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ORIGINAL ARTICLE

The Relationship between Serological Testing, Demographics, Clinical Presentation and RT-PCR Testing for COVID-19

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SUMMARY

Background: **Serological tests provide an important tool to diagnose previous exposure to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Herein we describe the relationship between the demographics, clinical characteristics, and molecular investigations and the presence of coronavirus disease 2019 (COVID-19) antibodies.**
Methods: **Three hundred and four participants, living in Gauteng, South Africa, were screened for COVID-19 antibodies between September 12, and December 12, 2020. Indications for serological testing included previous infection (n = 45, 14.80%), World Health Organization (WHO) symptoms (n = 122, 40.13%), positive household contact (n = 40, 13.16%), and/or positive close non-household contact (n = 80, 26.32%).**
Results: **There were 58 (19.08%) positive rapid antibody tests. Risk factors associated with a positive rapid antibody test included WHO symptoms, namely fever/chills (odds ratio [OR] 3.50, 95% confidence interval [CI] 1.50 to 8.19), loss of taste or smell (OR 8.66, 95% CI 3.27 to 22.94), and the presence of a household contact (OR 3.66, 95% CI 1.59 to 8.40).**
Conclusions: **The findings support the measures implemented to reduce the spread of infection.**
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INTRODUCTION

As of December 12, 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has affected over 76 million people in 218 countries [1]. South Africa's first case was reported on March 5, 2020. Since then, we have had a total of 852,965 cases and 23,106 fatalities [1]. Accurate and accessible diagnostic tools are the cornerstone of understanding the current coronavirus disease 2019 (COVID-19) pandemic. Molecular diagnostic tests such as real-time polymerase chain reaction (RT-PCR) are the reference method for the diagnosis of SARS-CoV-2 infection. Serological tests measure antibody responses to either

the spike (S) or nucleocapsid (N) protein and identify individuals who have mounted an adaptive immune response following SARS-CoV-2 infection. Screening data of defined populations indicate that approximately 31.00% (95% CI 26.00% - 37.00%) of SARS-CoV-2 infections are asymptomatic [2]. As such, it is likely that a third of infected persons would not have undergone molecular testing. Therefore, serological tests are necessary to accurately assess the magnitude of the pandemic. Serological test methods include enzyme linked immunosorbent assays and lateral flow immunoassays. Serological tests that include IgM and IgG antibody classes may allow for a more accurate assessment of timing of exposure. Antibodies are reported to rise around the first week of infection. IgM and IgG antibodies persist for approximately 21 days and four months, respectively, in the majority of patients [3]. However, these assays do not discriminate between binding and neutralizing antibodies. These assays provide information about previous exposure. Ongoing research is investigating the contribution of non-neutralizing antibodies and other immune functions to protective immunity [4-6]. In addition, serological tests are of value in the setting of a negative RT-PCR and clinical features which are highly suggestive of SARS-CoV-2 infection [7].

Several large population-based studies have been conducted on the immunological responses to SARS-CoV-2 from hotspots such as China, the United States of America, and Europe [8-13]. Variable rates have been reported. This is dependent on the timing of serological testing in relation to population demographics, clinical disease severity, and pandemic peak. As an example, a Swedish study reported a seroprevalence of 23.00% among employees of elderly care homes in contrast to the national seroprevalence of 7.20% [14]. Additionally, disease severity has emerged as a strong predictor of higher antibody levels [5]. In contrast, in mild or asymptomatic cases, antibody responses may wane rapidly following infection [15]. Furthermore, a meta-analysis of serological testing revealed a sensitivity of 96.00% (95% confidence interval 90.60 to 98.30) for IgG and/or IgM in participants tested 21 to 35 days after onset of symptoms [16]. Nonetheless, at the time of writing this manuscript, few studies have evaluated serological tests 35 days post-symptom onset [17].

The relationship between seropositivity and clinical characteristics in the South African setting, however, remains to be established. Cohort data across population groups severely affected by the COVID-19 pandemic are important in order to improve the understanding of the risk factors, demographics, clinical spectrum, and seroprevalence associated locally with COVID-19 infection. This study therefore aimed to determine the relationship between the demographics, clinical characteristics, and diagnostic investigations (RT-PCR) with the presence of COVID-19 IgG and IgM antibodies.

MATERIALS AND METHODS

Study design and population

A record review was conducted of participants living in Gauteng, South Africa, in order to investigate seropositivity for SARS-CoV-2. Participants were screened between September 12, and December 12, 2020. Written informed consent was obtained from the participants, and the study protocol was approved by the Institutional Review Board (M 201067).

Study protocol

Participants screened for COVID-19 antibodies for the following indications were included: identification of past exposure to SARS-CoV-2 as well as the diagnosis of COVID-19 in participants with suspected SARS-CoV-2 infection, who tested negative with RT-PCR. Patients who were tested for COVID-19 antibodies 21 days prior to symptom onset were excluded in order to improve the diagnostic sensitivity. Patient demographics, World Health Organization (WHO) clinical symptoms, a history of a close contact with COVID-19, a travel history, and laboratory investigations (RT-PCR for SARS-CoV2) were recorded on data collection sheets. Serological testing for COVID-19 IgG and IgM antibodies were performed using the Orient Gene Rapid Antibody Test kit (Orient Gene Biotech Co, Zhejiang, China). The assay is a qualitative lateral flow assay which is used to detect IgG and IgM antibodies against the receptor binding domain of the S protein. The manufacturer reported sensitivity of the assay is 97.20% for IgG and 87.90% for IgM and a specificity of 100.00% for both IgG and IgM, using RT-PCR as the gold standard. Testing was performed according to instructions from the local health authorities.

Statistical analysis

This study was conducted as an exploratory analysis. Data was analyzed using Statistica® software (Version 13.2, Palo Alto, CA, USA). The data was normally distributed. Continuous data were presented as mean ± standard deviation (SD). Statistical comparisons between antibody positive and antibody negative patient groups were performed using chi-squared or two-tailed Fischer's exact test for categorical variables. For statistical comparisons of continuous variables, the independent *t*-test or one-way ANOVA test were used. Unadjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine the predictors of seropositivity. A stepwise multiple logistic regression analysis was applied by including risk factors with a p-value of < 0.05. Adjusted OR and 95% CI were computed. Assumptions, such as goodness of fit, residuals normality, and absence of collinearity were checked. Statistical significance was set at a p-value of < 0.05.

RESULTS

Study population

During the study period, 304 participants from 219 different households were identified and underwent serological testing. Indications for serological testing included previous COVID-19 infection (n = 45, 14.80%), WHO symptoms (n = 122, 40.13%), positive household contact (n = 40, 13.16%), and/or positive close non-household contact (n = 80, 26.32%). RT-PCR testing was performed prior to serological testing in 125 participants. Of this subgroup (n = 125), 45 (36.00%) participants had a positive RT-PCR test at a mean ± SD of 88 ± 44 days prior to serological testing. The demographics and clinical characteristics of the participants according to antibody status are reflected in Table 1 and 2, respectively. The median (interquartile range) age of the participants was 41 [18] years (Figure 1S). With regards to clinical characteristics in the study population, 122 (40.13%) participants reported at least one of the WHO identified symptoms. The most common symptoms included fatigue or weakness (n = 65, 21.38%), unexplained body aches (n = 59, 19.41%), and sore throat (n = 51, 16.78%). Less common symptoms were cough (n = 44,14.47 %), fever/chills (n = 41, 13.49%), shortness of breath (n = 36, 11.84%), loss of taste or smell (n = 30, 9.87%), and nausea, vomiting or diarrhea (n = 29, 9.54%). These symptoms did not require hospitalization in the majority (n = 120, 98.36%) of symptomatic participants (n = 122). In the subgroup with a previous positive RT-PCR test (n = 45), 7 (15.56%) reported all three WHO symptoms (fever, cough, and loss of taste or smell), 15 (33.33%) reported at least two WHO symptoms and 13 (28.89%) reported at least one WHO symptom.

Serological testing

There were 58 (19.07%) positive rapid antibody tests. Thirty-two (55.17%) participants tested positive for both IgM and IgG, at a mean of 83 ± 43 days post-onset of symptoms. Twenty-six (44.83%) tested positive for IgG alone, at a mean of 84 ± 45 days post-onset of symptoms. No participants tested positive for IgM alone. In participants with a prior positive RT-PCR (n = 45), the proportion of participants with a positive rapid antibody test was 75.56% (n = 34) at a mean of 81 ± 41 days from molecular to serological testing (Figure 2S). This time period was not significantly different from recovered participants with a negative rapid antibody test (n = 11, 24.44%) at a mean of 84 ± 37 days (p = 0.747) from molecular to serological testing. The number of participants who were asymptomatic with a positive rapid antibody test was 12/58 (20.69%). Risk factors associated with a positive rapid antibody test on univariate logistic regression analysis included a previous RT-PCR test, clinical symptoms such as unexplained body aches, fever/chills, cough, shortness of breath, loss of taste or smell, and fatigue or weakness, and the presence of a household contact (Table 1S). Multiple logistic regression identified fever/chills, loss of taste or smell, and the presence of a household contact as risk factors (Table 3).

DISCUSSION

We report results of COVID-19 rapid antibody tests performed in 304 participants living in Gauteng, South Africa. The main indications for screening were identification of past exposure to SARS-CoV-2 in participants with a personal history of WHO symptoms (40.13%) followed by a positive close non-household contact (26.32%). The SARS-CoV-2 seroprevalence was 19.08%. Of particular relevance, seroprevalence did not differ significantly according to gender, race group, occupation or age. This included susceptible population groups such as the elderly. The seroprevalence was highest in the age group of 18 - 39 years, in contrast to other published reports [11]. Furthermore, according to occupation, the seroprevalence among health care workers (n = 46), was 10.87%, which was not significantly increased when compared to other occupations, despite the greater exposure to SARS-CoV-2. Nonetheless, variable rates have been reported depending on adopted guidelines pertaining to appropriate use of personal protective equipment [18,19]. WHO symptoms significantly associated with seropositivity on multiple logistic regression analysis included fever/chills and loss of taste or smell. The presence of these symptoms may be useful for identification and isolation of new cases and their contacts. This is important in the control of the spread of infection. In addition, the majority of the study population did not require hospitalization. This highlights the potential role for home-based care by qualified caregivers to decrease the burden on hospital facilities. It is interesting to note that the seropositive rate among asymptomatic participants was 20.67%. This compares with a recent systematic review and meta-analysis of 79 studies, which also found a proportion (20%, 95% CI 17% - 25%) of reported infections across all study settings are asymptomatic [2]. Surveillance seroprevalence studies at a population level have commenced in South Africa in order to estimate the proportion of asymptomatic infections. This has important implications for regulatory measures to reduce the risk of transmission. In addition to clinical symptoms, a positive household contact was associated with an approximately four-fold increased risk of seropositivity. This provides further evidence for implementation of appropriate isolation measures for household members of an infected person. Nonetheless, this poses a challenge in overcrowded urban settlements, correctional services, and institutions in South Africa.

To our knowledge this is one of the first studies to evaluate IgG and IgM COVID-19 antibody responses 35 days post-symptom onset in Gauteng, South Africa. Testing was performed at least 21 days after symptom onset and as such no patients tested positive for IgM

Table 1. Seroprevalence of SARS-CoV-2 by demographics.

Characteristics	Total (n = 304)	Rapid antibody test negative (n = 246)	Rapid antibody test positive (n = 58)	p-value
Demographics				
Gender				
Male, n (% of total)	**170 (55.92)**	**137 (80.59)**	**33 (19.41)**	**0.868**
Female, n (% of total)	**134 (44.08)**	**109 (81.34)**	**25 (18.66)**	
Age, years (median [IQR])	**41 [17]**	**40 [17]**	**41 [27]**	**0.798**
Race group				
White, n (% of total)	**259 (85.20)**	**216 (83.40)**	**43 (16.60)**	**0.008**
Black, n (% of total)	**36 (11.84)**	**22 (61.11)**	**14 (38.89)**	
Indian, n (% of total)	**9 (2.96)**	**8 (88.89)**	**1 (1.11)**	
Occupation				
Health care worker, n (% of total)	**46 (15.13)**	**41 (89.13)**	**5 (10.87)**	**0.131**
Other employment, n (% of total)	**207 (68.09)**	**169 (81.64)**	**38 (18.36)**	**0.868**
Unemployed, n (% of total)	**15 (4.93)**	**12 (80.00)**	**3 (20.00)**	**0.926**
Student, n (% of total)	**36 (11.84)**	**24 (66.67)**	**12 (33.33)**	**0.024**
Household Residents				
Elderly (> 60 y) per household, mean ± SD	**0 ± 1**	**0 ± 1**	**1 ± 1**	**0.025**
Children (< 14 y) per household, mean ± SD	**1 ± 2**	**1 ± 2**	**2 ± 2**	**0.126**

Table 2. Seroprevalence of SARS-CoV-2 by self-reported clinical characteristics.

Characteristics	Total (n = 304)	Rapid antibody test negative (n = 246)	Rapid antibody test positive (n = 58)	p-value
Clinical characteristics				
Symptoms > 21 days prior to serology				
Asymptomatic, n (% of total)	**182 (59.87)**	**170 (93.40)**	**12 (6.59)**	**< 0.001**
Symptomatic, n (% of total)	**122 (40.13)**	**76 (62.30)**	**46 (37.70)**	
WHO identified symptoms				
Unexplained body aches, n (% of total)	**59 (19.41)**	**41 (69.49)**	**18 (30.51)**	**0.014**
Fever/chills, n (% of total)	**41 (13.49)**	**19 (46.34)**	**22 (53.66)**	**< 0.001**
Cough, n (% of total)	**44 (14.47)**	**25 (56.82)**	**19 (43.18)**	**< 0.001**
Shortness of breath, n (% of total)	**36 (11.84)**	**21 (58.33)**	**15 (41.67)**	**< 0.001**
Sore throat, n (% of total)	**51 (16.78)**	**39 (76.47)**	**12 (23.53)**	**0.377**
Loss of taste or smell, n (% of total)	**30 (9.87)**	**8 (26.67)**	**22 (73.33)**	**< 0.001**
Nausea, vomiting or diarrhea, n (% of total)	**29 (9.54)**	**23 (79.31)**	**6 (20.69)**	**0.817**
Fatigue or weakness, n (% of total)	**65 (21.38)**	**39 (60.00)**	**26 (40.00)**	**0.002**
Hospitalized				
No, n (% of total)	**302 (99.34)**	**246 (81.46)**	**56 (18.54)**	**0.004**
Yes, n (% of total)	**2 (0.66)**	**0 (0)**	**2 (100)**	
RT-PCR status				
Not done, n (% of total)	**179 (58.89)**	**161 (89.94)**	**18 (10.06)**	**< 0.001**
Negative, n (% of total)	**80 (26.32)**	**74 (92.50)**	**6 (7.50)**	
Positive, n (% of total)	**45 (14.80)**	**11 (24.44)**	**34 (75.56)**	
Travel history				
No, n (% of total)	**281 (92.43)**	**226 (80.43)**	**55 (19.57)**	**0.448**
Yes, n (% of total)	**23 (7.57)**	**20 (86.96)**	**3 (13.04)**	
Contact with a confirmed case				
Household contact, n (% of total)	**40 (13.16)**	**21 (52.50)**	**19 (47.50)**	**< 0.001**
Non-household close contact, n (% of total)	**80 (26.32)**	**60 (75.00)**	**20 (25.00)**	**0.089**

Key: WHO - world health organization, RT-PCR - real-time polymerase chain reaction.

Table 3. Multiple logistic regression analysis of positive SARS-CoV-2 rapid antibody tests.

Variable	Multiple logistic regression		
	Adjusted OR	95% CI	p-value
Fever/chills	**3.50**	**1.50 to 8.19**	**0.004**
Loss of taste or smell	**8.66**	**3.27 to 22.94**	**< 0.001**
Household contact	**3.66**	**1.59 to 8.40**	**0.002**
AUC 0.78, p = 0.214			

Key: OR - odds ratio, CI - confidence interval.

alone. Nonetheless, 55.17% of seropositive participants were positive for both IgM and IgG at a mean of 83 ± 43 days prior to onset of symptoms. This is in contrast to other coronaviruses where IgM antibodies suggest current or recent infection. The findings of this study, rather, suggest that IgM does not add diagnostic value in determining time from infection. In this study, 11 (24.44%) of the participants, with a prior positive RT-PCR, had a negative rapid antibody test at a mean of 84 ± 37 days. This may be attributed to false negative rapid antibody test results, waning antibody levels, or a robust innate immune response. Various studies have reported a decline in antibody levels in the months following infection [5,6,17,20]. It is therefore recommended to conduct seroprevalence testing as close as possible to the peak of the pandemic when most previously infected individuals will continue to have circulating antibodies. The results of this study must be interpreted in light of certain limitations. First, this was a study of a single province. As such the demographics do not accurately reflect the age, gender, race, and employment status of the general population. The results of ongoing large, broad-spectrum, population based serological studies are anticipated in order to estimate exposure rates, especially in under-resourced areas in South Africa. Second, only three of the independent variables could be included in the multiple logistic regression analysis due to the small sample size which limits the precision of the risk estimates. Third, this study included participants who fulfilled South African regulations for COVID-19 antibody testing. As such, population seroprevalence data cannot be extrapolated reliably from these results. This is a result of referral/selection bias which could have resulted in an overestimation of individual risk factors. Recall bias could also have resulted in inaccurate assessment of individual risk factors. Finally, as a result of the cross-sectional design of the study, the persistence of positive rapid antibody tests could not be determined.

CONCLUSION

This exploratory study performed in Gauteng, South Africa, in the midst of this pandemic, found a seropositive rate of 19.08% at a mean of 84 ± 43 days prior to the onset of symptoms. We identified WHO symptoms, namely fever/chills, loss of taste or smell, and the presence of a household contact, as independent predictors of seropositivity with rapid serological testing. This supports the regulatory measures employed to reduce the risk of transmission. Further studies from these participants at follow-up time-points are required in order to determine the longevity of antibody responses to SARS-CoV-2.

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The author(s) declare no conflicts of interest with respect to the authorship and/or publication of this article. The authors declare the manuscript has not been published nor submitted elsewhere.

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