Original Article

Sero-prevalence of anti-SARS-CoV-2 Antibodies in Addis Ababa, Ethiopia

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Abstract

Background: Anti-SARS-CoV-2 antibody tests are increasingly used for sero-epidemiological purposes to provide a better understanding of the extent of the infection in the community, and to monitor the progression of the COVID-19 epidemic. A sero-prevalence study was conducted to estimate prior infections with SARS-CoV-2 in Addis Ababa.

Methods: A cross-sectional study was conducted from April 23 to 28, 2020 among 301 randomly selected residents of Addis Ababa; sub-city health offices, health facilities and health extension workers were contacted, to obtain a population profile and to conduct the random selection of study participants. Participants were selected, who had not been in direct contact with people who had contracted COVID-19, to maintain consistency among the study population. Interviews on socio demographic and behavioural risk factors, followed by serological tests were performed for SARS-CoV-2 IgM, and IgG antibodies, using the COVID-19 IgG/IgM Rapid Test Cassette. Based on the manufacturer information, the test has a sensitivity of 87·9% and specificity of 100% for lgM; and a sensitivity of 97·2% and specificity of 100% for IgG. A Polymerase chain reaction (RT-PCR) test was also done on combined nasopharyngeal and oropharengeal swabs.

Findings: The unadjusted antibody-based crude SARS-CoV-2 prevalence was 7·6% and the adjusted (weighted average) SARS-CoV-2 prevalence was estimated at 8·8% (95% CI 5·5%-11·6%) for the study population. Higher sero-prevalence were observed for males (9.0%), age below 50 years (8.2%), students and unemployed (15.6%), as well as those with primary education (12.1%), educated above high school (37·9%), non- smokers (78·7%), with no history of regular alcohol (53·8%), no chat (70·8%), and no shisha use (94·7%). According to the findings, a significantly higher number of individuals had been infected in Addis Ababa as compared to what was being detected and reported by the RT-PCR test, which is suggestive of community transmission. [*Ethiop. J. Health Dev.* 2021; 35(4) 367-374]

Keyword: Sero-prevalence, Ethiopia, Addis Ababa, SARS-CoV-2, Antibody Testing

Introduction

Testing for SARS-CoV-2 is done in two ways, by detecting the virus itself (RT-PCR) and by detecting the host's response to the virus (serology) (1). The World Health Organisation (WHO) recommends the RT-PCR test as a gold standard for COVID-19 case identification (2,4). The clinical course of SARS-CoV-2 infection ranges from asymptomatic to fatal (5,6) making the identification of cases very complex. The response plan to the pandemic requires epidemiological data that indicates the true magnitude of the disease, and the serology test can be used for this purpose (7). However, sensitivity is reported to be as low as 64% (8-10). Differences in the detection limits of the test kits used, low initial viral load due to timing as well as improper specimen collection (11-13) may account for the decreased sensitivity.

The lower sensitivity or false negative RT-PCR results and/or the "immune passport" led to the rapid development of serologic assays (14-16). The serologic tests in the markets have different formats (lateral flow immunoassays, ELISA, and chemiluminescent immunoassays), which detect different classes of antibodies (total antibody, IgG, IgM, and IgA), target different antigens (recombinant nucleocapsid protein [NP], subunit 1 of the spike glycoprotein [S1], and the Spike glycoprotein receptor-binding domain [RBD]) and accept different types of the specimen (whole blood, serum, and plasma) (16). Data generated from

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population-based serology can be used for various purposes including estimation of community transmission rates and to assess the impact of non-pharmacological interventions (14). Despite the potential of the serology test, equally, the availability of a serology test with excellent sensitivity and specificity is crucial.

Since the report of the first confirmed SARS-CoV-2 inflection in Ethiopia on March 13, 2020, the rate of increment has been significantly slow at the beginning. This required a more precise estimation of the magnitude of SARS CoV-2 infection, apart from the estimates which were based on routine RT-PCR testing. Therefore, a goal was set to conduct a series of sero-epidemiological studies. Hereunder are the reports from the first findings on anti-SARS-CoV-2 antibody among permanent residents in Addis Ababa, which were conducted to estimate the extent of the infections of SARS-CoV-2 amongst the population.

Methods

Study design and participants

A cross-sectional community-based study was conducted from April 23-28, 2020. The study was undertaken in Addis Ababa, the capital city of Ethiopia, with an estimated total population of 4,793,699 (17).

The study population were adults (≥ 18 years), residents of Addis Ababa, who had not been in direct contact with people who had confirmed COVID-19 cases and no recent history of travel out of Ethiopia in the past four months prior to the period of data collection. Individuals who were unable to consent, those with an unstable mental state and who were suspected to have acute SARS-CoV-2 infections were excluded.

Sample size was calculated using a samples size formula for single population proportion, by assuming the proportion of people with immunity among community members. With no direct contact with SARS-CoV-2 Infected individuals, immunity was estimated to be at 14% with a 5% margin of error at a 95% confidence level based on earlier European studies conducted during the time of the study (18), power was set at 80% and 99% the confidence level was 99%. The Epi Info/Open Epi program was used for the sample size calculation (19). Accordingly**,** the sample size calculated was set at 319 participants.

A List of the community members were compiled through community level health extension workers and used as a sampling frame and study participants were randomly selected from the compiled list through sub-city health extension focal personnel. All randomly selected individuals from all the sub-cities in Addis Ababa and who fulfilled study inclusion criteria (stable mental state, not suspected to have acute SARS-CoV-2 infection permanent residents, and willing to provide consent) were invited to participate in the study. They were approached and received an invitation through health extension workers (HEWs) from the respective sub-city. All study participants were briefed about the study and provided written consent before proceeding to be interviewed. Participants of the cross-sectional sero-prevalence were recruited from contacts of cases, those with travel history, workplaces, and households. Both government and public health care facilities were recruited from all the sub-cities in Addis Ababa.

Data collection

Socio-demographic and clinical data were collected through interviewer administered questionnaires. Substance use related variables such as smoking, drinking habits, chat chewing, and shisha smoking were assessed. Complete blood samples for antibody testing and combined nasopharyngeal and oropharengeal swab for RT-PCR testing was collected from each participant. A questionnaire comprising of socio-demographic characteristics such as age, sex, marital status, and place of residence; a history of illness in the past three to five months like signs and symptoms of pneumonia and/ or influenza-like illness; and perceived severity of illness, etc was recorded. Prior to data collection, the questionnaire was pre-tested and necessary adjustments were made. Peripheral blood (4ml) was collected from each participant by experienced phlebotomist by venipuncture using Ethylenediaminetetraacetic acid (EDTA) tubes and were transported to the Ethiopian Public Health Institute (EPHI) Influenza laboratory for further analysis. Plasma was extracted using centrifugation at 3000rpm for 10 minutes in a level-II bio-safety cabinet. Plasma was stored using cryovials in refrigerator at 2-8^{0}C until the next day. The leftover plasma was stored at -80^{0}C for future use at EPHI biobank. A combined nasopharyngeal and oropharengeal swab was collected from each participant using Dacron or polyester flocked swabs (*KANGJIAN Medical* Apparatus *Co*., *Ltd*.), which was done by trained personnel, and in a Viral Transport Medium (VTM) (Longsee, LAKEbio) transported to EPHI for RT-PCR analysis of SARS-CoV-2 in a cold chain (2-8^0 C) and processed immediately or stored for 1 to 3 days at -80^{0}C.

Laboratory Analyses of collected samples

Serology

Analysis for the presence of plasma IgM and/or IgG antibodies was done using a commercially available immunolateral flow immunoassay kit (Zhejiang Orient Gene Biotech Co Ltd, Huzhou, Zhejiang, China) following manufacturer's instructions. The interpretation of the test was made by two experts (microbiologists and laboratory technologist). According to the manufacturer the test has a sensitivity and specificity of 87.9 and 100 % respectively. Another validation study had reported the test had a lower sensitivity (69% for IgM and 93.1% for IgG and the specificity was found to be 100% for IgM and 99.2% for IgG using RT-PCR assay as a comparator (20).

Dependent and independent variables list

The dependent variable was a sero positive/negative status while the independents variables include socio demographic characteristics (sex, age, marital status, occupation, educational status). The following options, (do you smoke (yes/no), do you drink alcohol (yes/no),

do you chew chat (yes/no) and do you have shisha smoking habits (yes/no) were used to assess substance use.

RNA extraction and RT-PCR analysis of COVID-19

The combined swab (nasopharyngeal and oropharyngeal) was transferred into a lysis buffer which contained a guanidinium-based inactivating agents and viral RNA was extracted using a Nucleic Acid Isolation Kit (Da'an Gene Corporation) based on the manufacturer's instructions. Briefly a 200 µL of combined swab in VTM was used for viral RNA extraction and viral RNA was eluted with a 60 µL elution buffer. A Real-time reverse transcriptional polymerase chain reaction (RT-PCR) reagent of Da'an Gene cooperation was employed for SARS-CoV-2 detection following the manufacturer's protocol. Briefly, two PCR primer and probe sets, which target the open reading frame 1a/b (ORF 1a/b) (FAM reporter) and nucleocapsid protein (N) genes and N (VIC reporter) genes were added in the same reaction mixture. In each run, positive and negative controls were included. Samples were positive when both sets produced a reliable signal (<40CT value).

Data processing and Analysis

All demographic, epidemiologic and laboratory data were cleaned and entered EPI DATA using a controlled and programmed data entry format. The data was coded, and the anonymized data were merged to laboratory data which were then exported into SPSS for windows version 20. Descriptive statistics was used to summarise key findings. An estimation of true values for sero-prevalence for the given levels of sensitivity and specificity were computed via a web-based statistical software and epidemiologic calculator, which included EPI Tools (21). Binary logistic regression was used to identify factors associated with the outcome variable. Variables whose p value was less than 0.05 at bivariate analysis were included in the multivariable analysis.

Ethical Considerations

The research proposal is approved by the Institutional Review Boards (IRB) of both the College of Health Sciences of the Addis Ababa University and EPHI. Written informed consent was obtained from all study participants. All study participants were informed of their RT-PCR test results as per the national testing protocol.

Results

Characteristics of the study participants

A total of 301 participants were included in the study with a response rate of 94.4%. Most of the study participants were in the age group 18-30 years (48.2%) (with a median age of 30 years ± 10.9 years), males (62·5%), single (43·9%), and health professionals (25·6%), educated above high school (37·9%), non-smokers (78·7%), with no history of regular alcohol (53·8%), no chat (70·8%), no shisha (94·7%) use (Table1).

Table 1: **Socio demographic and behavioural characteristics of participants in Addis Ababa Ethiopia, May 2020 (n=301)**

Socio-demographic and behaviour factors		Number	Percent
Sex	Male	188	62.5
	Female	113	37.5
	18-29	145	48.2
Age group	30-49	122	40.5
	above 49	34	11.3
	No formal education	16	5.3
Educational status	1-4 grade	16	5.3
	5-8 grade	75	24.9
	9-12 grade	80	26.6
	12 and above	114	37.9
	Married	133	44.1
Current marital status	Single	132	43.9
	Divorced//widowed	36	12.0
	Daily labourers/ Petty trading	114	37.9
Occupation	Health care worker	77	25.6
	(Government/NGO)	48	15.9
	Student/ Unemployed	31	10.3
	(Housewife /Retired)	15	5.0
	Driver/parking/mechanics	8	2.6
	Bank/ supermarket/ shop/Hotel	8	2.6
	Yes	64	21.3.
Smoked a cigarette	No	237	78.7
	Yes	139	46.2
Consumed alcohol	No	162	53.8
	Yes	88	29.2
Chewed chat	No	213	70.8
	Yes	6	3.3
Used shisha	No	295	94.7

SARS-CoV-2 Sero-prevalence

Out of the 301 individuals included in the study, 23 (7·6%) tested positive for anti-SARS-CoV-2 antibodies with an unadjusted antibody-based crude prevalence of 7·6%. Accordingly, true prevalence was adjusted for the test sensitivity and specificity was estimated at 8·8% (95% CI 5·5%-11·6%). Higher sero-prevalence were observed for Males (9.0%), age below 50 years (8.2), students and unemployed (15.6), those with primary education (12.1), smokers (7.8), alcohol consumers (8.6), chatt-chewers (13.6%) and shish smokers (18.8%). (Table 2)

Table 2: Sero- prevalence study participants by demographic and behavioural characteristics, Addis Ababa Ethiopia, May 2020

Socio-demographic and behaviour factors		Total	Positive	Sero prevalence
Sex	Male	188	17	9.0
	Female	113	6	5.4
Age group	Less the 50 years	267	22	8.2
	50 Years and above	34	1	2.9
Educational status	No formal education	16	1	6.3
	Primary (1-8)	91	11	12.1
	High School and Above	194	11	5.7
	Married	133	13	9.8
Current marital status	Single	132	9	6.8
	Divorced//widow	36	1	2.8
Occupation	Daily labourers/ Petty trading	110	11	10.0
	Health care worker	78	4	5.1
	(Government/NGO)	48	1	2.1
	Student/ Unemployed	32	5	15.6
	Housewife /Retired	15	0	0
	Bank/ supermarket/	9	1	12.5
	shop/Hotel	8	1	11.1
	Driver/parking/mechanics			
Smoked a cigarette	Yes	64	5	7.8
	No	237	18	7.6
Consumed alcohol	Yes	139	12	8.6
	No	162	10	6.2
Chewed chat	Yes	88	12	13.6
	No	213	11	5.2
Used shisha	Yes	16	3	18.8
	No	285	21	7.4

Factors Associated with SARS-CoV-2 Seropositive tests

On binary logistic regression there was a negative association between chat chewing and SARS Co2 Seropositivity (OR=0·35, 95% CI: 0·15, 0·82. Associations were lost on adjusted logistic regression. Government/NGO workers were found to have a protective effect for SARS-CoV-2 sero status (OR=0·052, 95% CI: 0.01, 0·57) (Table 3)

Table 3: **Demographic and behavioural factors associated with Sero prevalence in bivariate and multivariable analysis in Addis Ababa Ethiopia, May 2020**

Socio-demographic and behaviour factors		Negative		Positive			
		Number	Percent	Number	Percent	COR(95CI%)	AOR(95CI%)
Sex	Male	171	61.5%	17	73.9%	0.56 (0.03,2.45)	1.01(0.31,3.26)
	Female	107	38.5%	6	26.1%		
Age group	Less the 50 years	245	88.1%	22	95.7%	2.96(0.39,22.71)	0.18(0.02,2.07)
	50 Years and above	33	11.9%	1	4.3%	1.00*	
	Median age = 30 years ± 10.9 years						
Educational status	No formal education	15	5.4%	1	4.3%	1.11(0.13,9.18)	3.47(0.28,42,89)
	Primary (1-8)	80	28..7%	11	47.8%	2.29(0.95,5.50)	1.73(0.54,5.58)
	High School and Above	183	65.8%	11	47.8%	1*	
Current marital status	Married	120	43.2%	13	56.5%	3.79(0.48, 30.00)	4.56(0.47,44.73)
	Single	123	44.2%	9	39.1%	2.56(0.31, 20.91)	2.09(0.21,21.32)
	Divorced//widow	35	12.6%	1	4.3%	1.00*	
Occupation	Health care worker	74	26.6%	4	17.4%	0.29(0.073,1.17)	0.29(0.06, 1.34)
	Driver/parking/mechanics	7	2.5%	1	4.3%	0.77(0.077,7.71)	0.15(0.01, 1.99)
	Bank/ supermarket/ shop/Hotel	8	2.9%	1	4.3%	0.66(0.069,6.65)	0.36(0.03, 3.99)
	Daily labourers/ Petty trading	99	35.6%	11	47.8%	0.60(0.192.1.88)	0.22(0.05, 1.02)
	Government and NGO	47	16.9%	1	4.3%	0.11(0.013,1.03)	0.052(0.01, 0.57)
	Student/ Unemployed	27	9.7%	5	21.7%	1.00*	
Smoked a cigarette	Yes	59	21.2%	5	21.7	0.97 (0.34,2.72)	
	No	219	78.8	18	78.3%	1.00*	
Consumed alcohol	Yes	126	45.3	12	54.5%	0.69 (0.29,1.65)	0.93(0.34,2.55)
	No	152	54.7	10	45.55	1.00*	
Chewed chat	Yes	76	27.3%	1211	52.2%	0.35(0.146,0.815)	0.39(0.13,1.18)
	No	202	72.7%	1112	47.8%	1.00*	
Used shisha	Yes	13	4.7%	2	8.7%	0.52(0.11,2.45)	
	No	264	95.3%4	21	91.3%		

Discussion

As per the review of literature, this was the first SARS-CoV-2 sero-prevalence study in Ethiopia which sheds light on the extent of SARS-CoV-2 in a time point when there was a range of concerns in terms of the true extent of the infections in Africa due to the slow trajectory of cases. The findings of the study indicated that there is a proportionally high level of SARS-CoV-2infection in the community based on the Sero-prevalence findings.

Viral antigen tests (RT-PCR) are primarily done to identify new cases while antibody tests are used for the estimation of past infections who already have recovered without possibly realizing it. This helps to understand the spread of the disease within the community and to possibly predict its progression during the coming months. It also helps to identify those who have already been infected and developed immunity (22). The duration of the exposure depends on the type of antibody. However, it is not known how long the antibodies last. Reports have documented that antibody detection offers vital clinical information during SARS-CoV-2 infections with potential application in the diagnosis and management of COVID-19 patients (23).

It is recommended that adjustments be made on the crude estimates of prevalence values which are based on screening tests. Based on this, the true estimate adjusts the values to the levels of sensitivity and specificity of the screening tests employed. The COVID-19 IgG/IgM Rapid Test Cassette screening test used in the study has 87% sensitivity and 100% specificity as is reported by the manufacturer. The true value estimate using an EPI Tool indicated an overall sero-prevalence of 8·8% (95% CI 5·5%-11·6%) Validation conducted for the same test in Sweden has documented relatively lower values of sensitivity (69% for IgM and 93·1% for IgG), while kept the specificity high (100% for IgM and 99·2% for IgG) (20). This reflects the fact that the actual estimates of sero-positivity in the study subjects could be even higher, when adjusted to the lower values of sensitivity.

Sero-prevalence studies conducted in other countries during the period of the study reported varied magnitudes of SARS-CoV-2 sero-prevalence: Spain health workers (9·3%) (24) and community (5%) (18);

Germany (14%) (25), Switzerland (5·5%) (25), Massachusetts (30%) (25), Los Angeles (4·1%) (25), Santa Clara (1·5%) (25), New York (13%) (25), Iran (22%) (26), and Brazil (4%) (27). The differences between the findings of this study and the mentioned reports could be explained by technical and methodological provisions of the tests, the risk levels of the tested population and the extent of SARS-CoV-2 infection on the community.

The findings signify the importance of documenting the true extent of SARS-CoV-2 infection in Addis Ababa. Based on the official reports of cases in Ethiopia, there were 126 confirmed cases (as of April 28, 2020) (28) of which the majority were from Addis Ababa. On the other hand, based on SARS-CoV-2 modelling for Addis Ababa, the total number of infected and recovered individuals would have been 430 cases in the same week corresponding to our study. According to the estimates of our study and adjusted true values of 8·8% (95% CI 5·5%-11·6%) the estimated total number of individuals who were possibly infected and recovered would be 421,845 (95% CI, 254,066 - 584,831) (17). This is way higher than the official RT-PCR report and the projected estimates released by EPHI (29).

The possible reasons for the very high levels of estimated numbers of infected and recovered individuals beyond the available estimates could indicate issues around the adequacy of RT-PCR testing, predominantly asymptomatic individuals, and finally, the presence of cases already in the city long before the official reports.

Timing of the first cases in Addis Ababa: Similar discrepancies have been documented elsewhere between the number of cases reported and the estimates based on serological testing.

Even though the first case of SARS-CoV-2 was reported on March 13, 2020, as per the findings in the study it is possible that infections were in the community prior to the study. There are anecdotal reports that there have been cases with severe respiratory signs and symptoms in late December to early January, about the time when official reports of cases were made in China. Owing to the frequent air traffic between various Chinese cities in Addis Ababa, over 10 times per week through Ethiopian airlines, makes it difficult to rule out the possibility of such early cases in Addis Ababa. Furthermore, a review of health facility reports of respiratory illnesses in Addis Ababa indicated a two-fold increase in the 6-month period (September 2019 to February 2020), compared to the levels of the previous year (30).

The RT-PCR testing issues: If the magnitude reported based on the current serology estimate is closer to the true estimate, then why was it not possible to detect this by active surveillance. It is known that the RT-PCR test detects active cases and not past infections and recovered patients. If individuals were already infected and recovered, they will not be detected by the RT-PCR. It is also possible that the RT-PCR misses some active cases due to some factors which are prone to the decreased sensitivity. Apart from the inherent nature of the test, the yield depends on the total number of individuals tested and what population groups are being targeted for testing. In the Ethiopian setting until recently about 70-75% of the cases nationally reported are those returning from other countries. This is followed by the contacts of those individuals. The national testing strategy used to focus on testing these groups and suspected individuals making it almost impossible to detect any possible asymptomatic cases in the community which would require extensive testing of representative targets in the community. On top of that in a community where the majority have already been infected and recovered, decreased RT-PCR positivity is expected, especially if they already have some level of immunity. After the communication of the preliminary findings at Ministry of Health, the testing focused more on the community levels and the number of confirmed cases had increased since then.

According to the widely reported natural history of SARS-CoV-2 infection, the majority (80-85%) would have a mild variant of the disease. Even though there is no close to exact estimates, there are increasing reports that there are significant portions of asymptomatic or pre-symptomatic SARS-CoV-2 infections. This could be determined by some factors such as age, co-morbidities, immune status, and possibly ecological factors related to the viral strain and virulence. The Addis Ababa population is predominantly young, and the prevalence of chronic diseases is relatively low compared to western countries.

In the presence of such a big burden of cases in a big city like Addis Ababa, why was there no increase or surge in the number of severe cases in the health facilities? Asymptomatic cases could explain this owing to the predominantly young population and lower levels of non-communicable diseases in the Ethiopian population. However, this study did not get any supporting evidence to the looming theory of the protective association between BCG Vaccination and SARS-CoV-2 infection.

As the first antibody testing among the public in Addis Ababa, the study signified the need for extensive serological evidence to make comprehensive estimates of the true extent of SARS-CoV-2 infection in the population especially in a situation where we have the limited testing capacity for RT-PCR. Comparative advantages of antibody testing include cost-effectiveness, easy technology, and point of care testing. This makes serological estimates very appealing to the low-resource settings. However, in the given circumstances, serological tests are taken as complementary rather than independent in generating epidemiologic evidence for SARS-CoV-2 infection. This is indicative of the need for more precise population-based sero prevalence and surveillance, to have a better sense of the extent of the epidemic and to monitor its progress over time, which further guides the design and implementation of targeted interventions. As this is the first of a series of studies planned, the researchers intend to conduct nationwide sero-

prevalence studies and possibly set-up sentinel sero-prevalence longitudinal studies.

Such serological estimates provide a substantial and comprehensive input to the epidemiologic projections of SARS-CoV-2 especially in a setting where extensive and reliable viral antigen tests are limited. In addition, the current and subsequent data will provide evidence for policy makers to make decisions on locally generated evidence.

While this is possibly the first SARS-CoV-2 sero-prevalence study in Ethiopia (possibly in Africa, there are several limitations. The test kit utilized was not validated in our population and the target antigen of the kit is not described by the company. In addition, the relatively smaller number of subjects included in the study may affect generalisability. The test kit was validated in Sweden and used for population-based studies in Spain and China. In-country validation of the study is currently being done by EPHI but has not completed. The serological tests were not primarily done to detect a concurrent active infection with no contribution to SARS-CoV-2 case detection and management purposes.

Conclusion: The extent of sero-prevalence indicates that more individuals are infected by SARS-CoV-2 in Addis Ababa as compared to what is being detected and reported by RT-PCR testing, as well as what was estimated by current prediction models. The sero-prevalence results indicate community transmission has already been established in Addis Ababa.

Recommendations: The approach would serve as a baseline and a model of analysis for further serological investigation and its application in Ethiopia and other African countries. It is recommended that a more extensive sero-prevalence study be conducted with a more representative population sample to further establish substantial evidence on the extent of SARS-CoV-2infection in Addis Ababa and nationally. To establish a system for longitudinal sero-prevalence studies in Addis Ababa and other parts of the country to have a continued precise estimate of the extent of SARS-CoV-2 infection. The national estimate of cases should be complemented with appropriate serology tests in the community to diagnose and identify levels of community transmission and guide the containment strategies among different population groups while maintaining non-pharmacological interventions

Contributors

BN, GM, AB, AdA and ND contributed to the conceptualization of the study. TA, AbA, WAy, TH, RA, ZD, EK, MW, SA, GT, and LT contributed to further developments of the research idea and study design. BN, AdA, ND, TA, AbA, WAb, GM, WAm, Way, TH, RA, AB, ZD, BT, GT, MW, and LT were involved in the organization of data collection, data analysis and interpretations of the findings. AdA, BN, ND, AbA, TA, ZD, WAy, WAm and WAb were involved in drafting the initial version of the manuscript. All authors were involved in writing the final version of the manuscript.

Declaration of interests

All authors declare no competing interests.

Data sharing

Data analyzed would be made available based on reasonable request to the corresponding author.

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References

Patel R, Babady E, Theel ES, Storch GA, GA, Pinsky BA, St George K, et al. Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: Value of Diagnostic Testing for SARS-CoV-2/COVID-19. mBio. 2020; 11(2).PP

2. Shen M, Zhou Y, Ye J, Abdullah Al-Maskri AA, Kang Y, Zeng S, et al. Recent advances and perspectives of nucleic acid detection for coronavirus. J Pharm Anal. 2020.
3. Wan Z, Zhang Y, He Z, Liu J, Lan K, Hu Y, et al. A Melting Curve-Based Multiplex RT-qPCR Assay for Simultaneous Detection of Four Human Coronaviruses. Int J Mol Sci. 2016; 17(11).PP
4. Noh JY, Yoon SW, Kim DJ, Lee MS, Kim JH, Na W, et al. Simultaneous detection of severe acute respiratory syndrome, Middle East respiratory syndrome, and related bat coronaviruses by real-time reverse transcription PCR. Arch Virol. 2017; 162(6): 1617-23.
5. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. JAMA. 2020.
6. Inciardi RM, Adamo M, Lupi L, Cani DS, Di Pasquale M, Tomasoni D, et al. Characteristics and outcomes of patients hospitalized for COVID-19 and cardiac disease in Northern Italy. Eur Heart J. 2020; 41(19): 1821-9.
7. WHO. Advice on the use of point-of care immunodiagnostic tests for COVID-19 Scientific Brief: April 8, 2020. In: WHO, editor. Geneva: WHO; 2020.
8. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019

(COVID-19) in China: A Report of 1014 Cases. Radiology. 2020: 200642.
9. Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, et al. Sensitivity of Chest CT for COVID-19: Comparison to RT-PCR. Radiology. 2020: 200432.
10. Xiao AT, Tong YX, Gao C, Zhu L, Zhang YJ, Zhang S. Dynamic profile of RT-PCR findings from 301 COVID-19 patients in Wuhan, China: A descriptive study. J Clin Virol. 2020; 127: 104346.
11. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. N Engl J Med. 2020; 382(12): 1177-9.
12. Li Y, Yao L, Li J, Chen L, Song Y, Cai Z, et al. Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19. J Med Virol. 2020.
13. He JL, Luo L, Luo ZD, Lyu JX, Ng MY, Shen XP, et al. Diagnostic performance between CT and initial real-time RT-PCR for clinically suspected 2019 coronavirus disease (COVID-19) patients outside Wuhan, China. Respir Med. 2020; 168: 105980.
14. Winter AK, Hegde ST. The important role of serology for COVID-19 control. Lancet Infect Dis. 2020.
15. Drame M, Teguo MT, Proye E, Hequet F, Hentzien M, Kanagaratnam L, et al. Should RT-PCR be considered a gold standard in the diagnosis of Covid-19? J Med Virol. 2020.
16. Theel ES, Slev P, Wheeler S, Couturier MR, Wong SJ, Kadkhoda K. The Role of Antibody Testing for SARS-CoV-2: Is There One? J Clin Microbiol. 2020.
17. WorldPopulationReview. World Population Review. 2020 [cited 2020 May 30]; Available from: https://worldpopulationreview.com/
18. Carreño B. Spanish antibody study suggests 5% of population affected by coronavirus, https://www.reuters.com/article/us-health-coronavirus-spain/spanish-antibody-study-suggests-5-of-population-affected-by-coronavirus-idUSKBN22P1IK, Accessed on May 20, 2020. May 2020 [cited 2020 May 28, 2020]; Available from:
19. Dean A, Arner T, Sunki G, Friedman R, Lantinga M, Sangam S, et al. Epi Info™, a database and statistics program for public health professionals.: CDC, Atlanta, GA, USA; 2011.
20. Hoffman T, Nissen K, Krambrich J, Rönnberg B, Akaberi D, Esmaeilzadeh M, et al. Evaluation of a COVID-19 IgM and IgG rapid test; an efficient tool for assessment of past exposure to SARS-CoV-2. Infect Ecol Epidemiol. 2020; 10(1): 1754538.
21. Sergeant, ESG. Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au.; 2018.
22. Dimeglio C, Loubes J-M, Deporte B, Latour J, Mansuy J-M, Izopet J. The SARS-CoV-2 seroprevalence is the key factor for deconfinement in France. Journal of Infection. 2020; doi.org/10.1016/j.jinf.2020.04.031.
23. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019 Clinical Infectious Diseases. 2020; ciaa344, https://doi.org/10.1093/cid/ciaa344.
24. Garcia-Basteiro AL, Moncunill G, Tortajada M, Vidal M, Guinovart C, Jimenez A, et al. Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospital Medrxiv: Pre-Print 2020; https://doi.org/10.1101/2020.04.27.20082289
25. Levesque J, Maybury DW. A note on COVID-19 seroprevalence studies: a meta-analysis using hierarchical modelling. Medrxiv: Pre-Print. 2020; doi: https://doi.org/10.1101/2020.05.03.20089201
26. Shakiba M, S Nazari eSH, Mehrabian F, Rezvani SM, Ghasempour Z, Heidarzadeh A. Seroprevalence of COVID-19 virus infection in Guilan province, Iran. Medrxiv: Pre-Print. 2020; doi: https://doi.org/10.1101/2020.04.26.20079244
27. Filho LA, Landmann C, Oliveira Sd, Carlos A. Seroprevalence of IgG and IgM anti-SARS-CoV-2 among voluntary blood donors in Rio de Janeiro, Brazil preprintsscielo. 2020; https://doi.org/10.1590/SciELOPreprints.404
28. MOH. Ministry of Health Daily COVID-19 Briefings: April 28, 2020. 2020.
29. EPHI. COVID 19 epidemic trajectory and social distancing; modeling results for Ethiopian and Addis Ababa. 2020.
30. AARHB. Causes of Health Facility Visit in Addis Ababa. 2020.