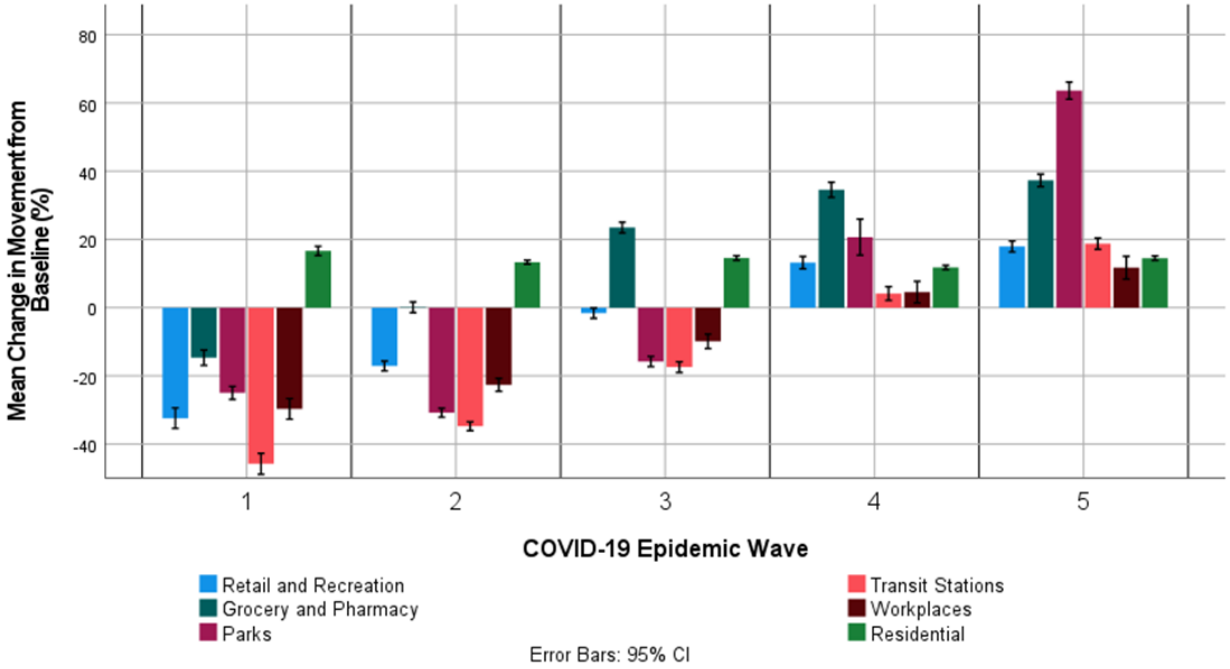
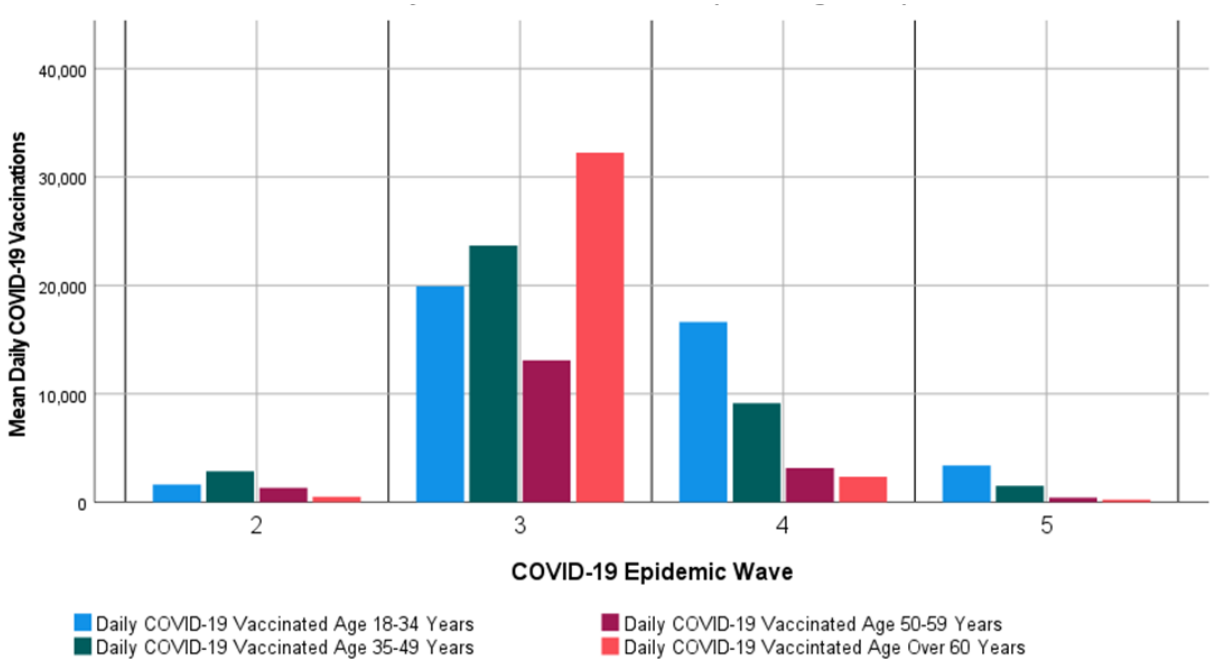


Figure 2. Mean daily number of COVID-19 vaccinated people per age group during the first, second, third, fourth, and fifth COVID-19 epidemic wave in South Africa.



Descriptive Statistics and Paired Samples *t* tests of South African COVID-19 Epidemiological Data

COVID-19 Detection in South Africa

Table 2 shows the descriptive statistics for COVID-19 active and daily positive COVID-19 tests for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa.

The daily positive COVID-19 tests indicate the transmissibility of COVID-19 based on the detection rate of COVID-19 and account for covariance in the testing rate for statistical comparison between the epidemic wave periods. The mean daily positive COVID-19 tests in South Africa's first and second COVID-19 epidemic waves were not statistically different (P=.97). The mean daily positive COVID-19 tests in South Africa's third, fourth, and fifth COVID-19 epidemic waves were 15.7%, 18.4%, and 24.3% more than those of the first and second COVID-19 epidemic wave periods, respectively. The difference among the mean daily positive COVID-19 tests in South Africa's first, second, third, fourth, and fifth COVID-19 epidemic wave periods can also be observed in Figure 3. A paired *t* test of the daily positive COVID-19 tests between the first and second COVID-19 epidemic wave periods (pair 1)

showed no significant difference at a 95% CI between these COVID-19 epidemic periods with $P=.97$ (Table 3). The paired *t* test of the daily positive COVID-19 tests between test pairs 2 to pair 7 showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods (Table 3).

Table 2. Statistical sample number (n), range, and mean (SD) of COVID-19 active cases and daily positive COVID-19 tests in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

Parameter and COVID-19 epidemic wave	Values, n[a]	Values, mean (SD; range)
COVID-19 active cases		
1	237	45,851 (53,975; 0-173,590)
2	208	66,178 (53,878; 19,809-239,799)
3	197	89,171 (63,613; 16,243-211,052)
4	137	75,527 (65,996; 10,849-216,947)
5	82	35,833 (22,323; 9644-81,174)
Daily positive COVID-19 tests (%)		
1	203	11.5 (8.58; 0-34.0)
2	201	11.5 (8.45; 2.7-33.7)
3	191	13.3 (9.72; 0-31.6)
4	133	13.3 (9.91; 0-60.7)
5	61	13.3 (8.49; 0-41.6)

[a]Values, n represents the number of observations or records pooled for the mean sample calculations. All other values represent absolute descriptive statistical values.

Figure 3. The mean daily positive COVID-19 tests (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.

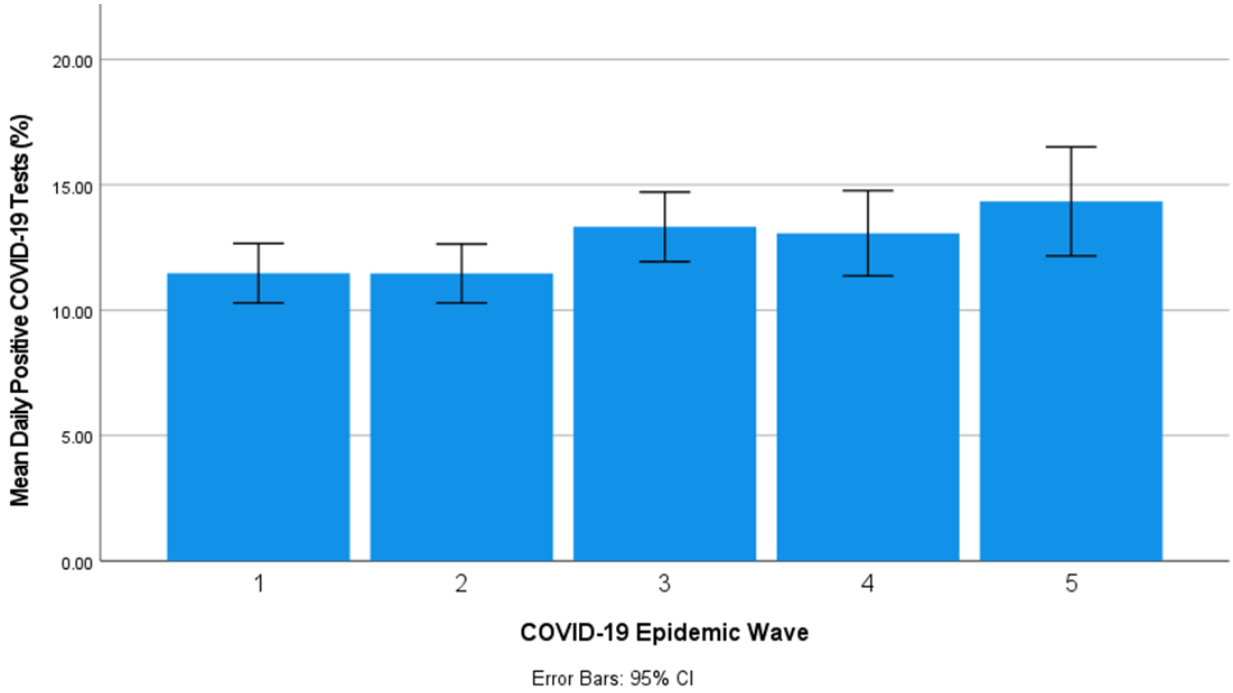


Table 3. Mean paired differences, SD of paired differences, SE of mean (SE mean), 95% CI of the upper and lower difference, t value, df, and *P* value (significance, 2-tailed) for the daily positive COVID-19 tests in pair 1 to pair 7 (paired samples *t* test).

Sample *t* test pairing	Paired variables	Paired differences, mean (SD)	*t* test (*df*)	Significance (2-tailed), *P* value
Pair 1	Daily positive COVID Test1—daily positive COVID Test2	−0.03 (13.26)	−0.04 (198)	.97
Pair 2	Daily positive COVID Test1—daily positive COVID Test3	−2.78 (14.42)	−2.65 (187)	.009
Pair 3	Daily positive COVID Test1—daily positive COVID Test4	−5.00 (15.10)	−3.66 (121)	<.001
Pair 4	Daily positive COVID Test1—daily positive COVID Test5	−10.8 (10.67)	−7.39 (52)	<.001
Pair 5	Daily positive COVID Test2—daily positive COVID Test3	−1.20 (5.95)	−2.80 (191)	.006
Pair 6	Daily positive COVID Test3—daily positive COVID Test4	5.22 (14.87)	3.96 (126)	<.001
Pair 7	Daily positive COVID Test4—daily positive COVID Test5	2.71 (8.85)	2.37 (59)	.02

COVID-19 Hospital Admissions in South Africa

Table 4 shows the descriptive statistics for the COVID-19 HA for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. The second COVID-19 epidemic wave period in South Africa had the highest number of COVID-19 HA, followed by the third COVID-19 epidemic wave period. The mean paired difference of the COVID-19 HA in pairs 1, 2, 3, 4, 5, 6, and 7 was −14.0%, −10.6%, −4.62%, −7.72%, 2.31%, 4.02%, and −2.34%, respectively (Table 5). The difference between the mean number of COVID-19 HA in South Africa's first, second, third, fourth, and fifth COVID-19 epidemic wave periods can also be observed in Figure 4. Paired *t* tests of the COVID-19 HA among pairs 1, 2, 3, 4, 5, 6, and 7 showed a significant difference at a 95% CI between the respective COVID-19 epidemic periods with $P<.001$ (Table 5).

Multimedia Appendix 4, Multimedia Appendix 5, and Multimedia Appendix 6 show the COVID-19 HA and vaccinated people in the first half period of the third, fourth, and fifth COVID-19 epidemic wave in South Africa, respectively. The first half period of the COVID-19 epidemic wave is the period from the first case to the peak of the epidemic wave. Multimedia Appendices 4-6 show that the number of COVID-19 HA decreased while the number of COVID-19 vaccinated people increased in the first half period of the third, fourth,' and fifth COVID-19 epidemic wave in South Africa.

Table 4. Statistical sample number (N), minimum, maximum, and mean (SD) of COVID-19 hospital-to-active cases in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

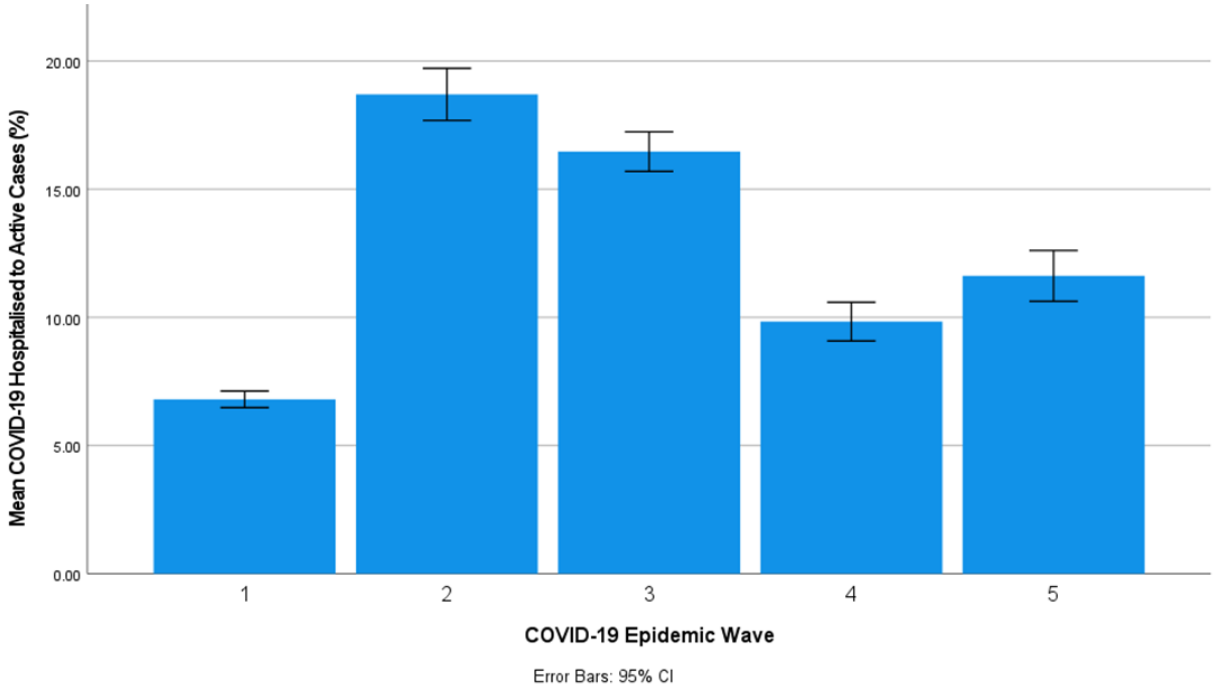
Parameter and COVID-19 epidemic wave	Values, N[a]	Values, mean (SD; range)
COVID-19 hospital-to-active cases (%)		
1	126	6.80 (1.82; 4.02-12.7)
2	189	18.7 (7.08; 0-32.4)
3	202	16.5 (5.56; 9.28-29.0)
4	138	9.84 (4.48; 3.65-24.2)
5	113	4.48 (5.32; 3.73-21.7)

[a]Values, n represents the number of observations or records pooled for the mean sample calculations. All other values represent absolute descriptive statistical values.

Table 5. Mean paired differences, SD of paired differences, SE of mean (SE mean), t value, df, and *P* value (significance, 2-tailed) for the COVID-19 hospital-to-active (HA) cases in pair 1 to pair 7 (paired samples *t* test).

Sample *t* test pairing	Paired variables	Paired differences, mean (SD)	*t* test (*df*)	Significance (2-tailed), *P* value
Pair 1	HA1—HA2	−14.0 (8.59)	−18.3 (126)	<.001
Pair 2	HA1—HA3	−10.6 (5.99)	−19.7 (122)	<.001
Pair 3	HA1—HA4	−4.62 (4.41)	−7.48 (50)	<.001
Pair 4	HA1—HA5	−7.72 (4.01)	−11.2 (33)	<.001
Pair 5	HA2—HA3	2.31 (7.34)	4.33 (189)	<.001
Pair 6	HA3—HA4	4.02 (5.21)	9.07 (137)	<.001
Pair 7	HA4—HA5	−2.34 (3.04)	−8.42 (119)	<.001

Figure 4. The mean COVID-19 hospitalized-to-active cases (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.



COVID-19 Hospital Admission Status in South Africa

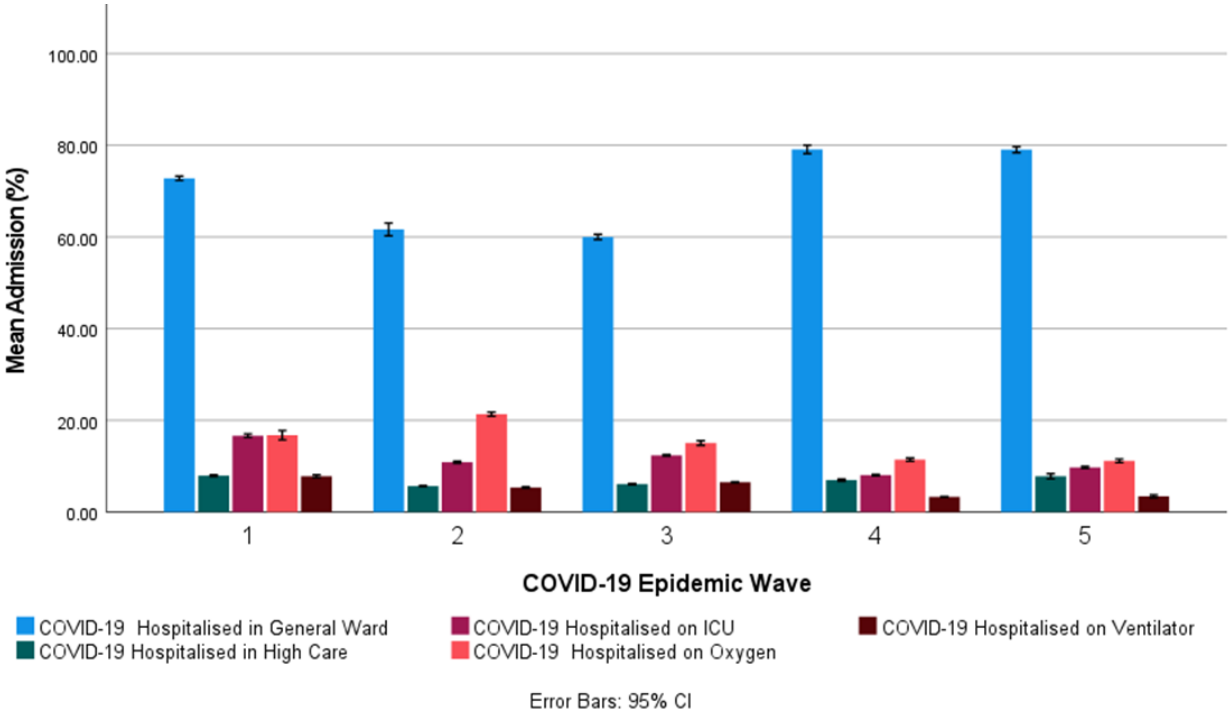
Table 6 shows the descriptive statistics for the COVID-19 hospital admission status for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Figure 5 shows the COVID-19 hospital admission status profiles in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Figure 5 shows that most COVID-19 hospitalized cases in South Africa were hospitalized in the general ward (60.0%-79.1%). Figure 5 also shows that the COVID-19 patients on oxygen were the second-largest admission status (11.2%-16.8%), followed by patients with COVID-19 in the ICU (8.07%-16.7%). The number of hospitalized COVID-19 cases admitted to the general ward was highest in the fourth and fifth COVID-19 epidemic wave periods, followed by the first COVID-19 epidemic wave period. The number of South African patients with COVID-19 admitted in high care, in the ICU, and on ventilators was highest in the first COVID-19 epidemic wave. The number of patients with COVID-19 on oxygen was highest in the second COVID-19 epidemic wave. The general trend showed a decrease in patients admitted on oxygen, in the ICU, and on a ventilator in the fourth and fifth COVID-19 epidemic waves compared with previous COVID-19 epidemic waves. Patients in high care have remained relatively in the mean range of 5.65% (SD 0.80%) to 7.93% (SD 1.03%) throughout the COVID-19 epidemic in South Africa. Paired *t* tests of the mean COVID-19 hospital admission status in pairs 1, 2, 3, 4, 5, 6, and 7 showed a significant difference at a 95% CI between the respective COVID-19 epidemic periods with *P*<.001 (Table S9 in Multimedia Appendix 1). Except for the COVID-19 hospitalized cases admitted in the general ward, in high care, on oxygen, and on ventilator between the fourth and fifth COVID-19 epidemic waves (pair 7), whose difference was found not to be statistically significant (Table S9 in Multimedia Appendix 1).

Table 6. Statistical sample number (n), range, and mean (SD) of COVID-19 hospitalized cases in the general ward, in high care, in the intensive care unit (ICU), on oxygen, and on ventilator in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

Parameter and COVID-19 epidemic wave	Values, n[a]	Values, mean (SD; range)	Variance
COVID-19 hospitalized general ward (%)			
1	126	72.8 (2.62; 68.7-78.1)	6.9
2	187	61.7 (9.61; 52.6-86.2)	92.4
3	202	60 (4.10; 50.9-70.7)	16.8
4	138	79.1 (5.45; 63.8-84.4)	29.7
5	126	79.1 (3.71; 56.9-84.3)	13.8
COVID-19 hospitalized high care (%)			
1	126	7.93 (1.03; 5.07-10.5)	1.07
2	187	5.65 (0.80; 4.09-7.81)	0.647
3	202	6.09 (0.81; 4.51-7.80)	0.651
4	138	6.96 (1.33; 4.66-11.0)	1.76
5	126	7.81 (3.22; 4.29-30.0)	10.34
COVID-19 hospitalized ICU (%)			
1	126	16.7 (2.17; 11.9-20.6)	4.69
2	187	10.9 (1.49; 7.96-14.1)	2.21
3	202	12.4 (1.12; 9.65-15.1)	1.25
4	138	8.07 (0.89; 6.33-10.1)	0.789
5	126	9.74 (1.28; 6.97-11.8)	1.646
COVID-19 hospitalized on oxygen (%)			
1	126	16.8 (5.76; 10.6-30.8)	33.2
2	187	21.4 (3.12; 14.7-27.7)	9.72
3	202	15.1 (3.64; 8.43-23.9)	13.3
4	138	11.4 (2.02; 7.66-16.3)	4.07
5	126	11.2 (2.18; 8.81-26.3)	4.75
COVID-19 hospitalized on ventilator (%)			
1	126	7.81 (1.79; 4.87-10.8)	3.20
2	187	5.34 (1.17; 3.28-6.93)	1.38
3	202	6.49 (0.68; 3.86-8.08)	0.458
4	138	3.29 (0.45; 2.47-4.50)	0.205
5	126	3.41 (1.74; 2.33-16.3)	3.013

[a]Values, n represents the number of observations or number of records pooled for the mean sample calculations. All other values represent the absolute descriptive statistical values.

Figure 5. The mean COVID-19 hospitalized admission status (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.



COVID-19 Hospital Admission Age Profile in South Africa

Table 7 shows the descriptive statistics for the COVID-19 hospital admission age profiles for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa.

Figure 6 shows the COVID-19 hospital admission age profile in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Figure 6 shows that most COVID-19 hospitalized cases in South Africa's first, second, third, and fourth COVID-19 epidemic wave periods were in the ages of 40 to 49 years (16.8%-20.4%) and 50 to 59 years (19.8%-25.3%), respectively. Patients admitted owing to COVID-19 in the age groups of 0 to 19 years were relatively low (1.98%-4.59%) and highest in the fourth and fifth COVID-19 epidemic wave periods. Figure 6 shows that the mean age profile for COVID-19 hospital admissions for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods had a similar relative normal distribution within the admitted age groups. In general, COVID-19 hospital admissions in South Africa for the age groups between 0 and 29 years increased after each consecutive COVID-19 epidemic wave, while those for age groups between 30 and 79 years decreased.

Paired *t* tests of the COVID-19 hospital admission age profile between the COVID-19 epidemic waves for all age groups showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with $P<.001$ (Table S10 in Multimedia Appendix 1), except for the following:

- Age groups between 20 and 29 years, 50 and 59 and 60 and 69 years between COVID-19 epidemic waves 2 and 3 ($P=.12$, $P=.08$, and $P=.68$, respectively).

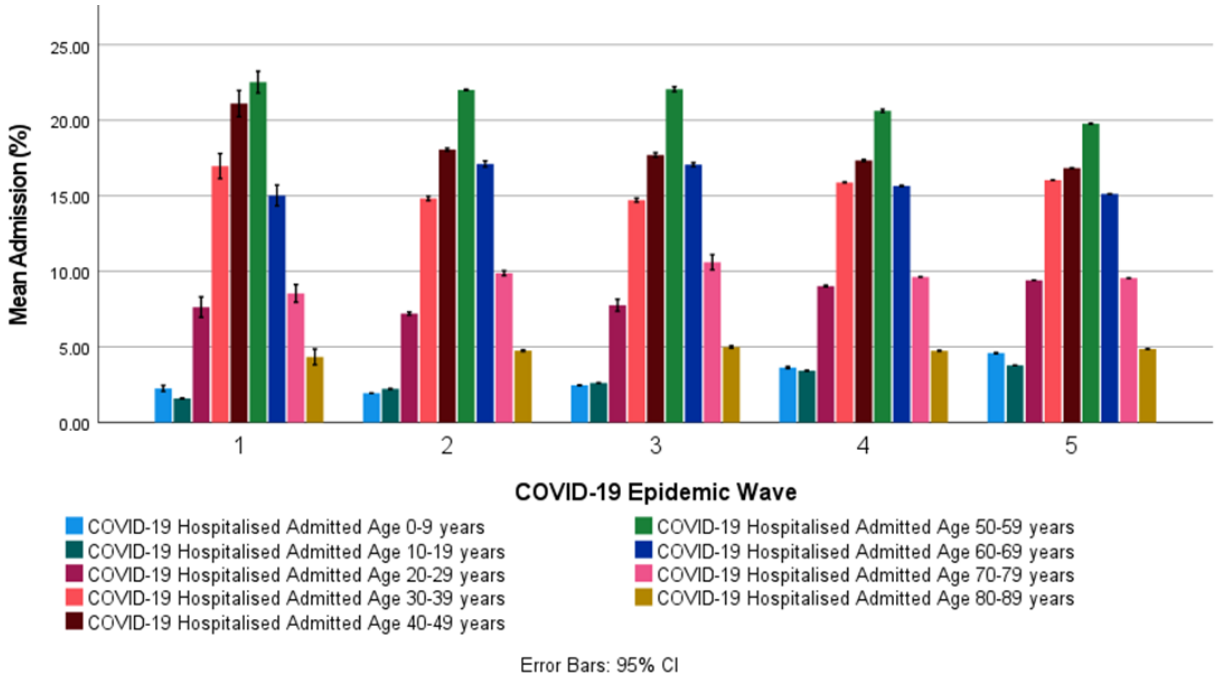
Table 7. Statistical sample number (n), range, mean, and SD of COVID-19 hospitalized cases in the ages of 0 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, 60 to 69, 70 to 79, and 80 to 89 years in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

Parameter and COVID-19 epidemic wave	Values, n[a]	Values, mean (SD; range)	Variance
COVID-19 hospitalized admitted (age 0-9 years; %)			
1	110	2.32 (0.58; 1.68-3.96)	0.34
2	187	1.98 (0.25; 1.81-3.66)	0.06
3	202	2.47 (0.19; 1.14-3.48)	0.03
4	138	3.63 (0.40; 2.92-4.14)	0.16
5	126	4.59 (0.21; 4.21-4.83)	0.05
COVID-19 hospitalized admitted (age 10-19 years; %)			
1	108	1.75 (0.22; 1.23-2.62)	0.05
2	179	2.23 (0.18; 1.98-2.56)	0.03
3	202	2.62 (0.20; 1.19-3.68)	0.04
4	138	3.43 (0.18; 3.09-3.66)	0.03
5	126	3.78 (0.03; 3.71-3.81)	0.00
COVID-19 hospitalized admitted (age 20-29 years; %)			
1	126	8.04 (2.11; 0.04-14.6)	4.46
2	187	7.39 (1.19; 0.63-13.8)	1.41
3	202	7.78 (2.83; 3.30-47.3)	8.02
4	138	9.03 (0.34; 8.29-9.32)	0.11
5	126	9.42 (0.01; 9.40-9.43)	0.00
COVID-19 hospitalized admitted (age 30-39 years; %)			
1	126	18.1 (3.27; 11.4-29.8)	10.68
2	187	15.3 (2.49; 13.0-30.5)	6.20
3	202	14.8 (0.98; 6.36-18.9)	0.96
4	138	15.9 (0.16; 15.5-16.0)	0.02
5	126	16.0 (0.05; 16.0-16.1)	0.00
COVID-19 hospitalized admitted (age 40-49 years; %)			
1	75	20.4 (2.64; 18.8-27.4)	6.99
2	181	18.17 (1.52; 17.2-36.5)	2.31
3	201	17.73 (1.11; 7.70-22.6)	1.23
4	138	17.3 (0.40; 17.0-18.6)	0.16
5	126	16.8 (0.13; 16.7-17.0)	0.02
COVID-19 hospitalized admitted (age 50-59 years; %)			
1	126	25.3 (4.07; 3.54-38.4)	16.58
2	186	22.5 (2.57; 21.0-39.1)	6.61
3	201	22.1 (1.21; 9.76-27.2)	1.47
4	138	20.6 (0.64; 19.4-21.8)	0.41
5	126	19.8 (0.19; 19.6-20.1)	0.03
COVID-19 hospitalized admitted (age 60-69 years; %)			
1	125	17.46 (2.73; 10.5-24.3)	7.47
2	186	17.32 (1.91; 14.9-28.1)	3.64
3	202	17.11 (1.03; 7.65-22.5)	1.06

Parameter and COVID-19 epidemic wave	Values, n[a]	Values, mean (SD; range)	Variance
4	138	15.66 (0.29; 15.3-16.7)	0.08
5	126	15.13 (0.07; 15.1-15.3)	0.01
COVID-19 hospitalized admitted (age 70-79 years; %)			
1	126	10.17 (1.98; 4.56-16.3)	3.91
2	183	9.90 (1.16; 8.08-13.9)	1.33
3	202	10.6 (3.61; 5.44-60.5)	13.04
4	138	9.63 (0.08; 9.47-9.85)	0.01
5	126	9.55 (0.07; 9.45-9.63)	0.00
COVID-19 hospitalized admitted (age 80-89 years; %)			
1	125	5.498 (1.54; 0.88-9.43)	2.38
2	183	4.77 (0.45; 3.74-7.16)	0.20
3	202	5.01 (0.57; 2.41-7.16)	0.32
4	138	4.74 (0.19; 4.44-4.92)	0.04
5	126	4.87 (0.12; 4.69-5.01)	0.01

[a]Values, n represents the number of observations or number of records pooled for the mean sample calculations. All other values represent the absolute descriptive statistical values.

Figure 6. The mean COVID-19 hospitalized admission age profile (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.



COVID-19 Hospital Deaths Age Profile in South Africa

Table 8 shows the descriptive statistics for the COVID-19 hospital death age profiles for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Table 8 also shows the cumulative COVID-19 death age risk ratio, with age groups of 0 to 9 years as the reference.

Figure 7 shows the mean COVID-19 hospital death age profiles in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Figure 7 shows that most COVID-19 hospitalized deaths in South Africa in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods were in the ages of 50 to 59 years (15.8%-24.8%), 60 to 69 years (15.9%-29.5%), and 70 to 79 years (16.6%-20.7%). COVID-19 hospitalized deaths in the age groups of 0 to 29 years were relatively low (0.227%-4.89%). Figure 7 also shows that the COVID-19 hospitalized death age profiles for the first, second, and third COVID-19 epidemic wave periods were similar in distribution, while the distributions in the fourth and fifth COVID-19 epidemic waves were also similar. The mean COVID-19 hospitalized deaths in the fourth and fifth COVID-19 epidemic waves for the age groups of 0 to 49 years and 80 to

89 years were significantly higher than those observed in the first, second, and third COVID-19 epidemic waves, while the age groups of 50 to 69 years were significantly lower than the respective COVID-19 epidemic waves. General trends show an increase in COVID-19 hospitalized deaths in the age groups of 0 to 49 years and 80 to 89 years after each consecutive COVID-19 epidemic wave and a decrease in the age groups of 50 to 79 years. The cumulative risk of death in COVID-19 hospitalized deaths increased with increasing age groups. The cumulative risk of death in COVID-19 hospitalized deaths in the age groups above 40 years was significantly lower in the fourth and fifth COVID-19 epidemic waves when compared with prior COVID-19 epidemic waves.

Paired *t* tests of the COVID-19 hospital admission age profile between the COVID-19 epidemic waves for the age groups of 10 to 39 years and 50 to 89 years showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with $P<.001$ (Table S11 in Multimedia Appendix 1). Paired *t* tests of the COVID-19 hospital admission age profile between the COVID-19 epidemic waves for the age groups of 40 to 49 years showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with $P<.001$, except for the paired *t* tests among the first, fourth, and fifth COVID-19 epidemic waves. The differences were statistically insignificant with $P=.34$ and $P=.32$ (Table S11 in Multimedia Appendix 1).

Table 8. Statistical sample number (n), range, mean, SD of COVID-19 hospitalized deaths in the ages of 0 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, 60 to 69, 70 to 79, and 80 to 89 years in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

Parameter and COVID-19 epidemic wave	Values, n[a]	Values, mean (SD; range)	Variance	CFARR[b,c]
COVID-19 hospitalized deaths (age 0-9 years; %)				
1	126	0.227 (0.154; 0-1.15)	0.024	Reference 1
2	174	0.23 (0.082; 0-0.56)	0.007	Reference 2
3	198	0.837 (5.3; 0.12-75)	28.1	Reference 3
4	138	1.88 (1.043; 0.16-7.85)	1.087	Reference 4
5	126	1.23 (1.193; 0.08-7.69)	1.424	Reference 5
COVID-19 hospitalized deaths (age 10-19 years; %)				
1	126	0.298 (0.17; 0-1.70)	0.029	1.31
2	182	0.309 (0.109; 0.23-1.45)	0.012	1.35
3	201	0.273 (0.076; 0-0.36)	0.006	0.33
4	134	1.01 (0.595; 0-5.25)	0.354	0.54
5	126	0.94 (0.711; 0.03-5.56)	0.506	0.76
COVID-19 hospitalized deaths (age 20-29 years; %)				
1	126	1.57 (0.65; 0-7.04)	0.422	6.90
2	183	1.58 (0.442; 1.14-4.93)	0.195	6.86
3	200	1.22 (0.26; 0.00-1.61)	0.068	1.45
4	138	4.68 (2.31; 0.94-21.45)	5.34	2.49
5	126	4.89 (4.21; 0.34-22.22)	17.73	3.99
COVID-19 hospitalized deaths (age 30-39 years; %)				
1	126	6.02 (2.01; 1.03-26.82)	4.02	26.5
2	183	4.51 (0.828; 2.93-8.75)	0.686	19.6
3	202	5.08 (1.51; 3.07-25)	2.27	6.06
4	138	11.9 (6.29; 4.05-51.78)	39.52	6.33
5	126	10.6 (3.23; 0.92-25.93)	10.45	8.65
COVID-19 hospitalized deaths (age 40-49 years; %)				
1	126	13.7 (4.8; 7.22-63.29)	23.04	60.2
2	183	8.78 (1.87; 4.19-13.75)	3.50	38.2
3	202	9.19 (1.59; 0-10.88)	2.54	11.0
4	138	14.2 (6.54; 11.17-59.95)	42.82	7.57
5	125	13.2 (3.26; 1.58-18.73)	10.64	10.7
COVID-19 hospitalized deaths (age 50-59 years; %)				
1	76	24.8 (2.68; 14.78-37.50)	7.21	109
2	179	17.1 (3.15; 9.29-19.43)	9.93	74.6
3	202	20.3 (1.7; 17.14-25)	2.88	24.2
4	138	15.8 (7.21; 12.03-70.04)	52.0	8.42
5	126	16.1 (4.38; 1.90-25.78)	19.2	13.1
COVID-19 hospitalized deaths (age 60-69 years; %)				
1	125	29.5 (4.11; 23.80-39.86)	16.9	130
2	183	23.5 (4.62; 13.13-36.67)	21.32	103
3	201	26.9 (2.6; 24.81-36.97)	6.78	32.1

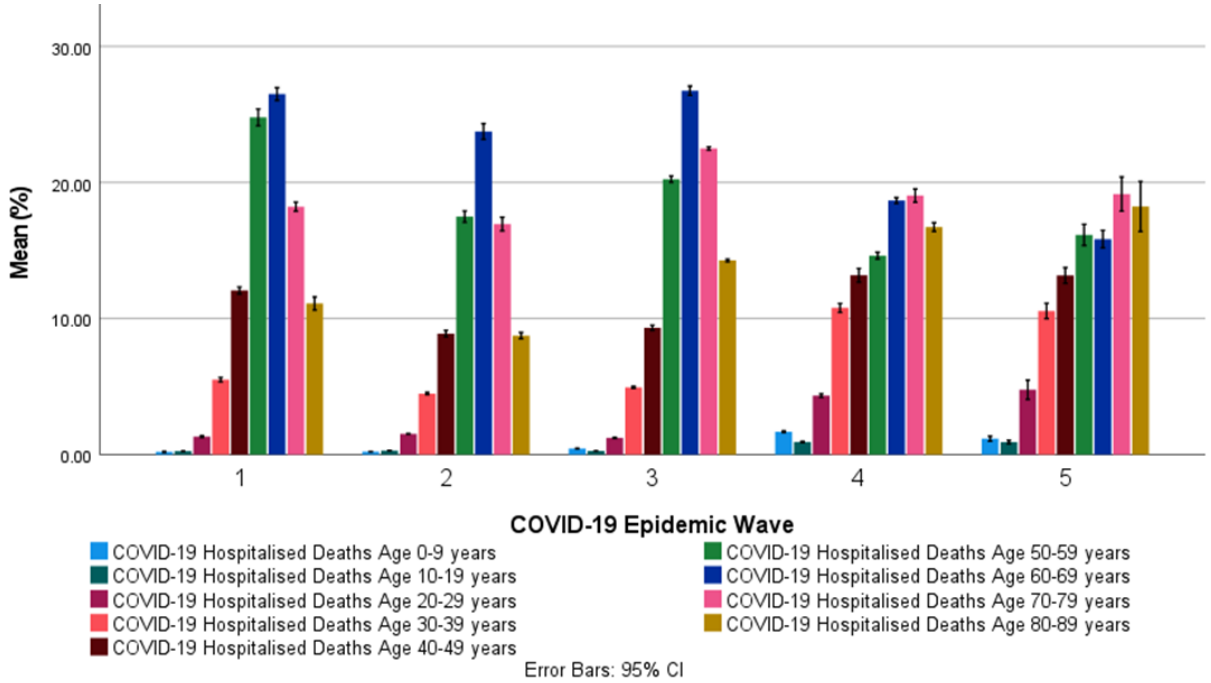
Parameter and COVID-19 epidemic wave	Values, n[a]	Values, mean (SD; range)	Variance	CFARR[b,c]
4	134	18.5 (2.05; 0-24.09)	4.22	9.82
5	126	15.9 (3.61; 1.49-22.22)	13.01	12.9
COVID-19 hospitalized deaths (age 70-79 years; %)				
1	125	20.7 (3.33; 11.75-25.45)	11.11	91.3
2	183	16.6 (3.7; 8.28-22.28)	13.7	72.4
3	202	22.6 (2.18; 15.13-50)	4.74	27.0
4	138	20.1 (10.5; 6.67-102)	110	10.7
5	126	19.1 (7.1; 1.56-91.57)	50	15.6
COVID-19 hospitalized deaths (age 80-89 years; %)				
1	125	13.5 (3.44; 2.16-18.18)	11.8	59.6
2	183	8.77 (2.22; 4.95-27.54)	4.93	38.2
3	202	14.3 (1.03; 11.74-25)	1.06	17.1
4	136	17.7 (8.8; 5.13-87.65)	77.5	9.39
5	126	18.2 (10.38; 1.11-92.06)	107.8	14.8

[a]Values, n represents the number of observations or records pooled for the mean sample calculations. All other values represent absolute descriptive statistical values.

[b]CFARR: cumulative COVID-19 death age risk ratio.

[c]CFARR with COVID-19 epidemic wave references, reference 1, reference 2, reference 3, reference 4, and reference 5 as the mean COVID-19 hospitalized deaths (age 0 to 9 years; %) for the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa, respectively.

Figure 7. The mean COVID-19 hospitalized death age profile (with 95% CI error bars) in the first, second, third, fourth, and fifth COVID-19 epidemic wave period in South Africa.



COVID-19 CFR, DR, and ECDR in South Africa

Table 9 shows the descriptive statistics for the COVID-19 hospital CFR, hospital DR, natural deaths, excess natural deaths, weekly reported COVID-19 deaths, and weekly unreported ECDR for the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa. Paired *t* tests of the CFR between the COVID-19 epidemic waves showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with $P<.001$ (Table S12 in Multimedia Appendix 1). Except for the paired *t* tests among the third, fourth, and fifth COVID-19 epidemic waves. Paired *t* tests of the DR between the COVID-19 epidemic waves showed no statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with lowest $P=.27$ (Table S12 in Multimedia Appendix 1). Except for the

paired *t* tests between the third and fourth COVID-19 epidemic periods. Paired *t* tests of the ECDR between the COVID-19 epidemic waves showed no statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with lowest *P*=.69 (Table S12 in Multimedia Appendix 1). Paired *t* tests of the weekly COVID-19 deaths and excess deaths between the COVID-19 epidemic waves showed statistically significant differences at a 95% CI between the respective COVID-19 epidemic periods with *P*<.001 (Table S12 in Multimedia Appendix 1).

Table 9. Statistical sample number (n), range, mean, SD of COVID-19 hospital case fatality rate (CFR), hospital discharge rate (DR), weekly natural deaths, excess natural deaths, reported COVID-19 deaths, and the weekly unreported excess deaths (natural) to COVID-19 death ratio (ECDR) in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods in South Africa (descriptive statistics).

Parameter and COVID-19 epidemic wave	Values, n[a]	Values, mean (SD; range)
CFR		
1	121	2.06 (1.10; 0 to 6.9)
2	180	2.33 (1.59; 0 to 12.9)
3	199	1.76 (1.18; 0.07 to 10.1)
4	129	1.63 (7.57; 0 to 86.3)
5	117	0.99 (1.72; 0 to 18.1)
DR		
1	121	8.40 (4.89; 0 to 42.5)
2	180	8.03 (8.62; 0 to 77.6)
3	200	6.11 (2.90; 0.19 to 16.0)
4	137	9.30 (7.70; 0 to 63.2)
5	118	10.1 (10.7; 0 to 111.9)
ECDR		
1	22	1.88 (2.34; −0.37 to 6.43)
2	30	1.99 (1.38; 0.30 to 4.25)
3	29	2.78 (1.92; 0.53 to 8.96)
4	18	4.81 (5.58; −0.43 to 17.64)
5	18	13.3 (8.48; 5.34 to 30.30)
Weekly excess deaths		
1	22	2134 (2155; 35 to 6676)
2	30	3822 (4277; 752 to 16,123)
3	29	3856 (2837; 834 to 10,339)
4	19	1655 (954; 832 to 3571)
5	20	1197 (492; 383 to 2055)
Weekly natural deaths		
1	36	9975 (2387; 7819 to 15,865)
2	30	11,941 (4341; 9041 to 24,215)
3	29	12,751 (3262; 8863 to 19,959)
4	19	9558 (1168; 8495 to 11,891)
5	20	10,070 (624; 8945 to 11,197)
Weekly COVID-19 deaths		
Baseline	4	7884 (554; 7454 to 8662)
1	35	469 (574; 0 to 2042)
2	30	1258 (1123; 324 to 3942)
3	29	1228 (902; 155 to 2916)
4	18	577 (456; 85 to 1674)
5	20	101 (71; 0 to 232)

[a]Values, n represents the number of observations or number of records pooled for the mean sample calculations. All other values represent the absolute descriptive statistical values.

Discussion

Principal Findings

The values of the Pearson correlation coefficients obtained between the daily COVID-19 tests and cases in this study indicate a strong positive association between daily COVID-19 tests and cases in the 5 COVID-19 epidemic waves in South Africa. The *F* test values and standardized coefficients obtained between the respective parameters using ANOVA and the Univariate General Linear Model indicated that the residual error between the linear predicted values and the actual values was relatively small, showing a high degree of linearity. On the basis of the Probability Theory, an increase in testing results in an increased probability of the outcome of detection of a positive COVID-19 test. In this study, COVID-19 transmissibility was measured based on the magnitude of the mean and variance of daily COVID-19 positive tests (COVID-19 detection rate). Considering the linear positive association between daily COVID-19 tests and cases, the COVID-19 detection rate normalized the variance between the respective parameters. The mean daily positive COVID-19 tests in South Africa's first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 11.5% (SD 8.58%), 11.5% (SD 8.45%), 13.3% (SD 9.72%), 13.1% (SD 9.91%), and 14.3% (SD 8.49%), respectively. The mean daily positive COVID-19 test results in South Africa's first and second COVID-19 epidemic waves showed no significant difference at a 95% CI with $P=.97$. The mean daily positive COVID-19 tests in South Africa's third, fourth, and fifth COVID-19 epidemic waves were 15.7%, 18.4%, and 24.3% higher than those of the first and second COVID-19 epidemic wave periods, respectively, with statistically significant differences at a 95% CI. These results suggest that the COVID-19 transmission rates in the first and second COVID-19 epidemic waves in South Africa were similar, while the COVID-19 transmission rate was higher in the third, fourth, and fifth COVID-19 epidemic waves than in the first and second waves.

The negative mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, and workspaces gradually decreased during each consecutive COVID-19 epidemic wave as NPIs in South Africa were relaxed. The mean change in movement from baseline in residential locations was positive for the 5 COVID-19 epidemic waves. By the fourth and fifth COVID-19 epidemic waves, the mean change in movement from the baseline in all South African locations was positive, indicating greater movement than that observed before the COVID-19 pandemic (baseline, median values of movement in the respective locations from January 3 to February 6, 2020). The daily effective contact rate is the average number of adequate contacts per infective per day; it is directly proportional to the reproductive number [43]. Mabuka et al [38] showed through stochastic COVID-19 modeling that adjusting the NPIs by 1 Alert Level in South Africa translated into a reduction in the effective SARS-CoV-2 daily contact number by 4.13% to 14.6%. Thus, the relaxation of NPIs in South Africa after each consecutive COVID-19 epidemic wave could have possibly contributed to the increase in COVID-19 transmissibility in the COVID-19 epidemic waves.

The emerging dominant SARS-CoV-2 lineages in the South African SARS-CoV-2 genotypes collected during the first, second, third, fourth, and fifth COVID-19 epidemic waves were alpha (B.1.1.54, B.1.1.56, and C.1), beta (B.1.351), delta (B.1.617.2), and omicron B.1.1.529 and omicron BA.4, BA.2*, BA.5, BA.4, and BA.5.* SARS-CoV-2 variants or lineages, respectively. The beta, delta, and omicron SARS-CoV-2 lineages had major mutations in the spike protein. The beta (B.1.351) SARS-CoV-2 variant had 8 of its 17 mutations in the spike protein at Δ69-70 deletion, Δ144 deletion, N501Y, A570D, P681H, T716I, S982A, and D1118H [44-46]. The delta (B.1.617.2) SARS-CoV-2 variant had 10 mutations in the spike protein at T19R (G142D*), 156del, 157del, R158G, L452R, T478K, D614G, P681R, and D950N [47]. The omicron SARS-CoV-2 variant (B.1.1.529 lineage) had at least 34 mutations (30 amino acid substitutions, 3 small deletions, and 1 small insertion) in its genome. In total, 15 of the 30 amino acid substitutions in the omicron SARS-CoV-2 variant were in the receptor-binding domain. The key amino acid substitutions in the spike (S) protein were at A67V, del69-70, T95I, del142-144, Y145D, del211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F [48]. SARS-CoV-2 during infection binds to human angiotensin-converting enzyme (ACE) 2 receptors through the SARS-CoV-2 spike protein (S1). Some of the mutations observed in these lineages showed increased affinity by the spike protein in SARS-CoV-2 to the human ACE 2 receptors [49,50]. Thus, the increased COVID-19 transmission rate in the third, fourth, and fifth COVID-19 epidemic waves in South Africa could have been also a result of the mutations in the detected SARS-CoV-2 lineages in the respective COVID-19 epidemic waves.

The values of the Pearson, standardized coefficients, and coefficient of determination obtained between the number of COVID-19 active and hospital admission cases in this study indicated a strong linear positive association between COVID-19 active and hospital admission cases in the 5 COVID-19 epidemic waves in South Africa. This correlation was also well demonstrated by stochastic COVID-19 epidemiological models [38,51,52]. Considering this linear positive association between COVID-19 active and hospital admission cases, COVID-19 HA was used to understand the impact of SARS-CoV-2 lineages and COVID-19 vaccination on COVID-19 hospitalization in South Africa. The mean COVID-19 daily HA in South Africa's first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 6.80% (SD 1.82%), 18.7% (SD 7.08%), 16.5% (SD 5.56%), 9.84% (SD 4.48%), and 11.6% (SD 11.6%), respectively. The second COVID-19 epidemic wave period in South Africa had the highest number of COVID-19 HA, followed by the third COVID-19 epidemic wave period. The COVID-19 HA decreased during the first half period of the third, fourth, and fifth COVID-19 epidemic waves (Multimedia Appendices 4-6). The first half period of the COVID-19 epidemic wave is the period from the first case to the peak of the epidemic wave.

Most COVID-19 hospitalized cases in South Africa were hospitalized in the general ward (60.0%-79.1%). Patients with COVID-19 on oxygen were the second-largest admission status (11.2%-16.8%), followed by patients with COVID-19 in the ICU (8.07%-16.7%). The general trend showed a decrease in patients admitted on oxygen, in the ICU, and on a ventilator in the fourth and fifth COVID-19 epidemic waves compared with previous COVID-19 epidemic waves. The patients in high care remained relatively similar, with a mean range of 5.65% to 7.93% throughout the COVID-19 epidemic in South Africa. Most COVID-19 hospitalized cases in South Africa's first, second, third, and fourth COVID-19 epidemic wave periods were in the ages of 40 to 49 years (16.8%-20.4%) and 50 to 59 years (19.8%-25.3%). Patients admitted owing to COVID-19 in the age groups of 0 to 19 years were relatively low (1.98%-4.59%) and highest in the fourth and fifth COVID-19 epidemic wave periods. In general, COVID-19 hospital admissions in South Africa for the age groups between 0 and 29 years increased after each consecutive COVID-19 epidemic wave, whereas for age groups between 30 and 79 years, they decreased. Most COVID-19 hospitalized deaths in South Africa in the first, second, third, fourth, and fifth COVID-19 epidemic wave periods were in the ages of 50 to 59 years (15.8%-24.8%), 60 to 69 years (15.9%-29.5%), and 70 to 79 years (16.6%-20.7%). COVID-19 hospitalized deaths in the age groups of 0 to 29 years were relatively low (0.227%-4.89%). The worldwide incidence of COVID-19 deaths in children has been reported to be low [8]. The cumulative risk of death in COVID-19 hospitalized deaths increased with increasing age groups. The cumulative risk of death in hospitalized COVID-19 deaths in the age groups above 40 years was significantly lower in the fourth and fifth COVID-19 epidemic waves when compared with prior COVID-19 epidemic waves. General trends show an increase in COVID-19 hospitalized deaths in the age groups between 0 and 49 years and 80 and 89 years after each consecutive COVID-19 epidemic wave and a decrease in the age groups between 50 and 79 years.

By the end of the fifth COVID-19 epidemic wave, 20,323,729 COVID-19 vaccine doses had been administered in South Africa. The COVID-19 vaccines administered in South Africa were Pfizer-BioNTech (Comirnaty) and Johnson & Johnson or Janssen COVID-19 vaccines. The percentage of the population per age group in South Africa who had received at least 1 dose of the COVID-19 vaccine by the end of the fifth COVID-19 epidemic wave was 32.8%, 52.9%, 66%, and 71.6% of the total population in the age groups of 18 to 34 years, 35 to 49 years, 50 to 59 years, and above 60 years, respectively. The COVID-19 vaccination rate in South Africa had dropped drastically in all age groups since the third COVID-19 epidemic wave. The values of the Spearman correlation coefficients obtained between the daily cumulative COVID-19 vaccinated people and COVID-19 HA in the half period of the third, fourth, and fifth COVID-19 epidemic waves in this study indicated a strong negative monotonic association between the cumulative COVID-19 vaccinated people and COVID-19 HA. The Spearman correlation coefficients between daily cumulative COVID-19 vaccinated people and the percentage of hospitalized age groups of 18 to 34 years showed a significantly strong positive monotonic association, while the age groups of 50 years to above 60 years showed a significantly strong negative monotonic association. The values of the Spearman correlation coefficients obtained between the daily cumulative COVID-19 vaccinated people and change in daily COVID-19 cases in the half period of the third, fourth, and fifth COVID-19 epidemic waves in this study indicate a weak monotonic association between the daily cumulative COVID-19 vaccinated people and the change in daily COVID-19 cases. These results suggest that COVID-19 vaccination had an association with the reduction in COVID-19 hospital admission. Pfizer-BioNTech (Comirnaty) and Johnson & Johnson or Janssen COVID-19 vaccines have shown high efficacy against severe COVID-19 at 85% and 76.7%, respectively [53,54].

The mean COVID-19 hospital CFR in South Africa's first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 2.06% (SD 1.10%), 2.33% (SD 1.59%), 1.76% (SD 1.18%), 1.63% (SD 7.57%), and 0.99% (SD 1.72%), respectively. The mean COVID-19 hospital DR in South Africa's first, second, third, fourth, and fifth COVID-19 epidemic wave periods were 8.40% (SD 4.89%), 8.03% (SD 8.62%), 6.11% (SD 2.90%), 9.30% (SD 7.70%), and 10.05% (SD 10.73%), respectively. The CFRs in the third, fourth, and fifth COVID-19 epidemic waves in South Africa were lower than those observed in the first and second COVID-19 epidemic waves. The mean ECDR in South Africa's first, second, third, fourth, and fifth COVID-19 epidemic wave periods was 1.88% (SD 2.34%), 1.99% (SD 1.38%), 2.78% (SD 1.92%), 4.81% (SD 5.58%) and 13.3% (SD 8.48%), respectively. The ECDR values obtained in this study suggest that there was a relatively high number of deaths related to the COVID-19 pandemic which occurred outside South African hospitals and were unreported. Paired *t* tests of the ECDR between the COVID-19 epidemic waves showed statistically significant indifferences at 95% CI, indicating that the COVID-19 death rate occurring outside South African hospitals was similar in the 5 COVID-19 epidemic waves. According to the NICD, most hospitalized cases in the third, fourth, and fifth COVID-19 epidemic waves were mostly patients unvaccinated against COVID-19 (66.4%) [55,56].

Conclusions

The relaxation of COVID-19 NPI health policies in South Africa and the evolution of SARS-CoV-2 were associated with increased COVID-19 transmission and severity in the South African population. COVID-19 vaccination in South Africa was strongly associated with a decrease in COVID-19 hospitalizations and severity in South Africa.

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Conflicts of Interest

None declared.

Multimedia Appendix 1
SARS-CoV-2 lineages identified in genome samples and COVID-19 nonpharmacuetical intervention policies implemented in the South African COVID-19 epidemic waves; Pearson and Spearman correlation coefficients, ANOVA, univariate general linear model coefficients, and paired samples *t* tests for South African COVID-19 epidemiological data.
[DOCX File , 75 KB - xmed_v4i1e34598_app1.docx]

Multimedia Appendix 2
Mean change in movement from baseline in retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residences during the implementation of South African COVID-19 nonpharmacuetical intervention policies.
[PNG File , 125 KB - xmed_v4i1e34598_app2.png]

Multimedia Appendix 3
Maximum number of people with at least 1 dose of COVID-19 vaccine administered per total population age group during the first, second, third, fourth, and fifth COVID-19 epidemic wave in South Africa.
[PNG File , 74 KB - xmed_v4i1e34598_app3.png]

Multimedia Appendix 4
COVID-19 hospital-to-active cases and vaccinated people in the first half period of the third COVID-19 epidemic wave.
[PNG File , 102 KB - xmed_v4i1e34598_app4.png]

Multimedia Appendix 5
COVID-19 hospital-to-active cases and vaccinated people in the first half period of the fourth COVID-19 epidemic wave.
[PNG File , 87 KB - xmed_v4i1e34598_app5.png]

Multimedia Appendix 6
COVID-19 hospital-to-active cases and vaccinated people in the first half period of the fifth COVID-19 epidemic wave.
[PNG File , 94 KB - xmed_v4i1e34598_app6.png]

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Abbreviations

ACE: angiotensin-converting enzyme
CFR: case fatality rate
DR: discharge rate
ECDR: excess deaths (natural) to COVID-19 death ratio
HA: hospital-to-active cases
ICU: intensive care unit
NICD: National Institute for Communicable Diseases
NPI: nonpharmaceutical intervention
VOC: variant of concern
VOI: variant of interest
WHO: World Health Organization

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Predicting Waist Circumference From a Single Computed Tomography Image Using a Mobile App (Measure It): Development and Evaluation Study

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Abstract

Background: Despite the existing evidence that waist circumference (WC) provides independent and additive information to BMI when predicting morbidity and mortality, this measurement is not routinely obtained in clinical practice. Using computed tomography (CT) scan images, mobile health (mHealth) has the potential to make this abdominal obesity parameter easily available even in retrospective studies.

Objective: This study aimed to develop a mobile app as a tool for facilitating the measurement of WC based on a cross-sectional CT image.

Methods: The development process included three stages: determination of the principles of WC measurement from CT images, app prototype design, and validation. We performed a preliminary validity study in which we compared WC measurements obtained both by the conventional method using a tape measurement in a standing position and by the mobile app using the last abdominal CT slice not showing the iliac bone. Pearson correlation, student *t* tests, and Q-Q and Bland-Altman plots were used for statistical analysis. Moreover, to perform a diagnostic test evaluation, we also analyzed the accuracy of the app in detecting abdominal obesity.

Results: We developed a prototype of the app Measure It, which is capable of estimating WC from a single cross-sectional CT image. We used an estimation based on an ellipse formula adjusted to the gender of the patient. The validity study included 20 patients (10 men and 10 women). There was a good correlation between both measurements (Pearson R=0.906). The student *t* test showed no significant differences between the two measurements (P=.98). Both the Q-Q dispersion plot and Bland-Altman analysis graphs showed good overlap with some dispersion of extreme values. The diagnostic test evaluation showed an accuracy of 83% when using the mobile app to detect abdominal obesity.

Conclusions: This app is a simple and accessible mHealth tool to routinely measure WC as a valuable obesity indicator in clinical and research practice. A usability and validity evaluation among medical teams will be the next step before its use in clinical trials and multicentric studies.

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KEYWORDS

waist circumference; computed tomography; abdominal CT; mobile health; health apps; CT; CT scan; CT image; mobile app; app; application; waist; body; body mass; BMI; morbidity; mortality; clinical; tool; prototype; design; obesity; abdominal; usability; validity; medical

Introduction

Obesity is a major public health problem worldwide, and the reliance on BMI measurements alone has proven insufficient to help assess obesity-related health risks in patients [1]. Waist circumference (WC) is a simple method to evaluate abdominal adiposity that is easy to standardize. It is also an independent cardiovascular risk factor, with a higher predicting value than BMI [2,3]. However, this measurement is not routinely used in clinical practice.

Recently, a computed tomography (CT) scan estimation became a valid measure of standing WC [4,5]. This method is truly valuable in retrospective studies, where it can be difficult to obtain such measurements. Moreover, conventional WC assessment using a measurement tape can be challenging in patients with intellectual or motor disabilities. However, for a radiologist, this method may require time and training. Therefore, despite its widespread availability and limited cost, using CT images to assess WC is not routinely included in clinical and research practice.

Accordingly, the major aim of this study was to develop a mobile app to overcome these barriers and help clinicians routinely assess WC whenever a CT scan is available.

Methods

Overview

The development process involved three stages: determination of the principles of WC measurement from CT images, prototype design, and validation of the developed product.

As validated by Ciudin et al [6], the abdominal perimeter was estimated using the formula of the perimeter of an ellipse (Figure 1). In this previous study, there was a good correlation between conventional standing WC measurement and ellipse-estimated WC, with a Pearson test of 0.987 and a mean error of 0.4 cm.

We applied the same formula:

“a” being the anterior-posterior diameter and “b” being the transverse diameter. Afterward, we performed WC measurement on 10 healthy candidates using both the conventional tape method and the ellipse formula. We then used a simple linear regression analysis to adjust the final WC formula to the gender of the patient.

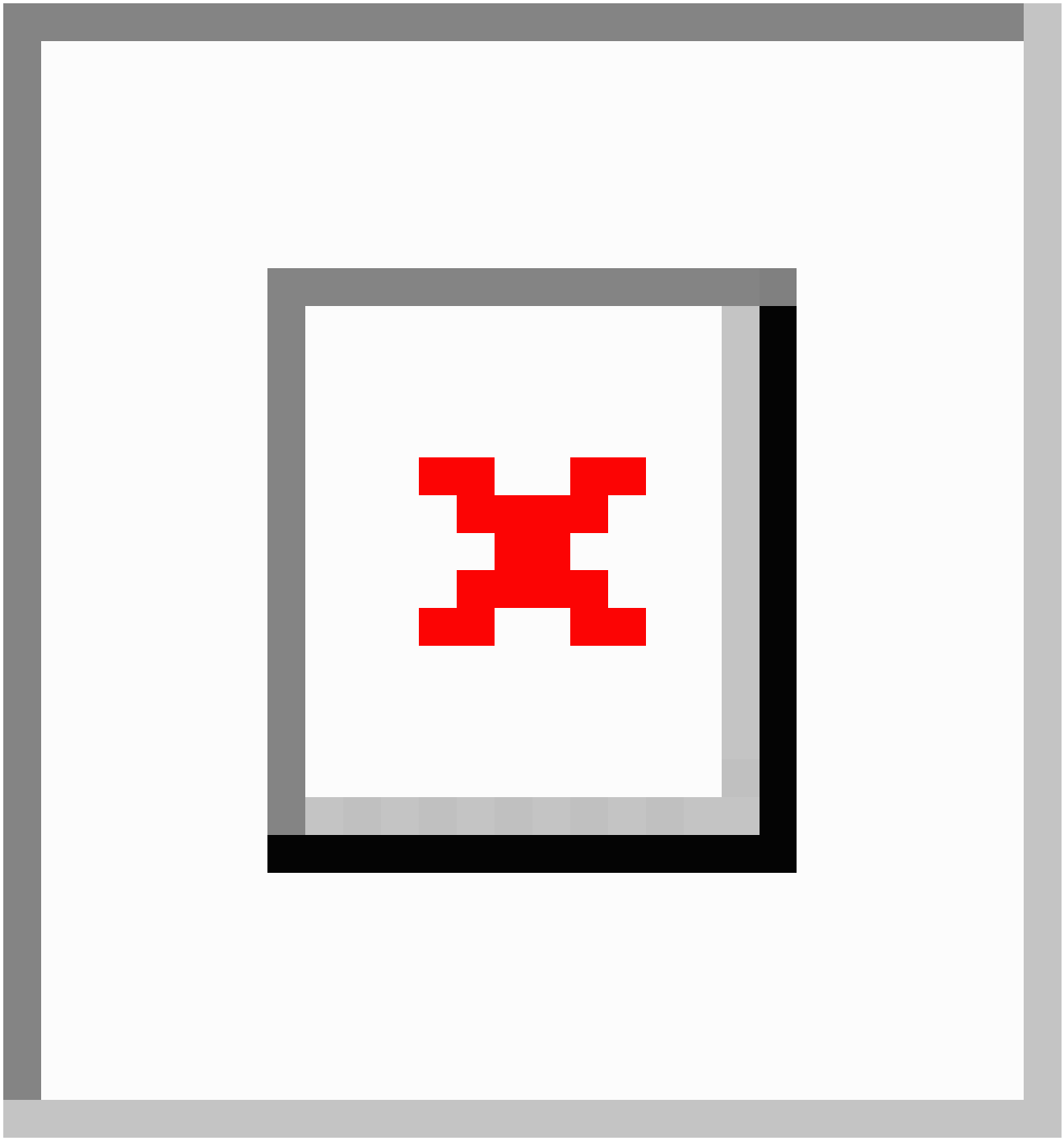
After confirming the app requirements (ellipse formula, required measurements, final formula applied to gender and the needed parameters, and organization of the steps required by the physician to ameliorate the user experience), we initiated the design and development of the app. After preparing the app prototype, we performed a preliminary validity study including 20 patients selected retrospectively based on the existence of a previous WC measurement and CT scan images in their file. We compared the conventional WC measurement (cWC) method to the mobile app–based WC measurement (mWC) method based on CT scan images.

The first measurement was done using a measuring tape placed horizontally around the patient’s abdomen just above the iliac crest as recommended by the National Institutes of Health National Institutes of Health National Institutes of Health guidelines [7]. It was done in a standing position, at the end of a normal expiration. The second measurement was performed with the mobile app. Using the camera of the phone, the app employused the last slice of the CT scan image, on the last slice, from cranial to caudal, not showing the iliac bone.

Measurements were expressed as mean ± (SD)standard deviation and range. Data were collected and analyszed using SPSS 20 software (SPSS Inc., Chicago, IL, USAIBM Corp). Abdominal obesity was defined by waist circumferenceWC measurements of >102 cm (~40 inches) and >88 cm (~35 inches) for men and women, respectively [8]. Student t test, Pearson correlation, Q-Q plot, and the Bland-Altman analysis were used. P values <inferior to 0.05 were considered statistically significant from a statistical point of view. In order tTo perform a diagnostic test evaluation, we also analyszed the mWC accuracy in detecting abdominal obesity.

At the end of the design stage, the app was demonstrated in several team meetings, which led to further modifications.

Figure 1. Evaluation of the waist circumference on a computed tomography image using an ellipse perimeter formula. (a) Anterior-posterior diameter, (b) transverse diameter, and (c) ellipse perimeter.



Patient and Public Involvement

The patients were not involved in setting the research question or the outcome measures, designing or implementing the study, or reporting or disseminating the research. Additionally, the public did not participate in the design, implementation, reporting, or dissemination plans of this study.

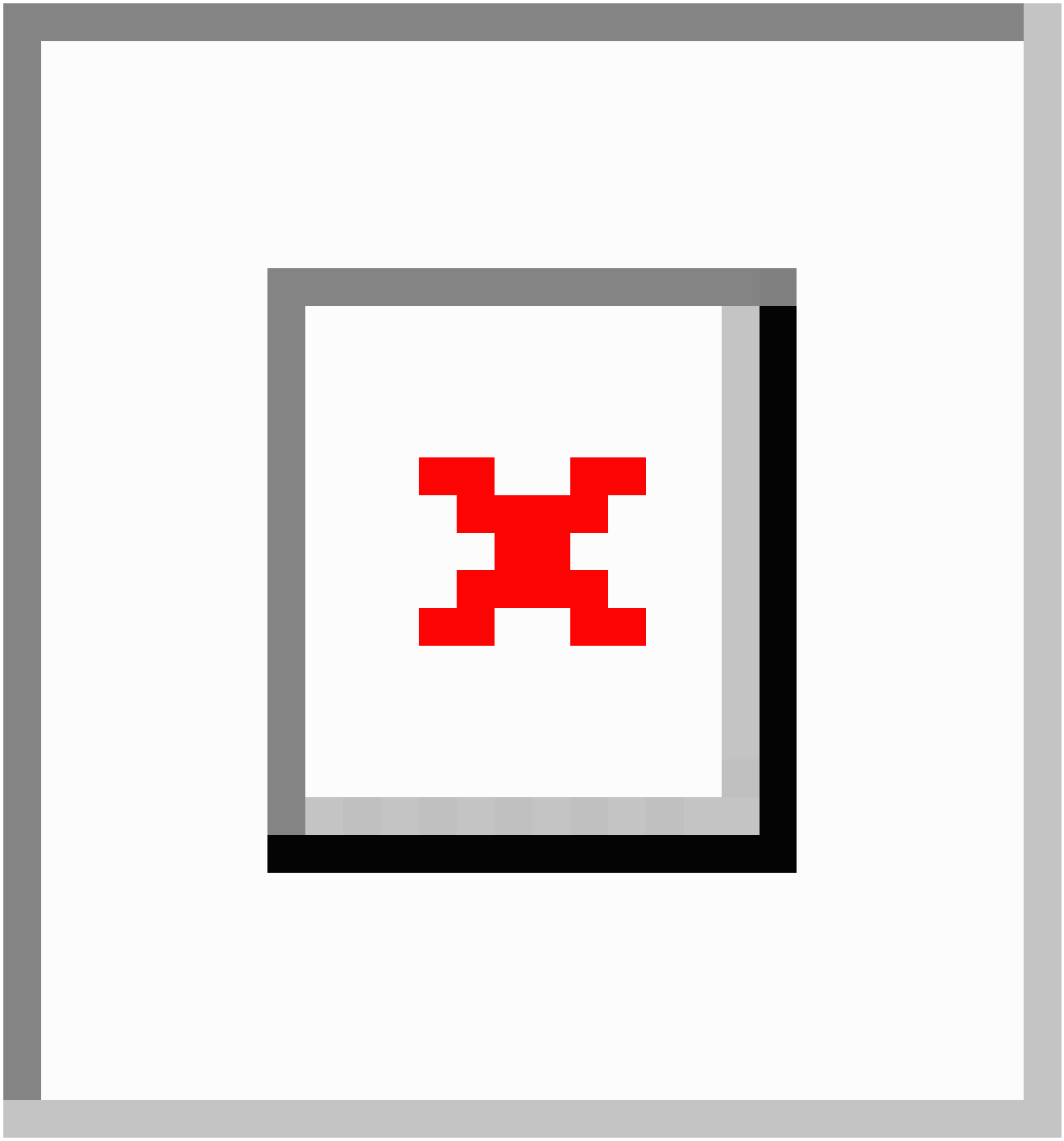
Ethical Considerations

Personal data have been respected. This study was approved by the ethics committee at Habib Bourguiba University Hospital in Sfax (Ref CE-03-2022).

Results

Following the design principles and requirements, the prototype of the app was developed and named Measure It. The flow of the app was designed to be simple and productive to ensure quality interaction between the app and the visitor (Figure 2). A demo video was provided in the app to facilitate its use.

Figure 2. Illustration of the flow of the app. WC: waist circumference.



The preliminary validation study included 20 patients. It included 10 men and 10 women. The mean age was 54 (SD 17) years. The mean BMI was 26 (SD 4; women: mean 27.8, SD 2.7; men: mean 24.2, SD 4.4). The mean cWC was 93.7 (SD 12.6, range 68-122; women: mean 95.1, SD 11.9; men: mean 92.3, SD 13.8) cm. The most common reason why patients (n=10) underwent an abdominal CT scan was for biliary stone disease.

The comparison between cWC and mWC showed a good correlation (Pearson R=0.90). The student t test showed no significant difference between the two measurements (P=.98). We also compared the two measurements using a Q-Q dispersion and a Bland-Altman plot. The analysis graphs can be found in Figures 3 and 4. The Q-Q plot showed good overlap with some dispersion of extreme values. The Bland-Altman analysis showed a mean difference of 0.03 cm (95% CI –2.46 to 2.53) between the two measurements ().

Figure 3. Q-Q plot of estimated WC versus measured WC. WC: waist circumference.

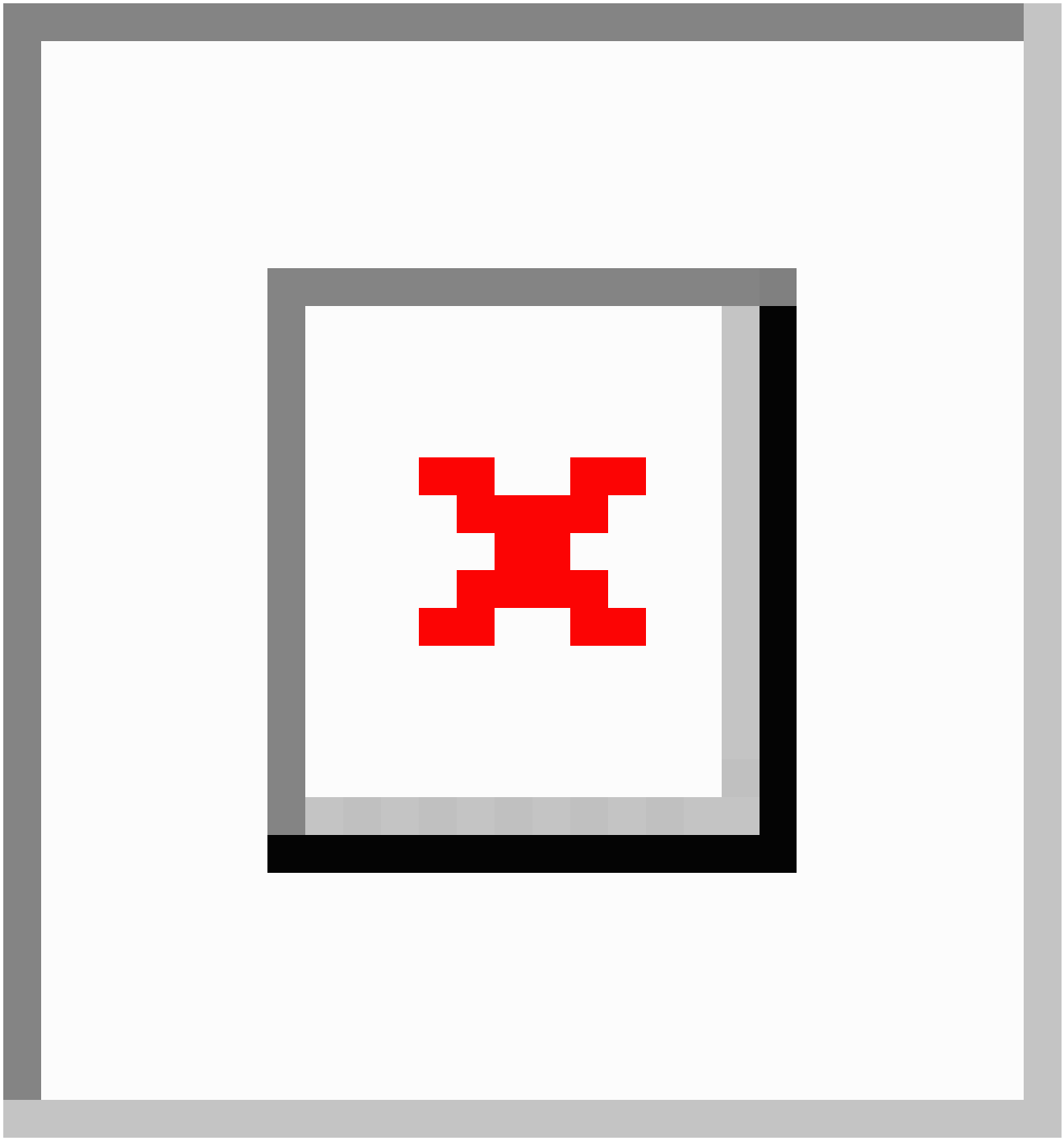
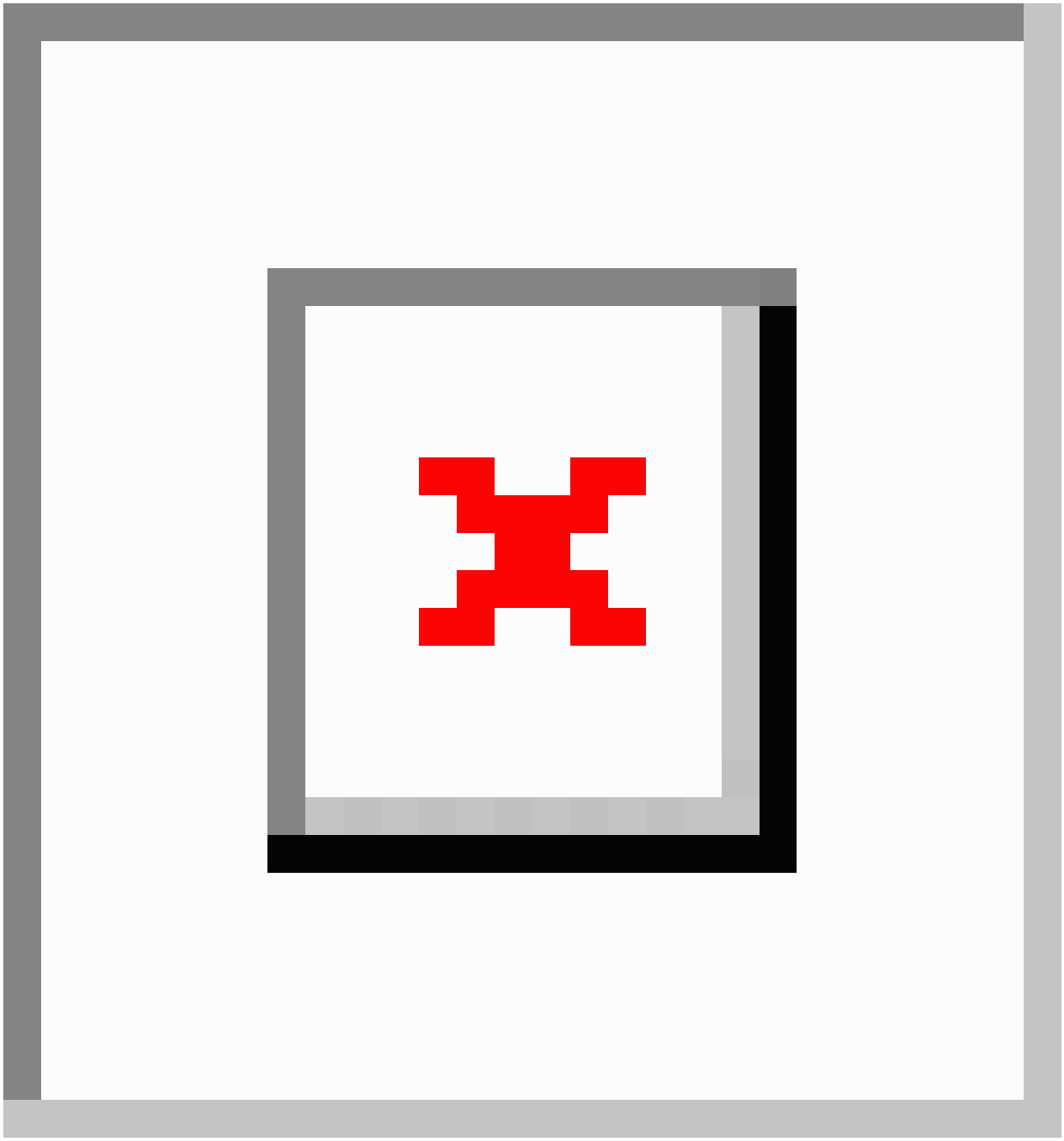


Figure 4. Bland-Altman plot of the differences between the standing and app-estimated waist circumferences.



We have also performed a diagnostic test evaluation regarding the accuracy of the mWC in detecting abdominal obesity. Results were adjusted to the prevalence of abdominal obesity (59%) [9]. The analysis showed an accuracy of 83% when using mWC to detect abdominal obesity (Table 1).

Table . Diagnostic test evaluation for mobile app waist circumference.

Statistic	Value (95% CI)
Sensitivity (%)	72.73 (39.03-93.98)
Specificity (%)	100.00 (66.37-100.00)
Positive predictive value[a] (%)	100.00
Negative predictive value[a] (%)	71.82 (49.26-86.99)
Accuracy[a] (%)	83.91 (60.82-96.28)

[a]These values are dependent on abdominal obesity prevalence [9].

Discussion

Principal Findings

Guidelines for the management of obesity from several professional societies recognize the importance of measuring WC in the context of risk stratification for future cardiometabolic morbidity and mortality [3,10-13]. Moreover, WC is gaining significant importance among surgeons since abdominal obesity has a growing value in preoperative risk assessment for morbidity and mortality in different surgeries [14-19].

We developed a prototype of the mobile app Measure It to accurately estimate WC using CT scan images. The app was developed based on a validated method [5,6] measuring WC using CT scan cross-sectional images. To our knowledge, this is the first mobile app that helps physicians estimate WC. The app was designed to be a simple and accessible tool with the purpose of routinely including this valuable obesity parameter in clinical and research practice. One of the most valuable advantages of our app is its usability in retrospective studies. WC measurements mostly do not exist in patient observations. However, CT scan slides or images are often available. Moreover, the simplicity of the app may reduce the time required for physicians to assess WC [20]. Conventional tape measuring is sometimes not possible, particularly for patients who are disabled. Additionally, for a radiologist, the conventional CT scan method requires training and can be more or less time-consuming. Eventually, being simple, accessible, and reproducible, the app may reduce the technology barriers for nontech physicians since smartphones are commonly available even in low- and middle-income countries [21].

As a screening tool for abdominal obesity, this attribute may be beneficial, especially in retrospective studies. With an accuracy of 83% compared to the conventional method, the mobile app method is reliable. However, the accuracy of WC measurement may be altered in some cases. This may be due to a measurement error in the conventional method or to particular body shapes and extreme values of WC.

One major problem with currently available mobile health apps is that few are established with strong research evidence [22,23]. Measure It is developed based on a strong statistical analysis, even though it needs to be validated in a prospective study.

The main limitation of this study is the small sample size used to validate the app. We consider this validity study as a preliminary validation that needs to be confirmed. Therefore, we would expect our app to be ready for clinical use to a certain degree.

Another limitation is that the small screen on the smartphone makes it difficult to precisely set reference scales and perform measurements on a CT scan image. However, the zooming functionality makes the app's accuracy very sufficient. To overcome this problem, we plan to develop a second prototype app with artificial intelligence technology to automatically detect the reference scale and make the essential measurements on the image without the user interfering.

Conclusions

In this study, we developed a prototype of a mobile app for estimating WC for physicians. Being simple, available, and reproducible, this app has the potential to positively affect the quality of data in future research. Usability and validity evaluation among medical teams will be the next step before its use in clinical trials and multicentric studies.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' Contributions

AM and AZ conceived the idea for the app and the document, and contributed to the writing and editing of the manuscript. MBA reviewed and edited the statistical analysis and the results in the manuscript. AB and KF reviewed and evaluated the app. MBA and S Boujelben reviewed the article before submission. All authors read and approved the final manuscript.

Conflicts of Interest

None declared.

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Abbreviations

CT: computed tomography
cWC: conventional waist circumference measurement
mHealth: mobile health
mWC: mobile app–based waist circumference measurement
WC: waist circumference

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Seroprevalence of SARS-CoV-2 in Niger State: Pilot Cross-Sectional Study

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Abstract

Background: The COVID-19 pandemic caused by SARS-CoV-2 is causing ongoing human and socioeconomic losses.

Objective: To know how far the virus has spread in Niger State, Nigeria, a pilot study was carried out to determine the SARS-CoV-2 seroprevalence, patterns, dynamics, and risk factors in the state.

Methods: A cross-sectional study design and clustered, stratified random sampling strategy were used to select 185 test participants across the state. SARS-CoV-2 IgG and IgM rapid test kits (colloidal gold immunochromatography lateral flow system) were used to determine the presence or absence of antibodies to the virus in the blood of sampled participants across Niger State from June 26 to 30, 2020. The test kits were validated using the blood samples of some of the Nigeria Center for Disease Control–confirmed positive and negative COVID-19 cases in the state. SARS-CoV-2 IgG and IgM test results were entered into the Epi Info questionnaire administered simultaneously with each test. Epi Info was then used to calculate the arithmetic mean and percentage, odds ratio, χ^2 statistic, and regression at a 95% CI of the data generated.

Results: The seroprevalence of SARS-CoV-2 in Niger State was found to be 25.4% (47/185) and 2.2% (4/185) for the positive IgG and IgM results, respectively. Seroprevalence among age groups, genders, and occupations varied widely. The COVID-19 asymptomatic rate in the state was found to be 46.8% (22/47). The risk analyses showed that the chances of infection are almost the same for both urban and rural dwellers in the state. However, health care workers, those who experienced flulike symptoms, and those who had contact with a person who traveled out of Nigeria in the last 6 months (February to June 2020) were at double the risk of being infected with the virus. More than half (101/185, 54.6%) of the participants in this study did not practice social distancing at any time since the pandemic started. Participants' knowledge, attitudes, and practices regarding COVID-19 are also discussed.

Conclusions: The observed Niger State SARS-CoV-2 seroprevalence and infection patterns meansuggest that the virus has widely spread, far more SARS-CoV-2 infections have occurred than the reported cases, and there is a high asymptomatic COVID-19 rate across the state.

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KEYWORDS

COVID-19; pandemic; SARS-CoV-2; seroprevalence; serology; epidemiology; Niger State; Nigeria; COVID-19 testing; social distancing

Introduction

The COVID-19 pandemic was caused by a novel coronavirus (SARS-CoV-2) that is believed to have crossed from bats to humans for the first time [1-3]. COVID-19 is an infectious disease of the respiratory system of humans and animals, and the virus can be transmitted through facial openings including the mouth, nostrils, and (maybe) eyes [2-4].

The first case of COVID-19 in Niger State, Nigeria, was announced by the Nigeria Center for Disease Control (NCDC) on April 10, 2020; this was about 6 weeks after the first confirmed case (index case) of COVID-19 in Nigeria was announced on February 27, 2020, when a foreigner in Lagos tested positive for SARS-CoV-2. Since then, many cases have been confirmed in the state, and this number is still increasing [5]. As part of the measures to curtail the spread of SARS-CoV-2, strict lockdown (restricting people to their homes except for essential needs, eg, medicine and food) was enforced in the state from March 25 to June 9, 2020. However, full compliance to the strict lockdown by the citizens of the state may not have been achieved or possible due to socioeconomic and cultural reasons, disbelief, and conspiracy theories. Many people would have to go out on a daily basis to work and provide for their families, and markets are usually open spaces bustling with large crowds of people. Many people did not believe in COVID-19, especially the highly contagious nature of the disease. There are also no efficient and robust housing and biometric data management systems where everyone is accounted for, especially for the purposes of employment, health, security, and social welfare. If these are available, foods and other goods purchased online can be sent to houses with ease. Additionally, utilities are not provided or are inadequately supplied in most cases. It is difficult for people to stay at home and comply with the strict lockdown in such situations. After the lockdown was eased, there has been enhanced enforcement of the compulsory use of face masks in public places and adherence to physical distancing in the state [5].

Niger State is one of the 36 states in Nigeria, with Minna being its capital. It has 25 local government areas that are fairly distributed among the three geopolitical zones of the state in terms of land mass and population. In terms of land mass, Niger State is the largest state (76,363 km^2) in Nigeria and has the 18th-highest (5,556,247 people) population [6,7]. However, as of December 21, 2020, Niger State is ranked 28th among the states in COVID-19 cases reported in Nigeria. The total number of reported COVID-19 cases in the state as of December 21, 2020, is 381, with 12 deaths, while for Nigeria (with a population of about 206,630,269), there have been 79,789 COVID-19 cases and 1231 deaths [5-8]. It is generally believed that the reported COVID-19 cases in the state and Nigeria are far below the actual SARS-CoV-2 infections in the populations. This may be due to polymerase chain reaction (PCR)–based SARS-CoV-2 test limitations in many states of Nigeria and unknown proportions of mild or asymptomatic COVID-19 cases that may not be diagnosed or reported. The presence and detection of antibodies to SARS-CoV-2 in the blood samples of participants likely indicate that they were infected at some point since the start of the pandemic. Therefore, serologic assays can be used to determine population-based estimates of SARS-CoV-2 infection, including those who had a mild or asymptomatic infection or who were never tested despite symptoms [9-11].

For COVID-19, like most infectious diseases, the isolation of the etiologic agent SARS-CoV-2 through the tissue or cell plate culture technique would be the gold standard method for the diagnostic test. However, plate culturing is usually laborious, time-consuming, complex, and costly, and is impossible to use, especially for epidemiological studies where large samples may be involved. Additionally, even though reverse transcriptase–PCR has been predominantly used to test for the agent of COVID-19 worldwide, including in Nigeria, it is also laborious, time-consuming, costly, and complex [12-14].

Infection by many pathogens including viruses does elicit the production of antibodies in humans and animals even if no symptoms manifested. The detection of the antibodies in the whole blood/serum/plasma of humans and animals has been used for preliminary diagnoses of infectious diseases [9-15]. Additionally, because of the relative ease of use and simplicity of the antigens and antibody test kits compared to cell/tissue cultures and PCR, they are mostly used in epidemiological

studies to determine infectious disease prevalence, patterns, dynamics, and risk factors [12-15]. Antigen and antibody test kits, unlike other methods, can detect previous exposure to infectious agents [9-15]. This information is important, especially for COVID-19, which has an assumed high rate of asymptomatic cases, to see how far the virus has spread, infection patterns, and the effectiveness of the enforced social distancing measures. This pilot study was aimed at determining the SARS-CoV-2 seroprevalence, patterns, dynamics, and risk factors for contracting COVID-19 in Niger State, Nigeria. It was also aimed at assessing the knowledge, attitudes, and practices of people regarding COVID-19 and related control measures in the state.

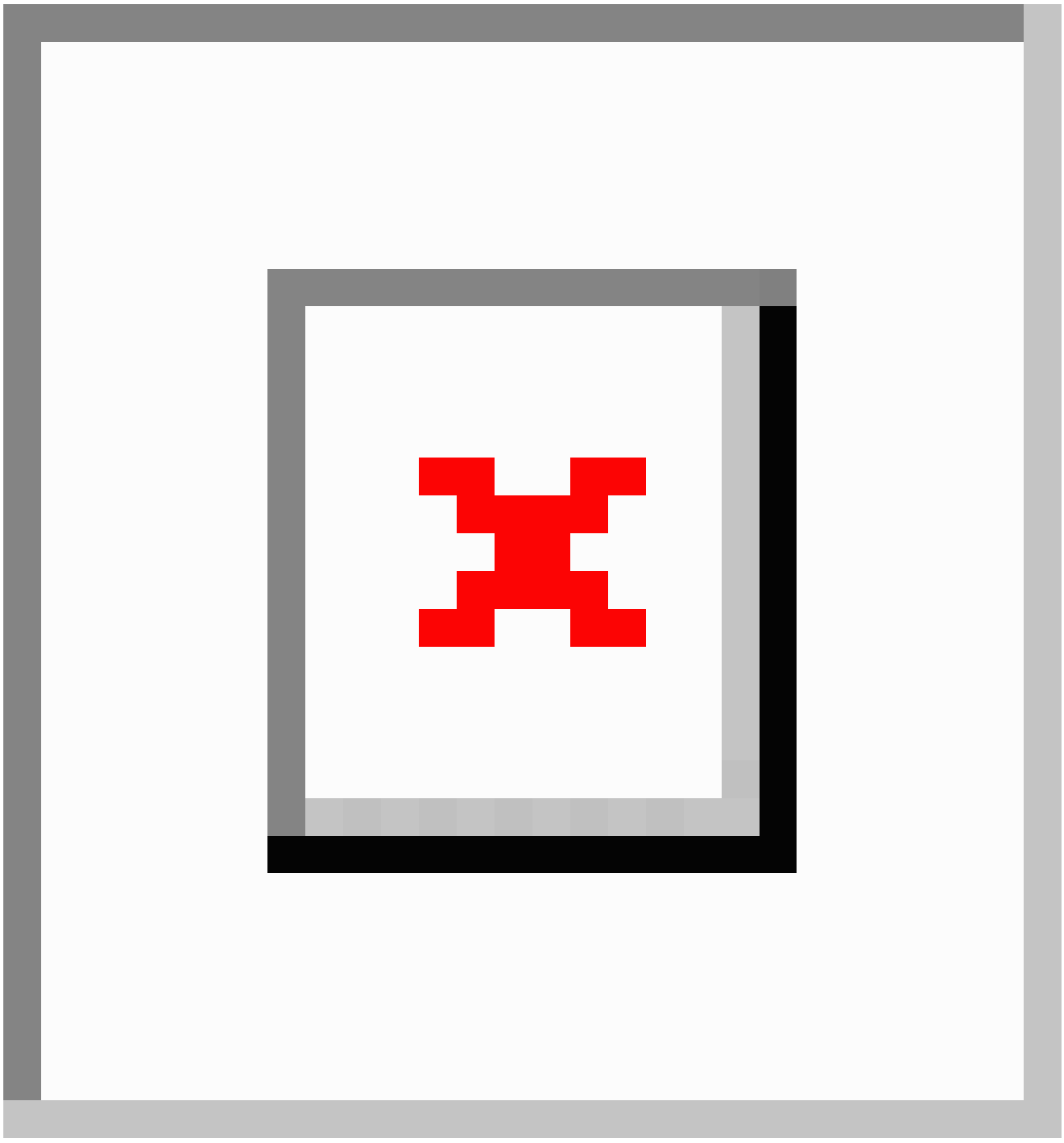
Methods

Study Design and Population

A cross-sectional study design and clustered, stratified random sampling strategy were used.

The study area was Niger State, and its residents were the study population (Figure 1). Niger State is one of the federating geopolitical states in Nigeria; its capital is Minna. Other major towns in the state are Bida, Kontagora, Suleja, New Bussa, Mokwa, Lapai, and Agaie. The three geopolitical zones (zone A, zone B, and zone C) in the state were covered (Figure 1). Place of residence (classified as urban or rural), gender, occupation (classified as health care worker or non–health care worker), and age group/range were the stratifications that were applied in the places chosen in each zone (Figure 1). The selected sampling points (Multimedia Appendix 1) were socioeconomic areas, such as hospitals and primary health care centers, motor parks, markets, village/community heads' households, sawmills, and schools. Considering the stratifications, people in the selected sampling points were randomly approached and recruited to participate in the study. OpenEpi Toolkit (Dean AG, Sullivan KM, Soe MM) was used to calculate the minimum sample size for the study. Since this is a pilot study and SARS-CoV-2 prevalence is not known in Niger State, which has a population of about 5 million people, we assumed that the overall SARS-CoV-2 prevalence would be about 50%, with 95% confidence in the estimate, 10% absolute precision, and a 1.0 design effect (for random cluster surveys); therefore, the minimum required sample size was 97 participants. A total of 185 participants were enrolled in this study. Among individuals approached for recruitment, the average acceptance/participation rate was 87.3% (185/212). From June 26 to 30, 2020, and with full consent to participate in the study, samples were taken randomly from 185 participants (with almost equal distribution among the three geopolitical zones) for SARS-CoV-2 IgG and IgM rapid tests, and the questionnaire (created by Epi Info 7.2.2.6; Centers for Disease Control and Prevention) was administered simultaneously.

Figure 1. Map of Niger State showing the sites where samples were taken, tests carried out, and questionnaires administered in the three geopolitical zones (zone A, zone B, and zone C) of the state for the pilot SARS-CoV-2 seroprevalence study carried out from June 26 to 30, 2020, in Niger State, Nigeria. The exact names, latitudes, and longitudes of the sampling points can be found in Multimedia Appendix 1.



Ethics Approval

Ethical approval for this study was given by the Research Ethics Committee of the Niger State Ministry of Health (STA/495/Vol/152). Consent was also sought from each of the participants prior to the test and questionnaire administration, and only individuals who gave full consent were included in the study. Parents/guardians were responsible for the consent of their wards who participated in the study and were younger than 18 years.

Specimen Type and SARS-CoV-2 IgG and IgM Rapid Test

SARS-CoV-2 IgG and IgM rapid tests were carried out using the whole blood of the participants. The test is a qualitative membrane-based immunoassay for the detection of COVID-19 antibodies in whole blood. The tests were carried out and interpreted according to the kit manufacturer's instructions. The test result of each participant was recorded and entered into the Epi Info questionnaire administered for that particular participant. The SARS-CoV-2 IgG and IgM rapid test kits were validated with the blood samples of those individuals that were confirmed by the NCDC through PCR as positive or negative

for COVID-19 in Niger State. All 10 NCDC-confirmed positive cases, tested positive for the IgG for SARS-CoV-2, while the 5 NCDC-confirmed negative individuals (that had never tested positive before) tested negative for the IgG and IgM for SARS-CoV-2. This means that 100% sensitivity and specificity were observed for the test kits used in the study.

Epi Info Questionnaire and Statistics

To be able to determine the COVID-19 prevalence, patterns, dynamics, and risk factors for contracting the disease in Niger State, a questionnaire (Multimedia Appendix 2) was designed and created using Epi Info 7.2.2.6. The questionnaire was designed to ask questions with categorical responses (yes or no) and to accommodate the test results of the participants; this allowed 2 × 2 statistics tables to be created and used for calculating the SARS-CoV-2 infection odds ratios and linear regression (multivariate analysis) for many scenarios. SARS-CoV-2 IgG and IgM test results were entered into the Epi Info questionnaire administered simultaneously with each test. Epi Info was used to calculate the arithmetic mean and percentage, odds ratio, χ^2, and regression at 95% CI of the data generated. Demographics; SARS-CoV-2 prevalence and COVID-19 asymptomatic rate; and the knowledge, attitudes, and practices of the participants were expressed as percentages. For the risk of contracting SARS-CoV-2 analyses (odds ratio and χ^2), the SARS-CoV-2 IgG status (prevalence) was the dependent variable, while the demographics and risks were the independent variables.

Results

Demographics; Knowledge, Attitudes, and Practices; Travel History; and Flulike Symptoms of the Participants

The demographic characteristics; knowledge, attitudes, and practices; travel history; and flulike symptoms of the participants in this study are shown in Table 1. More than half (n=101, 54.6%) of the 185 participants in this study had not practiced social distancing at any time since the pandemic started (January to June 2020), even as the lockdown was enforced in the state. The majority (n=114, 61.6%) of the participants practiced hand and face hygiene. Almost all (n=181, 97.8%) of the participants had not traveled out of Nigeria since the beginning of the year 2020 when the pandemic started. Only a few (n=4, 2.2%) of the participants traveled out of Nigeria in the last 6 months (January to June 2020) and returned. However, 24 (13%) participants had contact with someone who traveled out of the country in the last 6 months (January to June 2020). The majority (n=113, 61.1%) of the participants did not experience any flulike symptoms since when the pandemic started (January 2020 to June 2020). Only 72 (38.9%) participants experienced flulike symptoms (January to June 2020).

Table . Demographics; knowledge, attitudes, and practices; travel history; and flulike symptoms of the participants in a pilot SARS-CoV-2 seroprevalence study carried out from June 26 to 30, 2020, in Niger State, Nigeria.

Variable		Participants, n (%)	Exact 95% CI (%)
Age (years; n=185)			
	≤5	15 (8.1)	4.61-13.02
	6-17	26 (14.1)	9.39-19.91
	18-29	34 (18.4)	13.08-24.72
	30-41	45 (24.3)	18.33-31.16
	42-53	37 (20.0)	14.49-26.50
	54-65	20 (10.8)	6.73-16.20
	≥66	8 (4.3)	1.89-8.34
Gender (n=185)			
	Male	103 (55.7)	48.21-62.96
	Female	82 (44.3)	37.04-51.79
Resident (n=185)			
	Urban	115 (62.2)	54.75-69.17
	Rural	70 (37.8)	30.83-45.25
Occupation (n=185)			
	Health care workers	43 (23.2)	17.36-30.00
	Non–health care workers	142 (76.8)	70.00-82.64
Health care workers: gender (n=43)			
	Male	22 (51.2)	35.46-66.69
	Female	21 (48.8)	33.31-64.54
Knowledge (n=185)			
	Aware of COVID-19	151 (81.6)	75.28-86.92
	Not aware of COVID-19	34 (18.4)	13.08-24.72
Belief (n=185)			
	COVID-19 is in Niger State	109 (58.9)	51.46-66.08
	COVID-19 is not in Niger State	76 (41.1)	33.92-48.54
Hand and face hygiene (n=185) [a]			
	Yes	114 (61.6)	54.20-68.66
	No	71 (38.4)	31.34-45.80
Social distancing (n=185) [a]			
	Yes	84 (45.4)	38.09-52.87
	No	101 (54.6)	47.13-61.91
Travel history (n=185) [a]			
	Traveled overseas	4 (2.16)	0.59-5.44
	Did not travel overseas	181 (97.84)	94.56-99.41
Contact history (n=185) [a]			
	Contact with overseas returnee	24 (12.97)	8.49-18.69
	No contact with overseas returnee	161 (87.03)	81.31-91.51

Variable		Participants, n (%)	Exact 95% CI (%)
Flulike symptoms (n=185) [a]			
	Experienced flulike symptoms	72 (38.92)	31.85-46.35
	Did not experience flulike symptoms	113 (61.08)	53.65-68.15

[a]Variables were for the period of 6 months (January to June 2020) prior to the study being conducted.

SARS-CoV-2 Seroprevalence, COVID-19 Asymptomatic Rate, and Infection Risks in Niger State

The SARS-CoV-2 seroprevalence for the 185 participants in Niger State was 25.4% (n=47) and 2.2% (n=4) for the positive IgG and IgM tests, respectively, as of June 26-30, 2020 (Table 2). The number of participants that did not experience flulike symptoms in the last 6 months (January to June 2020) and tested positive for SARS-CoV-2 IgG amounted to the COVID-19 complete asymptomatic rate in Niger State. The COVID-19 asymptomatic rate in the state was found to be 47% (22/47). SARS-CoV-2 seroprevalence among age groups, gender, and occupations varied widely. Among age groups, the SARS-CoV-2 seroprevalence was found to be 33.3% (15/45) for those 30-41 years, 32.4% (12/37) for those 42-53 years, 30% (6/20) for those 54-65 years, 25% (2/8) for those ≥66 years, 19.2% (5/26) for those 6-17 years, 17.7% (6/34) for those 18-29 years, and 6.7% (1/15) for those ≤5 years. A seroprevalence of 27.2% (28/103) was recorded for male participants and 23.2% (19/82) for female participants in the state. A SARS-CoV-2 seroprevalence of 37.2% (16/43) was recorded for health care workers in Niger State. Among the non–health care workers in the state, the SARS-CoV-2 seroprevalence recorded was 21.8% (31/142). The SARS-CoV-2 seroprevalence among the urban dwellers in the state stood at 27.8% (32/115), while for the rural dwellers, it was 21.4% (15/70). The same SARS-CoV-2 seroprevalence (1/4, 25%) was recorded among the overseas returnees and those that did not travel 25.41% (46/181). However, a higher SAR-CoV-2 seroprevalence (10/24, 41.7%) was recorded for those who had contact with the overseas returnees compared to those who did not have contact with the returnees (37/161, 23%).

Table . SARS-CoV-2 seroprevalence, infection risks, and COVID-19 asymptomatic rate as of June 26-30, 2020, in Niger State, Nigeria.

Variable		SARS-CoV-2 seropositivity for IgG (n=47), n (%)	SARS-CoV-2 seroprevalence, n/N (%)	Exact 95%	Positive COVID-19 IgG test			
					2 × 2 statistics (univariate analysis)		Linear regression (multivariate analysis)	
					Odds ratio	*P* value (χ^2)	Coefficient	*P* value
Overall (IgG)		47 (100)	47/185 (25.4)	19.30-32.31	—[a]	—	—	—
Overall (IgM; n=4)		4 (100)	4/185 (2.2)	0.59-5.44	—	—	—	—
Age (years)				—	—	—	—	—
	≤5	1 (2.1)	1/15 (6.7)					
	6-17	5 (10.6)	5/26 (19.2)					
	18-29	6 (12.8)	6/34 (17.7)					
	30-41	15 (31.9)	15/45 (33.3)					
	42-53	12 (25.5)	12/37 (32.4)					
	54-65	6 (12.8)	6/20 (30.0)					
	≥66	2 (4.3)	2/8 (25.0)					
Gender				—	0.81	.65	0.00	.96
	Male	28 (60.0)	28/103 (27.2)					
	Female	19 (40.4)	19/82 (23.2)					
Resident				—	1.41	.43	0.00	.97
	Urban	32 (68.1)	32/115 (27.8)					
	Rural	15 (31.9)	15/70 (21.4)					
Occupation				—	2.21	.07	0.01	.91
	Health care workers	16 (34.0)	16/43 (37.2)					
	Non–health care workers	31 (66.0)	31/142 (21.8)					
Knowledge				—	—	—	—	—
	Aware of COVID-19	43 (91.5)	43/151 (28.5)					
	Not aware of COVID-19	4 (8.5)	4/34 (11.8)					
Beliefs				—	—	—	—	—
	COVID-19 is in Niger State	37 (78.7)	37/109 (33.9)					
	COVID-19 is not in Niger State	10 (21.3)	10/76 (13.2)					
Behavior (A)				—	1.90	.12	–0.07	.50
	Hand and face hygiene	34 (72.3)	34/114 (29.8)					
	No hand and face hygiene	13 (27.7)	13/71 (18.3)					

Variable		SARS-CoV-2 seropositivity for IgG (n=47), n (%)	SARS-CoV-2 seroprevalence, n/N (%)	Exact 95%	Positive COVID-19 IgG test			
					2 × 2 statistics (univariate analysis)		Linear regression (multivariate analysis)	
					Odds ratio	*P* value (χ^2)	Coefficient	*P* value
Behavior (B)				—	2.16	.04[b]	0.10	.31
	Social distancing	28 (59.6)	28/84 (33.3)					
	No social distancing	19 (40.4)	19/101 (18.8)					
Travel history				—	0.98	>.99	–0.19	.41
	Traveled overseas	1 (2.1)	1/4 (25.0)					
	Did not travel overseas	46 (97.9)	46/181 (25.4)					
Contact history				—	2.39	.09	0.16	.15
	Contact with overseas returnee	10 (21.3)	10/24 (41.7)					
	No contact with overseas returnee	37 (78.7)	37/161 (23.0)					
Flulike symptoms				—	2.20	.03[b]	0.14	.04[b]
	Experienced flulike symptoms	25 (53.2)	25/72 (34.7)					
	Did not experience flulike symptoms	22 (46.8)	22/113 (19.5)					

[a]Not applicable.

[b]Values are significantly different (*P*=.05).

To determine the risk factors of SARS-CoV-2 infection and the effectiveness of COVID-19 preventive measures enforced in the state, 2 × 2 statistics tables were used to calculate the odds ratios for many scenarios. When the gender of the participants and positive COVID-19 IgG results were cross-tabulated, the risk ratio recorded for female participants was 0.85 (Table 2).

The risk analyses showed that the chances of infection are almost the same for both urban and rural dwellers in the state even though COVID-19 seroprevalence among urban dwellers was a little higher than that of rural dwellers. Health care workers, those who experienced flulike symptoms, and those that had contact with a person that traveled out of Nigeria in the last 6 months (January to June 2020) are at double the risk of being infected with the virus. However, in linear regression multivariate analysis, only “experienced flu-like symptoms” was significant at 95% CI among them. The risk analyses showed that returning from overseas did not confer protection or pose any increased risk of contracting the virus (Table 2).

Discussion

Key Findings

This SARS-CoV-2 seroprevalence pilot study was carried out to understand how far the virus has spread in Niger State, Nigeria, and to determine the patterns, dynamics, and risk factors of COVID-19 in the state. We used a cross-sectional study design and clustered, stratified random sampling strategy to select 185 study participants across three geopolitical zones of the state; this was an effort to have a fair representation of the state even though the sample size was small.

The life expectancy in Nigeria is currently 55.8 years [8]. The gender of the participants reflected the ratio of males to females in Nigeria, which is currently 50.6% males to 49.4% females (Table 1) [7]. In Nigeria, currently, 52% of the population lives in urban areas, while 48% are in rural areas [8].

Before COVID-19 vaccines became available, other ways of preventing the transmission of SARS-CoV-2 (the causative agent of the COVID-19 pandemic) among humans are

social/physical distancing measures and good sanitation and hygiene practices. Adherence to these COVID-19 preventive measures will be impacted by the knowledge and beliefs of people about the disease since the measures involve some behavioral changes and practices. People can only believe what they know (or are aware of) and can only practice when they believe.

There are many reasons why people did not observe social distancing (Table 1). The first is poverty. The level of poverty in society is high, and many people have to go out on a daily basis to work to feed their families. Markets are usually open spaces bustling with large crowds, and most transactions are done with the physical exchange of cash, which prevents people from social distancing. Additionally, poverty causes people to gather in places where food and money are distributed and where physical distancing and other required COVID-19 control measures may not be observed or enforced [13].

The second reason is the prevalence of disbelief, myths, and conspiracy theories. Many people did not believe in COVID-19 (Table 1), especially regarding the highly contagious nature of the disease. This may be the chief reason why many people did not care to observe social/physical distancing (Table 1) when not enforced on them at ATMs, markets, religious gatherings, motor parks, shops, supermarkets, etc. Additionally, myths and conspiracy theories, such as COVID-19 not affecting Black people, high environmental temperature and weather killing off the virus, or COVID-19 being for rich people and elites, are some of the reasons why people are slow to accept the enormity of the pandemic and, therefore, take observance of social and physical distancing measures lightly [13].

Third, there are no efficient and robust housing and biometric data management systems where everyone is accounted for, especially for the purposes of employment, health, security, and social welfare. If these are available, foods and other goods purchased online can be sent to houses with ease. In addition, utilities such as power, water, or internet are not provided or are inadequately supplied in most cases. It is difficult for people to stay at home and observe social/physical distancing in such situations [13].

Participants in this study were asked whether they traveled out of Nigeria or had contact with someone that traveled out of Nigeria since the pandemic started (last 6 months; January-June 2020). The first confirmed case (index case) of COVID-19 in Nigeria was announced on February 27, 2020, when a foreigner in Lagos tested positive for SARS-CoV-2. Soon after, many people, including the contacts of the index case and those who returned to the country and their contacts, tested positive for the virus. Although overseas travel prior to the border closures and lockdowns in Nigeria was associated with the increased chance/risk of contracting COVID-19, this might have changed over time due to more community transmission of the virus (Table 2).

Looking at the dynamics and trajectory of COVID-19 in Nigeria in the early days of the pandemic when COVID-19 cases were reported already in urban areas in Nigeria, it was supposed to take a few weeks before the virus reached rural areas [5]. Since preventive measures such as social/physical distancing (lockdown) and use of face masks were enforced in these early days in most states of Nigeria including the Niger State, living in the rural areas of Niger State and other states of Nigeria ought to have been a protective factor against COVID-19 if the preventive measures were strictly observed. More than half (101/185, 54.6%) of the participants in this study did not practice social distancing (Table 1) at any time since the pandemic started, even as the lockdown was enforced in the state; this may be the reason why the risk of infection of the virus was the same for the urban and rural dwellers, who may be less observant of the preventive measures (Table 2). It is also an indication of community spread of SARS-CoV-2 in Niger State.

Additionally, the participants were asked whether they have had flulike experiences in the last 6 months (January to June 2020) since the COVID-19 index case was announced in Nigeria; this helped to deduce the COVID-19 asymptomatic rate in Niger State, which was 47% (22/47) (Table 2). Other SARS-CoV-2 serosurveys worldwide reported similar high asymptomatic rates of COVID-19 [16-18]. It has been reported that the majority of people infected with SARS-CoV-2 (about 50%-75%) are usually asymptomatic [19,20].

The seroprevalence of SARS-CoV-2 in Niger State was found to be 25.4% (47/185) and 2.2% (4/185) for positive IgG and IgM results, respectively. The observed seroprevalence was higher than in most of the SARS-CoV-2 serosurveys carried out around the same time in other parts of the world [17,18,21], and only 1 study in India reported a higher seroprevalence of 54.1% [22]. However, considering the 25.4% (47/185) SARS-CoV-2 seroprevalence observed in Niger State and the reported COVID-19 cases for Niger State and Nigeria as of June 30, 2020 (when this study was conducted) and December 21, 2020, SARS-CoV-2 infections occurred far more than the reported cases in the state and Nigeria [5]. Our data suggest that there are over 5000 times more SARS-CoV-2 infections than the number of reported cases in Niger State and over 900 times more SARS-CoV-2 infections than the number of reported cases in Nigeria. The high SARS-CoV-2 seroprevalence observed in this study may be due to many factors including a high COVID-19 asymptomatic rate and the lack of social distancing adherence in the state as observed in this study. An unknown high proportion of asymptomatic COVID-19 cases may not be diagnosed or reported, so our observed SARS-CoV-2 seroprevalence in the state will be more reliable and closer to the true prevalence of the disease than the official reported cases. As of December 21, 2020, and based on the reported COVID-19 cases and deaths, the fatality rates for COVID-19 in Niger State and Nigeria stood at 3.15% and 1.54%, respectively [5]. However, when the observed 25.4% (47/185) SARS-CoV-2 seroprevalence was considered, the fatality rates drastically reduced to 0.0009% and 0.024% for Niger State and Nigeria, respectively.

Usually, IgM becomes detectable in the whole blood/serum/plasma of patients 2-3 days from the onset of COVID-19 symptoms or after 10 days in cases of asymptomatic COVID-19 [10,15]. The IgM level in the blood peaks after 14 days of the SARS-CoV-2 infection and disappears after 28 days of infection [10,15]. However, IgG production starts after 14 days of infection and remains in the blood for long-term

immunity [10,15]. The timeline for production and disappearance of IgG and IgM are useful in the interpretation of the COVID-19 IgG and IgM rapid test. The test kit detecting only IgM means that the participant/patient is at the early stage of the infection, while the kit detecting only IgM means that the participant/patient had a past infection and recovered. However, the test kits detecting both the IgG and IgM at the same time means the participant/patient may be in the recovery stage of the infection. In this study, IgG and IgG plus IgM were observed. This means that the overwhelming majority of the participants who tested positive (positive IgG only) on the tests had past infections and recovered (Table 2). Only a few patients tested positive for both IgG and IgM and recovering from the infection (Table 2).

The SARS-CoV-2 seroprevalence among the age groups and gender correlated with the most mobile/active of the age groups and gender in our society (Table 2). The age groups 30-41 years, 42-53 years, and 54-65 years were the most mobile of the age groups, while men were more mobile than women and could, therefore, contract the virus easily. We observed less likely odds of contracting COVID-19 among females compared to males (Table 2). This means that being a female is a protective factor against the infection of SARS-CoV-2 in Niger State. This also correlated with the COVID-19 seroprevalence recorded among male and female participants (Table 2). The lower risk of infection for females in this study may be due to physical attributes such as less mobility and activity compared to males in society. Generally, the case fatality of COVID-19 varied widely worldwide (1%-20%), with more cases and fatalities observed in males compared to females [23,24].

High SARS-CoV-2 seroprevalence (16/43, 37%) and doubled odds of contracting COVID-19 among health care workers (Table 2) were observed. It is expected that health care workers have a higher COVID-19 prevalence compared to non–health care workers because they are the frontline workers responsible for the diagnosis, treatment, and management of patients, including those with symptomatic and asymptomatic COVID-19 [25-27]. These enormous essential tasks for controlling the COVID-19 pandemic together with the inadequate or lack of personal protective equipment in some instances and the high asymptomatic rate of COVID-19 among people put health care workers at greater risk of contracting and transmitting the disease. The double odds of being positive for SARS-CoV-2 were also observed for the participants who experienced flulike symptoms and observed social distancing since the pandemic started (January to June 2020; Table 2). The double odds for flulike symptoms were expected and in line with our findings that about 50% of the SARS-CoV-2 infections in the state were asymptomatic (Table 2). However, the double odds for observing social distancing are not correct and may be due to confounding issues; this was confirmed in the linear regression multivariate analysis (Table 2).

Limitations

The study has some limitations. First, selection bias may exist as it was more difficult to recruit participants who were 5 years or younger. Second, the sample size used in this pilot study and the number of SARS-CoV-2 rapid test kits used for validation were small. The SARS-CoV-2 rapid test kits that are suitable for epidemiological studies are costly; this limits the sample size of this pilot study. Third, to get a quick understanding of the levels of knowledge, attitudes, and practices about COVID-19, we kept the questionnaire short and simple, which might have limited the depth of the study.

Conclusions

To the best of our knowledge, this study is the first pilot SARS-CoV-2 seroprevalence data for Nigeria. The study revealed SARS-CoV-2 seroprevalence, patterns, dynamics, and risk factors in Niger State, Nigeria. The seroprevalence of SARS-CoV-2 in Niger State was found to be 25.41% (47/185) and 2.16% (4/185) for the positive IgG and IgM test results, respectively. Seroprevalence among age groups, genders, and occupations varied widely due to the differences in mobility and activity as well as the occupational exposures and hazards. The COVID-19 asymptomatic rate in the state was found to be 46.8% (22/47). The risk analyses showed that the chances of infection are almost the same for both urban and rural dwellers in the state. However, health care workers, those that experienced flulike symptoms, and those that had contact with a person that traveled out of Nigeria in the last 6 months have a doubled risk of being infected with the virus. More than half (101/185, 54.59%) of the participants in this study did not practice social distancing at any time since the pandemic started. The observed Niger State SARS-CoV-2 seroprevalence and infection patterns mean that the virus has widely spread, more SARS-CoV-2 infections have occurred than have been reported, and there is a high asymptomatic COVID-19 rate across the state. Our data suggest that >5000 times more SARS-CoV-2 infections occurred than the number of reported cases in Niger State and >900 times more than the number of reported cases in Nigeria.

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Conflicts of Interest

None declared.

Multimedia Appendix 1

Names, latitudes, and longitudes of the of the sampling and testing points.
[PDF File, 76 KB - xmed_v4i1e29587_app1.pdf]

Multimedia Appendix 2
Epi Info questionnaire administered simultaneously with the SARS-CoV-2 rapid IgG/IgM test to each of the participants in the study.
[PDF File, 127 KB - xmed_v4i1e29587_app2.pdf]

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Abbreviations

NCDC: Nigeria Center for Disease Control
PCR: polymerase chain reaction

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Original Paper

Angiotensin Converting Enzyme 1 Expression in the Leukocytes of Adults Aged 64 to 67 Years

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Abstract

The renin angiotensin system is composed of several enzymes and substrates on which angiotensin converting enzyme (ACE) 1 and renin act to produce angiotensin II. ACE1 and its substrates control blood pressure, affect cardiovascular and renal function, hematopoiesis, reproduction, and immunity. The increased expression of ACE1 has been observed in human monocytes during congestive heart failure and abdominal aortic aneurysm. Moreover, T lymphocytes from individuals with hypertension presented increased expression of ACE1 after in vitro stimulation with angiotensin II (ATII) with the highest ACE1 expression observed in individuals with hypertension with low-grade inflammation. Our group and others have shown that aging is associated with comorbidities, chronic inflammation, and immunosenescence, but there is a lack of data about ACE1 expression on immune cells during the aging process. Therefore, our aim was to evaluate the levels of ACE1 expression in nonlymphoid cells compared to lymphoid that in cells in association with the immunosenescence profile in adults older than 60 years. Cryopreserved peripheral blood mononuclear cells obtained from blood samples were used. Cells were stained with monoclonal antibodies and evaluated via flow cytometry. We found that ACE1 was expressed in 56.9% of nonlymphocytes and in more than 90% of lymphocytes (all phenotypes). All donors exhibited characteristics of immunosenescence, as evaluated by low frequencies of naïve $CD4^+$ and $CD8^+$ T cells, high frequencies of effector memory re-expressing CD45RA $CD8^+$ T cells, and double-negative memory B cells. These findings, in addition to the increased C-reactive protein levels, are intriguing questions for the study of ACE1, inflammaging, immunosenescence, and perspectives for drug development or repurposing (Reviewed by the Plan P #PeerRef Community).

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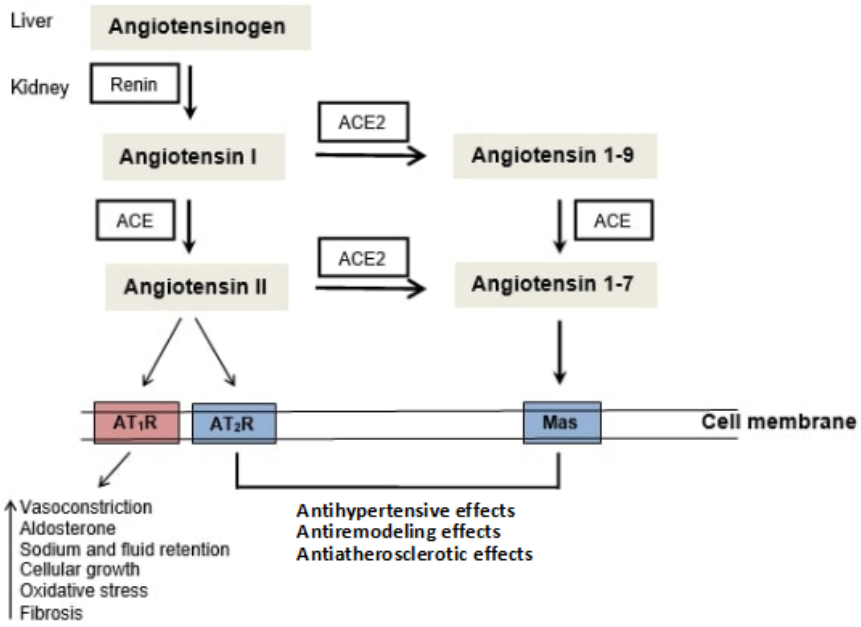
KEYWORDS

aging; angiotensin converting enzyme; lymphocytes; immunosenescence; inflammaging

Introduction

Angiotensin converting enzyme (ACE1, also known as CD143) and renin are components of the renin angiotensin system (RAS) acting to produce angiotensin II. In a simplistic definition, RAS is composed of a vasoconstrictor, proinflammatory ACE1/angiotensin II (ATII)/ATII receptor type 1 (AGTR1) axis, and a vasodilating anti-inflammatory ACE2/angiotensin-(1-7) [Ang-(1-7)]/Mas receptor axis (Figure 1). In addition to blood pressure control, ACE1 and its peptide substrates affect cardiovascular and renal function, hematopoiesis, reproduction, and the immunity [1,2]. Thus, it seems crucial that the RAS presents an inflammatory axis and an anti-inflammatory axis for adequate regulation of the immune response. ACE1 expression has been not only observed in tissues, but also its soluble form has been found in urine, serum, seminal fluid, amniotic fluid, and cerebrospinal fluid [3].

Figure 1. The renin angiotensin system. ACE: angiotensin converting enzyme; ACE1: angiotensin converting enzyme 1; AGTR1: angiotensin II type 1 receptor; AGTR2: angiotensin II type 2 receptor.



The expression of ACE1 in cells from the immune system has been reported in health and disease. Costerousse et al [4] observed, via reverse transcriptase–polymerase chain reaction and southern blot analysis, the expression of ACE1 in monocytes, macrophages, and T cells but not in B cells in healthy adult donors. In addition, ACE1 activity was very low in monocytes, whereas it was high in macrophages (monocytes driven to differentiation). T cells presented intermediary ACE1 activity and B cells expressed no activity [4]. In patients with type 1 diabetes (median age 29 years, normotension), higher *ACE1* and lower *ACE2* expression were observed when compared to healthy controls (median age 32 years, normotension) [5]. Coppo et al [6] found that T cells in culture had increased mRNA expression of *ACE1* and *AGTR1* in individuals with obesity with low-grade inflammation (high-sensitivity C-reactive protein [CRP] level of >3 mg/dL). ACE1 activity was also increased in the supernatant of a T cell culture in individuals with obesity with a high-sensitivity CRP level of >3 mg/dL. Moreover, expression of RAS genes in T cells and levels of inflammatory cytokines in the serum were oppositely associated with serum levels of insulin [6,7]. Ulrich et al [8] have shown that the increased expression of ACE1 in monocytes was associated with kidney and cardiovascular disease progression, suggesting that circulating leukocytes can modulate local immune responses via their own RAS components [8-10].

Considering that aging has been associated with comorbidities, low-grade chronic inflammation, and altered frequency or function of immune cells [11-14], it seems reasonable to suggest that ACE1 play an important role in the aging process. ACE1 has been suggested to influence age-related diseases (ie, Alzheimer disease, sarcopenia, and cancer) but the associated mechanisms are still under investigation. *ACE1* polymorphisms have been correlated with susceptibility to Alzheimer disease [15,16]. In addition, it was shown recently that in normal aging, ACE1 expression is increased in brain homogenates, and this expression is unchanged in early stages of Alzheimer disease [17]. Regarding sarcopenia, Yoshihara et al [18] found a weak correlation between *ACE1* polymorphism and physical function. In cancer (gastric or colorectal), patients presented higher expression of ACE1 in tumors than in healthy tissues [19,20]. In hematopoietic stem/progenitor cells isolated from peripheral blood, Joshi et al [21] showed that aging is associated with decreased ACE2 and increased ACE1 protein expression. This imbalance suggests a bias to the detrimental proinflammatory axis of the local RAS. Considering the scarce information about ACE1 expression in the phenotypes of T and B cells, we aimed to investigate ACE1 expression in cells from the immune system and parameters of immunosenescence in adults older than 60 years. Results herein show different levels of expression of ACE1 in nonlymphoid versus lymphoid cells, with expression being higher in lymphoid cells.

Methods

Overview

Blood was collected from adults (n=6, four females and 2 males) aged 64-67 years in 2015. Peripheral blood mononuclear cells were isolated using a Ficoll–Hypaque density gradient (Amersham Biosciences) and centrifugation. Viable cells were counted, adjusted to 2×106/100 µL in 80% fetal bovine serum and 20% dimethylsulfoxide (Sigma), and frozen stored until the phenotyping. In 2021, cells were thawed, checked for viability, and stained with monoclonal antibodies to the T cell phenotypes CD4 PerCP Cy5.5, CD8 APC Cy7, CD27 APC, CD45RA PE; B cell phenotypes CD19 PE, CD27 APC, IgD PE Cy5.5 (eBioscience), and ACE CD143 fluorescein isothiocyanate (R&D Systems). After 30 minutes of incubation with monoclonal antibodies in the dark at 4 °C, the cells were washed with phosphate-buffered saline and centrifuged. Living cells (based on forward and side scatter) were acquired in the BD FACSCanto II flow cytometry system using the DIVA software (Becton Dickinson).

For assessing metabolic parameters, the serum of studied individuals was previously isolated through centrifugation and frozen stored until use. Measurement of metabolic parameters was performed in the Laboratório Central–Hospital São Paulo, Federal University of São Paulo.

Statistical Analysis

Data are presented as mean (SD) values. To test the normality of data, we used the Shapiro-Wilk test. We considered *P* values for interindividual differences in each variable, since individuals were aged differently (biological aging) and thus, physiological parameters could be affected by genetics, lifestyle, nutrition, and comorbidities. A *P* value less than .05 was considered significant.

Ethics Approval

The Ethics Committee of the Federal University of São Paulo approved all procedures (protocol 10904).

Results

Table 1 shows that older adults are heterogeneous for some physiological parameters such as glucose, urea, glycated hemoglobin, and CRP.

Table 2 and Figures 2-4 show that CD143 (ACE1) is expressed in almost 100% of lymphocytes, whereas it is expressed in 56.9% (SD 20.6%) of nonlymphocytes. $CD8^+$ T cells presented the highest expression (98.4%), followed by $CD19^+$ B cells (93.7%, SD 3.4%) and $CD4^+$ T cells (90.7%, SD 8.7%). In T cells, ACE1 is expressed in all phenotypes (naïve, central memory, effector memory, and effector memory re-expressing CD45RA [EMRA]). In B cells, ACE1 was expressed in naïve, unswitched memory, switched memory, and double-negative (DN) cells.

Table 3 shows that characteristics of senescent T cells were observed in both males and females, such as low expression in naïve $CD4^+$ and $CD8^+$ T cells and high expression in EMRA $CD8^+$ T cells.

Table 4 shows that aging adults with lower percentages of naïve B cells also presented a higher percentage of DN memory B cells.

Table 1. Physiological parameters observed in older adults.

	Cholesterol[a] (mg/dL)	Low-density lipoprotein[a] (mg/dL)	Triglycerides[a] (mg/dL)	Glucose[b] (mg/dL)	Urea[c] (mg/dL)	Creatinine[a] (mg/dL)	Albumin[a] (mg/dL)	Glycated hemoglobin[d] (mg/dL)	C-reactive protein[e] (mg/dL)
Individual participants' values	207, 253, 181, 223, 249, and 191	137, 176, 96, 150, 186, and 125	152, 152, 130, 149, 163, and 130	80, 86, 137, 83, 89, and 165	30, 40, 28, 28, 29, and 28	0.86, 0.73, 0.84, 0.68, 0.79, and 1.01	3.8, 4.1, 3.2, 4.2, 3.8, and 3.4	5.9, 6.2, 7.9, 5.5, 5.8, and 6.0	7.3, 4.1, 6.0, 23.1, 4.6, and 0.6
Overall, mean (SD)	217.3 (27.2)	145.0 (30.4)	146.0 (12.1)	106.7 (32.5)	30.5 (4.3)	0.82 (0.1)	3.8 (0.4)	6.2 (0.8)	7.6 (7.2)

[a]*P*>.10.

[b]*P*=.047.

[c]*P*=.02.

[d]*P*=.02.

[d]*P*=.03.

Table 2. CD143 (ACE1) expression in lymphocytes and nonlymphocytes.

	Lymphocytes (%)			Nonlymphocytes[a] (%)
	$CD4^+CD143^{+b}$	$CD8^+CD143^{+b}$	$CD19^+CD143^{+b}$	
Individual participants' values	84.8, 77.6, 96.9, 98.8, 87.8, and 98.3	97.1, 96.7, 99.0, 99.6, 98.5, and 99.6	90.5, 90.6, 91.4, 99.0, 95.7, and 94.9	74.6, 35.4, 47.7, 75.0, 32.9, and 75.9
Overall, mean (SD)	90.7 (8.7)	98.4 (1.3)	93.7 (3.4)	56.9 (20.6)

[a]*P*=.08.

[b]*P*>.15.

Figure 2. Flow cytometry gating strategy for B cell phenotypes and CD143 expression. (A) All cells and gates for lymphocyte (green) based on forward scatter (FSC-A) and side scatter (SSC-A); (B) exclusion of doublets (from the lymphocyte gate); (C) $CD19^+$ B cells (from the doublets exclusion gate); (D) CD143+ACE1 cells (from the $CD19^+$ B cells' gate); and (E) B cell phenotypes and CD143+-IgM+IgD+CD27- (naïve), $IgMlowIgD-CD27^+$ (memory-unswitched), IgM-IgD-$CD27^+$ (memory-switched), and IgM+IgD-CD27- (memory double-negative). DN: double-negative; FSC: forward scatter; Mem: memory; SSC: side scatter.

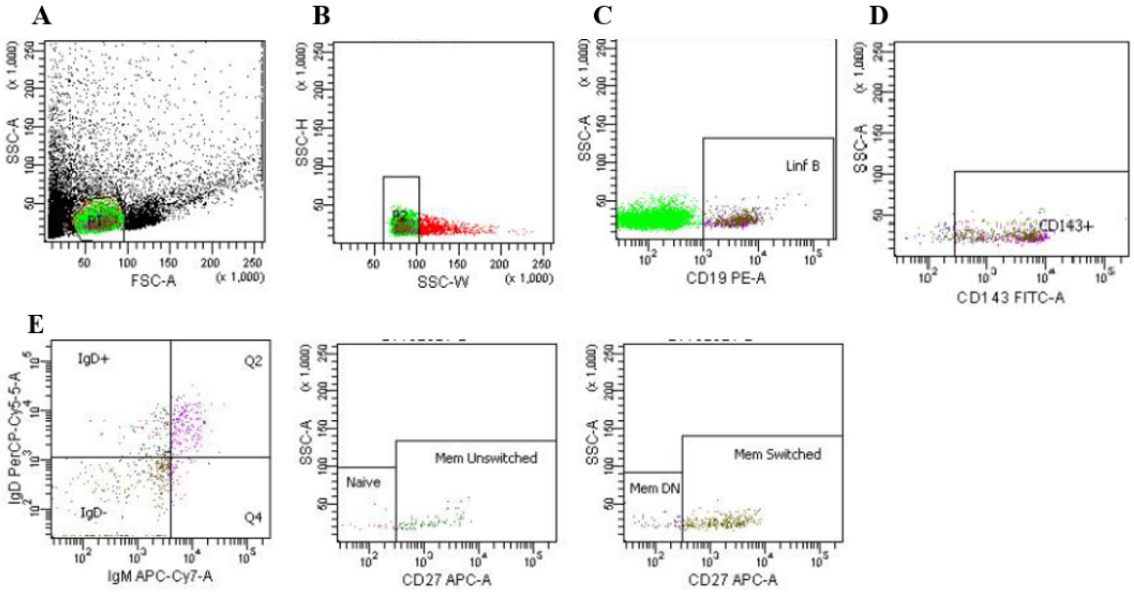


Figure 3. Flow cytometry gating strategy for T cell phenotypes and CD143 expression. (A) All cells and gates for lymphocyte (green) based on forward scatter (FSC-A) and side scatter (SSC-A); (B) exclusion of doublets (from the lymphocyte gate); (C) $CD4^+$ and $CD8^+$ T cells (from the doublets exclusion gate); (D) CD143+ACE1 cells (from the $CD4^+$ and $CD8^+$ T cells' gate); (E) T cell phenotypes and $CD143^+$, CD45RA+CD27- (naïve), CD45RA-$CD27^+$ (central memory), CD45RA-CD27- (effector memory), and CD45RA+CD27- (effector memory re-expressing CD45RA) cells. FSC: forward scatter; SSC: side scatter.

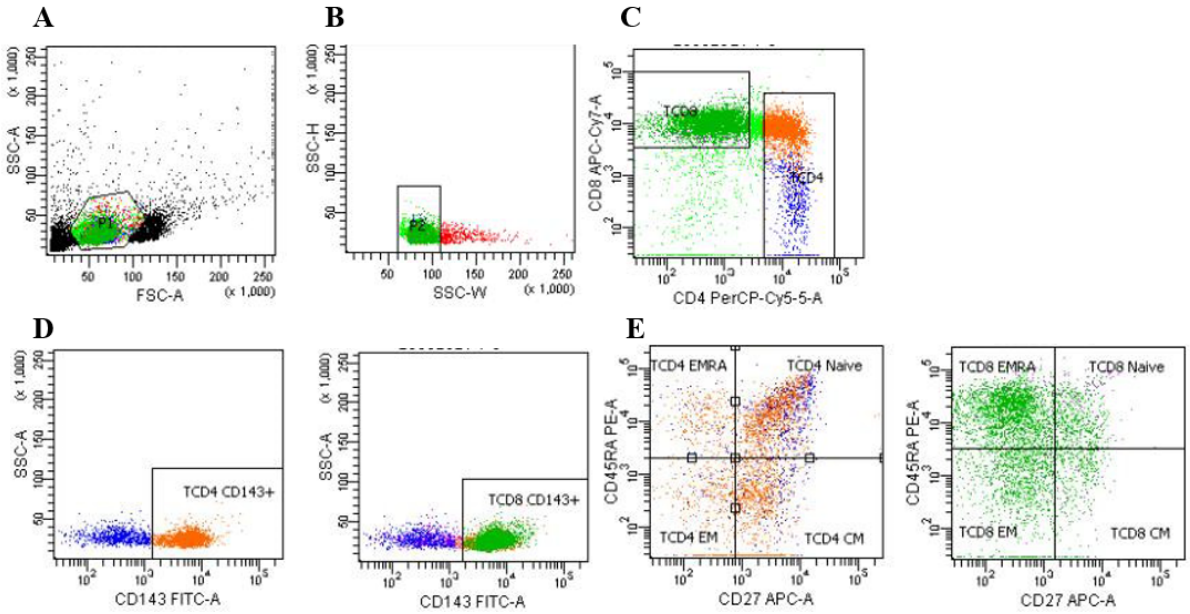


Figure 4. Flow cytometry gating strategy for nonlymphocytes and CD143 expression. (A) All cells and gates for lymphocytes (P1) and nonlymphocytes based on forward scatter (FSC-A) and side scatter (SSC-A) and (B) $CD143^+$ ACE1 cells (from the nonlymphocyte gate). FSC: forward scatter; SSC: side scatter.

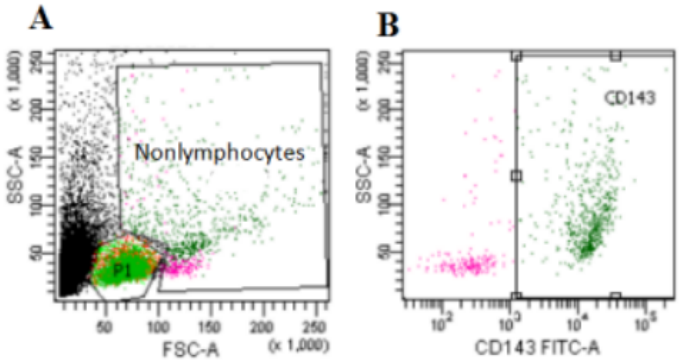


Table 3. Phenotypes of $CD4^+$ and $CD8^+$ T cells.

	$CD4^+$ T cells (%)				$CD8^+$ T cells (%)			
	Naïve[a]	Central memory[b]	Effector memory[a]	Effector memory re-expressing CD45RA[b]	Naïve[a]	Central memory[a]	Effector memory[a]	Effector memory re-expressing CD45RA[a]
Individual participants' values	27.6, 43.3, 13.4, 12.5, 24.8, and 32.6	55.9, 29.1, 55.4, 49.8, 55.3, and 25.4	12.4, 15.4, 29.2, 34.7, 18.3, and 19.7	4.1, 12.2, 2.0, 3.0, 1.5, and 22.4	17.3, 10.2, 13.6, 10.7, 12.8, and 11.7	26.5, 6.5, 10.3, 16.6, 11.5, and 18.3	20.1, 24.8, 13.6, 9.8, 27.6, and 20.4	36.0, 58.6, 62.5, 63.0, 48.1, and 49.6
Overall, mean (SD)	25.7 (11.7)	45.2 (14.1)	21.6 (8.6)	7.5 (8.3)	12.7 (2.6)	15.0 (7.1)	19.4 (6.7)	53.0 (10.4)

[a]$P>.10$.

[b]$P=.047$.

Table 4. Phenotypes of $CD19^+$ cells.

	Naïve[a] (%)	Unswitched memory[a] (%)	Switched memory[a] (%)	Double-negative memory[a] %
	73.8, 61.3, 28.6, 51.8, 35.9, and 67.7	6.3, 6.9, 4.1, 10.0, 7.9, and 3.5	4.0, 5.7, 31.4, 22.1, 18.5, and 9.8	15.9, 26.1, 35.8, 16.1, 37.7, and 19.0
Overall, mean (SD)	53.2 (17.9)	6.5 (2.4)	15.3 (10.6)	25.1 (9.8)

[a]*P*>.10.

Discussion

Our results show that for the studied population, chronological aging and biological aging are asynchronous. Even among individuals with a small chronological difference (64 to 67 years), there is heterogeneity in physiological parameters such as glucose, urea, glycated hemoglobin, and CRP. Changes in the same functional parameters have been reported by Carlsson et al [22] and Helmerson-Karlqvist [23] in healthy older adults. Carlsson's [22] study found that the CRP level was 2.6% with a coefficient variation of 1.4%, whereas in our study, we observed higher levels of CRP in 5 out of 6 individuals. Increased CRP levels have been associated with inflammaging, and our findings show that the study population has changes in functional parameters, which are likely associated with an inflammatory profile [24].

The link between the RAS and inflammation has been suggested but its role is not completely clear under physiological and pathological conditions [25,26]. In addition, the association between altered ACE1 expression in tissues (brain, muscle, heart, and vessels) and the development and progression of age-related conditions such as Alzheimer disease, sarcopenia, and cardiovascular disease has been suggested, but results are controversial [17,27-30].

There are few studies showing the association between ACE1 expression in cells from the immune system (monocytes and T cells) and the progression of kidney and cardiovascular disease [8,9,31,32]. Therefore, considering the lack of information on this issue, we questioned whether ACE1 (CD143) was highly expressed in cells from the immune system during the aging process. We found that ACE1 was expressed in almost 100% of T ($CD4^+$ and $CD8^+$) and B lymphocytes and in all phenotypes of these cells. In nonlymphoid cells, mean ACE1 expression was 56.9% (SD 20.6%). In agreement with our findings, independent studies showed that T cells from healthy donors and monocytes from patients with congestive heart failure expressed ACE1, but there has been no investigation on cell phenotypes [25,26]. Our study is the first to show that either inexperienced (naïve) or fully activated (memory) cells expresses ACE1. Our findings suggest that the expression of ACE1 in lymphoid and nonlymphoid cells reflects health status, since our studied population presented changes in physiological parameters and high levels of ACE1 expression in immune cells. Previous independent studies showed that patients with unstable angina [32] or acute myocardial infarction [33] presented higher expression of ACE1 in T cells and dendritic cells than control subjects. In addition, markers of cell (lymphoid and nonlymphoid) functional status, such as inflammatory or growth factor production, could be modulated by ACE inhibitors (ACEi). Accordingly, mononuclear leukocytes from healthy subjects incubated with an endotoxin exhibited high levels of tissue factor activity, which was reduced in the presence of captopril in a dose-dependent pattern. This result could be related to the antithrombotic effect of ACEi [34]. In patients with congestive heart failure, immune cells cultured with lipopolysaccharide secreted high levels of the proinflammatory tumor necrosis factor α, and these levels were significantly reduced in the presence of captopril [35].

It may be proposed that mechanistically, ATII is produced by mononuclear cells or lymphocytes and, at the same time, ATII induces immunologic activation in these cells. Therefore, the inflammatory axis ACE1/ATII/AGTR1 and the counterregulator ACE2/Ang-(1-7)/Mas receptor axis [36,37] could play a role in chronic diseases, inflammaging, and immunosenescence observed in older adults. Our studied population presented changes in some physiological parameters and increased levels of CRP. This inflammatory profile [24], in addition to more than 90% of T and B cells expressing ACE1 in our population of older adults, suggest a correlation among aging, inflammaging, and ACE1 expression. Independent of chronological age, inflammation (even if related to subclinical diseases) may be a contributor to disease progression when the balance with anti-inflammation is shifted [38]. In this context, the regulation of ACE1/ACE2 expression could be explored as a target for the balance of exacerbated inflammatory reactions. Considering that the equilibrium between ACE1 and ACE2 expression could play an important role in healthy aging, our subsequent studies will be focused on ACE1 and ACE2 expression in cells from the immune system.

The phenotype of T and B lymphocytes has been used to identify senescence in immune cells. $CD4^+$ T cells present changes during the aging process with a decrease in naïve phenotypes and an increase in effector memory phenotypes, whereas $CD8^+$ T cells show a decrease in the naïve phenotype and an increase in the effector memory and EMRA phenotypes [12,39,40]. It has been shown that the reduction in naïve B cells is accompanied by no change in memory-unswitched and memory-switched B cells but an increase in the percentage of double-negative B cells [41-44]. Using these phenotypes, we found a similar senescent phenotype in some of the studied aging adults. The reduction in naïve lymphocytes has been related to impaired antigen responsiveness, and for B cells, a decrease in the production of antibodies has been observed [45,46]. The increased percentage of DN memory B cells has been linked to autoimmune diseases [47,48]. We observed ACE1 expression in more than 90% of T cells and B cells and in all phenotypes. ACE1 was expressed in nonlymphocytes in a range of 32.9% to 75.9%. Our findings suggest that ACE1 could play

a role in several processes linked to aging, including the generation and activation of autoimmune cells, due to the experimental evidence that inhibitors of ACE1 suppress the autoimmune process in a number of autoimmune diseases such as experimental autoimmune encephalomyelitis, arthritis, autoimmune myocarditis [49].

This study is the first to compare the expression of the protein ACE1 between different cell types, both lymphoid cells ($CD4^+$ and $CD8^+$ T cells and B cells) and nonlymphocytes in older adults. It was also observed that even though the study participants were in the early stage of chronological aging (64 to 67 years), they presented heterogeneity in physiological parameters, signs of inflammaging (increased CRP levels), and immunosenescence, including low expression in naïve T and B cells in addition to the accumulation of terminally differentiated $CD8^+$ T cells and DN B cells. This study has limitations such as the small sample size and the lack of young adults for comparison. As an example, the subject presenting the highest CRP and albumin levels also exhibited a high percentage of ACE1 expression in T cells ($CD4^+$ and $CD8^+$), B cells, and nonlymphoid cells, in addition to the lowest percentage of $CD4^+$ naïve cells, and the highest percentage of $CD8^+$ terminally differentiated (EMRA) and DN B cells. However, due to the small sample size, it was not possible to associate the high expression of ACE1 in immune cells with inflammaging and immunosenescence. Correlation of physiological parameters and health status with ACE1 expression and investigating whether age and associated chronic diseases could lead to increased ACE1 expression would yield important information. Moreover, we only determined CRP as a marker of inflammaging, and interleukin 6 and tumor necrosis factor α would be desirable to complete our panel. Functional analyses are needed to clarify the impact of ACE1 expression on immune cells and whether ACEi and angiotensin receptor blockers administered to patients with hypertension somehow affect immunity. Recently, it was shown that membrane-bound ACE2 acts as a receptor for SARS-CoV-2, but the possible effects on RAS components [ATII, Ang-(1-7), ACE1, ACE2, AT1, and Mas] and whether ACEi and angiotensin receptor blockers interfere with the mitigation of COVID-19 require further investigation [50-54]. Therefore, it is important to emphasize the negative impact of chronic diseases on the outcomes of older adults during a viral infection and how ACE1 or ACE2 expression in immune cells could provide information regarding diagnosis and treatment.

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Conflicts of Interest

None declared.

Editorial Notice

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Abbreviations

ACE1: angiotensin converting enzyme
ACEi: angiotensin converting enzyme inhibitors
AGTR1: angiotensin II receptor type 1
Ang-(1-7): angiotensin-(1-7)
ATII: angiotensin II
DN: double-negative
EMRA: effector memory re-expressing CD45RA
RAS: renin angiotensin system

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Protocol

Postacute Sequelae of COVID-19 and Adverse Psychiatric Outcomes: Protocol for an Etiology and Risk Systematic Review

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Abstract

Background: The postacute sequelae of COVID-19 (PASC) is a syndrome characterized by persistent COVID-19 symptoms or the onset of new symptoms following recovery from the initial or acute phase of the illness. Such symptoms often occur 4 or more weeks after being diagnosed with COVID-19. Although a lot of work has gone into understanding the long-term mental health effects of PASC, many questions related to the etiology and risk of this condition remain.

Objective: This protocol is for a systematic review assessing the association between PASC and adverse psychiatric outcomes and whether people with PASC are at greater risk of developing an adverse psychiatric outcome than those without PASC.

Methods: Various medical literature databases (eg, PubMed and EMBASE) will be searched for eligible articles, using predefined search criteria. Gray literature will also be explored. Epidemiological observational studies and secondary analyses of randomized controlled trials that report a quantitative relationship between PASC and at least one adverse psychiatric outcome will be included. The Population, Exposure of interest, Comparator, and Outcome framework will be used as a standardized framework for the inclusion criteria. The Joanna Briggs Institute critical appraisal tools will be used to assess methodological quality and critically appraise the risk of bias in included studies. A random-effects meta-analysis will be conducted if possible. A formal narrative synthesis will be performed if a meta-analysis is impossible due to substantial heterogeneity across studies. The Grading of Recommendations Assessment, Development and Evaluation approach will be used to rate the cumulative certainty of the evidence for all outcomes. Ethical approval is not required. The study results will be published in a peer-reviewed journal.

Results: This study documents and addresses etiology, risk factors, and long-term symptoms of COVID-19 among people with PASC. It focuses on a key priority area for new evidence syntheses on the clinical management of COVID-19 and pandemic-related conditions. It will include evidence on nonhospitalized and hospitalized patients with a history of PASC.

Conclusions: Substantial heterogeneity across studies may limit the ability to perform a meta-analysis. Findings will inform disease prevention, decision-making, health care policy, and clinical research (Reviewed by the Plan P #PeerRef Community).

Trial Registration: PROSPERO CRD42022308737; https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=308737

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KEYWORDS

COVID-19; long COVID; post–COVID-19 condition; postacute sequelae of COVID-19; PASC; psychiatry; epidemiology; evidence-based medicine

Introduction

Overview

COVID-19 is a contagious illness caused by SARS-CoV-2. Persistent and long-lasting (>4 weeks) symptoms following infection with acute COVID-19 have given rise to a syndrome known as post-acute sequelae of COVID-19 (PASC) or post–COVID-19 condition [1-3]. Incidence and prevalence estimates for people with COVID-19 presenting with or reporting persistent psychiatric symptoms after months of initial infection range from 0.8% to 49% [1-5]. Among 44,759 people with no recorded history of psychiatric illness, the estimated overall probability of being diagnosed with new-onset psychiatric illness in the 90 days following a confirmed diagnosis of COVID-19 was 5.8% in a retrospective cohort study [6].

Similarly, clinical anxiety and depression, as well as other psychiatric sequelae, have been reported following diagnosis with COVID-19 in other studies [6-8]. Although sex and age are considered to be sociodemographic risk factors for PASC, there is no consensus on other baseline clinical features that act as independent predictors of PASC [9,10]. The prevalence of PASC symptoms is higher in women than in men [10].

Among people aged 35-49 years, the prevalence of PASC is 26.8% compared with 26.1% and 18% among people aged 50-69 years and 70 years or older, respectively [10]. Persistent symptoms occur weeks and months after infection irrespective of initial disease severity (mild, moderate, severe, and critical) [11,12]. Mendez et al [13] reported in their cross-sectional study that 2 months after discharge, neurocognitive impairment, psychiatric morbidity, and poor quality of life were markedly prevalent among 179 COVID-19 survivors who had been hospitalized [13]. Nevertheless, Vannorsdall and Oh [14] posit that current research on the postacute phase following hospitalization has been conflicting due to the absence of a detailed, standardized neuropsychological evaluation of patients with COVID-19 after hospitalization [14]. In addition, they stated that literature on PASC and adverse mental health outcomes are mostly limited to studies that cannot establish causal relationships or lack generalizability (eg, case reports, case series, and data obtained from cognitive screening instruments) [14]. Thus, more high-quality studies are warranted [15].

In a study where the short-term and long-term sequelae of COVID-19 were systematically evaluated, PASC was categorized as short-term (1 month), intermediate-term (2-5 months), and long-term (≥6 months) following COVID-19 diagnosis [13]. Clinical manifestations of PASC were classified into organ systems, including cardiovascular, dermatologic, digestive, ear, nose, and throat; mental health, neurologic, and respiratory; constitutional symptoms; and functional mobility [13]. The mechanisms leading to the postacute and chronic neuropsychiatric manifestations of COVID-19 may be due to the direct effect of the viral infection and the indirect effect on mental health due to social isolation, posttraumatic stress, and job loss. Specifically, correlations have been observed between COVID-19 posttraumatic stress scores, general distress, and sleep disruption [13,14]. Despite those correlations, Khubchandani et al [16] stated that the causal pathways and etiology of adverse mental health outcomes in people who were infected with COVID-19 are multidimensional and complex [16,17].

To clarify whether COVID-19 is a risk factor for psychiatric disorders and vice versa, an electronic health record network cohort study of 69 million people consisting of 62,354 people with a COVID-19 diagnosis compared the rates of psychiatric sequelae of health in the initial 4 months of the pandemic (January to April 2020) and subsequently (after April 2020) [8]. The study found that the rate of all diagnoses of psychiatric disorders and relapses was greater following COVID-19 infection than after control health events (eg, influenza infection, skin infection, other respiratory tract infections, and fracture) [8].

Likewise, a diagnosis of psychiatric disorder in the 12 months preceding the COVID-19 pandemic was associated with a 65% increased risk of COVID-19 (relative risk [RR] 1.65, 95% CI 1.59-1.71; $P<.001$) compared with a matched cohort of people with specific physical risk factors for COVID-19 without a psychiatric diagnosis [18]. Whereas these associations were partly attributed to illness severity and pandemic-related contextual factors (eg, social isolation, overwhelmed health care systems, and stigma), they do not adequately account for observed differences in psychiatric sequelae [18]. Moreover, the inability to conclusively determine why there were between 2- and 3-fold increases in the risk of neurologic and psychiatric complications following a COVID-19 infection, in this and other studies, calls for further examination of the association between COVID-19 and risk factors for psychiatric morbidity [8,16,18].

Rationale

With many long-term adverse mental health outcomes linked to COVID-19, effective interventions that optimize recovery and minimize relapse are needed. Such interventions may serve as appropriate tools to evaluate risk factors that may cause maladaptive psychiatric responses [19]. Furthermore, they may aid with the management of anxiety, fear, frustration, stigma, and paranoia by mitigating psychopathological symptoms and reducing contextual stress [19,20]. Interventions that have been assessed in patients with COVID-19 include web-based and physical psychotherapeutic approaches; for example, cognitive behavioral therapy, emotional freedom techniques, and ultrabrief psychological interventions; combined psychiatric and psychological interventions; technology and media; complementary and alternative therapies; self-care; spirituality and religion; and pharmacotherapies [21-23].

Evidence on the effectiveness of these interventions is mixed and not thoroughly synthesized, with quality inadequately assessed in earlier studies, and may vary depending on COVID-19 duration and severity. In a randomized controlled clinical trial of 51 people with COVID-19 consisting of an experimental group receiving progressive muscle relaxation technology for 30 minutes each day for 5 consecutive days and a control group receiving only usual care and treatment, participants in the experimental group reported lower depressive symptoms, lower anxiety levels, and better sleep quality than those in the control group [24]. Another randomized control trial of 30 hospitalized patients with COVID-19 assigned to an experimental or control group reported an improvement in all outcome measures among intervention group subjects compared to controls [24]. In that study, a short 4-session crisis intervention package tailored to cover COVID-19–specific guidance was delivered by clinical psychologists [24]. Topics covered included tension reduction, relaxation, adjustment, responsibility skills enhancement, and promoting resilience [24]. Outcome measures in the study were derived from the Depression, Anxiety, and Stress Scale, Symptom Checklist 25, and the abbreviated version of the World Health Organization Quality of Life assessment [24]. Lack of cultural specificity, methodological issues, small sample sizes, lack of follow-up, unadjusted confounding factors, and brief time spans in both studies limit their generalizability [23,24].

During the COVID-19 pandemic, digital interventions to deliver health care have gained widespread acceptance [25]. Remote care coordination and provision have been adopted to help reduce the risk of disease transmission [25]. Mobile apps have also been used for contact tracing and information dissemination [25]. Although an evidence synthesis of digital interventions to attenuate the adverse effects of the COVID-19 pandemic on the mental health of the public highlighted their importance in mental disorder prevention and mental health promotion; it noted that evidence on their cost-effectiveness, process quality, and long-term outcomes is sparse [26]. Furthermore, the negative impact and risks of the COVID-19 pandemic are sometimes more significant in vulnerable and clinically extremely vulnerable populations (eg, people older than 70 years, pediatric patients with cystic fibrosis, or people with developmental disabilities) who may be digitally disadvantaged [26-29].

Presently, it is unclear what duration of PASC, etiologies, and risk factors are most associated with the manifestation or persistence of adverse psychiatric outcomes (eg, depression, anxiety, substance use disorder, posttraumatic stress disorder, psychosis, dementia, nonsuicidal self-injury [self-harm], and suicide) compared with other health events. A prospective cohort study of patient-reported outcome measures 3 months after initial COVID-19 symptom onset noted impairment with self-care and anxiety or depression as being present in 13% and 22% of its 78 subjects, respectively, with at least 1 Charlson comorbidity at baseline compared to subjects without any Charlson comorbidities (4% and 9% respectively). Among subjects without any Charlson comorbidities, 70% reported an abnormal PROM, and 33% had at least 1 moderate issue in at least 1 EQ-5D assessment [30]. In addition, questions remain about the long-term (≥6 months) outcomes of COVID-19 [30].

Although some studies indicate that most people who acquire COVID-19 are at risk of psychiatric sequelae and their symptoms tend to improve over time, others suggest that symptoms may worsen over time or point to a different disease trajectory [29,30]. Research and any future recommendations about PASC and mental health should be guided by the best available evidence.

Few epidemiological studies have investigated the short- and long-term impact of COVID-19 and PASC on mental health. Thus, this study will examine the causes of adverse psychiatric outcomes and risk factors in people with PASC. Furthermore, prior studies on this and related topics report internal validity and generalizability (external validity) limitations due to evidence derived solely from electronic health records, single networks, or claims data. Because data on the psychiatric sequelae of PASC are conflicting and sparse, it is imperative to systematically summarize the evidence and combine the results of various scientific studies.

This study aims to generate a new hypothesis on causality and provide a more precise estimate of the risk factors underlying PASC and adverse psychiatric outcomes. An initial search of peer-reviewed and gray literature found no systematic reviews and meta-analyses on the topic. This protocol is for a systematic review that assesses the literature on PASC duration and risk factors that act as determinants (etiologies) of adverse psychiatric outcomes.

Objectives

The primary objective of this systematic review is to determine whether people with PASC are at greater risk of developing an adverse psychiatric outcome (depression, anxiety, substance use disorder, posttraumatic stress disorder, psychosis, dementia, nonsuicidal self-injury [self-harm], or suicide) than those without PASC.

Secondary review questions include the following:

1. Does the association between PASC and an adverse psychiatric outcome vary with age, sex, severity of COVID-19 (mild, moderate, severe, and critical), and duration of PASC (short-term [1 month], intermediate-term [2-5 months], and long-term [≥6 months] following COVID-19 diagnosis or hospital discharge)?
2. Is PASC an independent risk factor for an adverse psychiatric outcome?

Methods

This protocol has been drafted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance for protocols (PRISMA-P) [31]. The systematic review will explicitly report any amendments and modifications made to this protocol.

Eligibility Criteria

Study Design and Characteristics

The review will include observational studies, namely, retrospective studies, and prospective longitudinal cohort studies, case-control studies, cross-sectional studies, case series, and case reports. Secondary analyses of randomized controlled trials will also be included. Effect measures of risk factors, including the incidence rate ratio, risk difference, relative risk, odds ratio (OR), and hazard ratio central to the primary outcome, will be included. Risk factors predispose people with PASC to an adverse psychiatric outcome [32,33]. Such risk factors are associated with an increased probability of people with PASC having a negative mental health outcome [32,33]. Information on the relationship between risk factors and incidence of primary and secondary outcome measures will be included. Studies that do not report a quantitative relationship between PASC and at least one adverse psychiatric outcome will be excluded.

COVID-19 diagnosis must have been confirmed on the basis of clinical suspicion or with a positive nucleic acid amplification test such as reverse transcriptase–polymerase chain reaction, an antigen test, or a serologic test (eg, a rapid serology test or enzyme-linked immunosorbent assay) [34]. Studies will be included if subjects were longitudinally observed since the initial diagnosis of COVID-19; that is, during the acute phase or since the time of PASC onset (postacute or chronic phase) [34]. A follow-up time of at least 1 month since COVID-19 diagnosis is required [35]. Primary and secondary outcomes will encompass etiology, risk factors, symptom and illness severity, duration of PASC, and adverse events [34,35].

Participants

Studies with adult participants (aged 18 years or older) will be included. Pediatric and animal studies will not be included. There will be no sex, ethnicity, or race limitations. The search dates will range from December 2019 (date of the first confirmed case of COVID-19) until March 2023 (the anticipated completion date of the review). COVID-19 filters will be used, if necessary, to limit search results to COVID-19– and PASC-related articles.

Exposure

Primary Measure

PASC, for this review, is defined as a continuing symptomatic illness or the emergence of new symptomatic illness in people with a confirmed history of COVID-19 after recovery from the acute phase of the illness. PASC will be categorized as short-term (1 month), intermediate-term (2-5 months), and long-term (≥6 months) following a COVID-19 diagnosis or hospital discharge.

Secondary Measure

The severity of COVID-19 (mild [including asymptomatic], moderate, severe, or critical) will be considered.

Comparators (Controls)

Primary Measure

People with a confirmed history of COVID-19 without PASC will be considered.

Secondary Measure

The severity of COVID-19 (mild [including asymptomatic], moderate, severe, or critical) will be considered.

Outcomes

Primary Outcome Variable

Primary outcome variables will include adverse psychiatric outcomes such as depression, anxiety, substance use disorder, posttraumatic stress disorder, and psychosis.

Secondary Outcome Variables

Secondary outcome variables will include nonsuicidal self-injury (self-harm) and suicide.

Information (Evidence) Sources and Search Strategies

Information, including titles and abstracts extracted from evidence sources, will be initially screened against the review questions. Information deemed eligible for inclusion will undergo more comprehensive screening. Once an article, study, or review is considered suitable for inclusion, it will be placed in the list of included studies. The steps above will be carried out for each information source, after which duplicates will be removed. The study selection process will be described in a PRISMA flow diagram and reported in the systematic review. Author AE will develop the search strategy in consultation with a medical research librarian. The following databases and evidence sources will be searched: PubMed, Ovid MEDLINE, Embase, JBI EBP Database, CINAHL Plus, UpToDate, APA PsycInfo, Google Scholar, ProQuest Dissertations & Theses Global, Scopus, Web of Science, the University of Toronto COVID-19 Data & Statistical Sources, Centre for Addiction and Mental Health (CAMH) COVID-19 National Survey Dashboard reports, and COVID-END. Gray literature will also be considered where appropriate. Search strategies will be comprehensive and adapted for each information source. See Appendix 1 for a sample of the Embase search strategy.

The Covidence or JBI SUMARI software will be used during the systematic review process for screening, appraisal of evidence sources, data extraction, synthesis, and study completion.

Ethics and Dissemination

Ethical approval is not required for this study. The study findings will be disseminated via preprints, peer-reviewed publications, conference abstracts, posters, plain-language summaries, presentations, and infographics.

Patient and Public Involvement

Input on the review questions and outcomes was informally sought from patients and people who had been previously diagnosed with COVID-19 and PASC.

Results

Study Selection

Information, including titles and abstracts, extracted from information sources will be initially screened by AE and a second reviewer against the research questions. Information deemed eligible for inclusion will undergo more comprehensive screening. Once an article, study, or review is considered suitable for inclusion, it will be placed in the list of included studies. The steps above will be carried out for each information source, after which, duplicates will be removed. Disagreements on inclusion will be resolved through discussion or arbitration. The study selection process will be described in a PRISMA flow diagram and reported in the systematic review.

Data Extraction and Management

Data will be extracted on primary and secondary outcome measures following the PRISMA guideline for systematic reviews [36]. Outcome and effect size measures (eg, adjusted and ORs, risk ratios [RRs], hazard ratios, and SEs), *P* values, associated 95% credibility intervals, and associated 95% CIs. RRs for subgroups (eg, age, sex, duration of PASC, and COVID-19 severity) will be extracted if reported. The following data will also be extracted: authorship, publication year, journal name, study design, study location, sample size, baseline characteristics of the study participants, demographics (age, sex, ethnicity, or race of subjects), study population characteristics (eg, general population, prisoners, and health care workers), the definition of PASC, duration of PASC, comorbidities, other risk factors, duration of follow-up, list of adjusted and unadjusted colliders (eg, hospitalization, occupation, and symptom recognition) and a list of adjusted and unadjusted confounders (eg, age, sex, nature of exposure, and type of intervention), and propensity methods.

Two reviewers will conduct the data extraction. Discrepancies in data extraction will be resolved through discussion or arbitration.

Risk of Bias in Individual Studies

The JBI critical appraisal checklist will be used to determine the methodological quality and to critically appraise the risk of bias for included studies. Assessment will be done at the study and outcome level. Information related to a variable (exposure, outcome, or covariate), misclassification, confounders, participant selection, reverse causation, missing data, study power, and generalizability will be appraised. Two reviewers will initially pilot the checklist to enhance consistency, mitigate potential issues with mechanistic scoring, and mitigate performance bias in the overall risk-of-bias assessment. Studies that do not adequately report on statistical analyses or address confounders, biases (selection, performance, detection, or attrition) and other biases will be deemed lower-quality studies—that is, when they consistently have “no,” “unclear,” and “not applicable” ratings’ across relevant items.

Discussion

Summary treatment effects estimated as continuous outcomes will be converted to ORs, RRs, incidence rate ratios, risk differences, and number needed to treat with a 95% CI or 95% credibility interval (along with the baseline risk) for easier interpretation where possible. A random-effects meta-analysis will be conducted if possible. Statistical heterogeneity across studies will be explored using the Higgins I^2 and Cochran Q statistics. A Cochran Q test based on a chi-square statistic with a *P* value of <.05, and greater than the df, will indicate heterogeneity. The I^2 statistic will be interpreted as follows: 0%-40%, minimal heterogeneity; 30%-60%, moderate heterogeneity; 50%-90%, substantial heterogeneity; and 75%-100%, considerable heterogeneity. If there is substantial heterogeneity, subgroup analysis (based on the duration of PASC or COVID-19 severity) will be conducted. Subgroup effect sizes (Cohen *d* or Hedges *g*) and correlations will be assessed and compared with unadjusted values to interpret for meaningful effects. Observed effects will be considered robust if the effect estimates of the primary outcome remain consistent or there are no large differences in the magnitude of effect across subgroups. Subgroup analyses will not be performed if there is minimal or moderate heterogeneity. A formal narrative synthesis will be performed if meta-analysis is not possible. The reasons for not pooling data (eg, high statistical, methodological, and clinical heterogeneity) will be reported in the review.

A methodological quality-based sensitivity analysis presented as a summary table will be used to assess the robustness of the findings. Authors of included studies with missing information will be contacted for clarification. The Grading of Recommendations, Assessment, Development, and Evaluations approach will be used to rate the overall certainty of the evidence obtained from the study.

Authors' Contributions

AE conceived, designed, and drafted the study protocol in its entirety.

Conflicts of Interest

None declared.

Editorial Notice
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Abbreviations

OR: odds ratio
PASC: postacute sequelae of COVID-19
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RR: risk ratio

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