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Detection and comparison of SARS-CoV-2 antibody produced in naturally infected patients and vaccinated individuals in Addis Ababa, Ethiopia: multicenter cross-sectional study

Chala Bashea[1,2*], Addisu Gize[2], Tadesse Lejisa[1], Demiraw Bikila[1], Betselot Zerihun[3], Feyissa Challa[1], Daniel Melese[1], Alganesh Gebreyohanns[2], Kasahun Gorems[2], Solomon Ali[2], Gadissa Bedada Hundie[2], Habteyes Hailu Tola[4] and Wondewosen Tsegaye[2]

Abstract

Background Natural infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or vaccination triggers antibody production against key viral antigens. However, there is limited evidence on the levels of antibodies produced in naturally infected individuals compared to those vaccinated in Ethiopia. Therefore, we aimed to detect and compare SARS-CoV-2 antibodies produced by naturally infected and vaccinated individuals.

Materials and methods We conducted a multicenter cross-sectional study among a total of 355 naturally infected and 355 vaccinated individuals from November 2022 to April 2023 at 10 selected health facilities in Addis Ababa, Ethiopia. We enrolled the participants consecutively upon their arrival at health facilities until the required sample size was achieved. We used a structured questionnaire to collect data on the demographic and clinical characteristics of the participants. We also collected 3–5 ml of blood samples from all participants and tested for anti-Spike (anti-S) and anti-nucleocapsid (anti-N) antibodies using Cobas 6000. We utilized frequency, mean, or median to describe the data, the Mann-Whitney U test to compare groups, and a generalized linear regression model to assess factors associated with anti-S antibody concentration. We analyzed the data with SPSS version 26, and the level of significance was set at *P*-value < 0.05.

Results Of the naturally infected participants, 352 (99.5%) had anti-S antibodies and all (100%) had anti-N antibodies, whereas among vaccinated participants, all (100%) had anti-S antibodies, while 323 (91.6%) had anti-N antibodies. Anti-S antibodies produced by vaccinated individuals were significantly ($P < 0.001$) higher than those produced as a result of natural infection. Being young ($P = 0.004$), having hypertension ($P < 0.001$), and having diabetes ($P < 0.001$) were significantly associated with lower anti-S antibody levels, while being recently vaccinated and having a higher

*Correspondence:
Chala Bashea
chala.basha@gmail.com

Full list of author information is available at the end of the article

number of vaccine doses were significantly associated with higher anti-S antibody concentrations in vaccinated participants. Having diabetes ($P < 0.001$) were significantly associated with lower anti-S concentrations in participants who were naturally infected.

Conclusion There is a high seropositivity rate in both naturally infected and vaccinated individuals. However, vaccinated individuals had higher levels of SARS-CoV-2 antibodies than those who were naturally infected, which highlights the significant contribution of vaccination in increasing the protection of COVID-19 in Ethiopia.

Keywords COVID-19, SARS-CoV-2, Vaccine, Virology, Immunology, anti-SARS-CoV-2, Seroprevalence

Background

SARS-CoV-2 is a novel coronavirus that causes a respiratory disease called Coronavirus Disease 19 (COVID-19) and transmits through droplets and aerosols [1]. Since the end of 2019, the cases of COVID-19 have been detected in every country of the world. As of January 05, 2024, there have been more than 7 million COVID-19 deaths and over 700 million confirmed cases worldwide, while Ethiopia has reported 7,574 deaths and 501,087 cases [2]. SARS-CoV-2 is an emerging virus that has the possibility to re-emerge even after being controlled due to factors that favor its re-emergence, such as environmental aspects and viral behavior [3].

SARS-CoV-2 is a single-stranded, positive-sense, and enveloped RNA virus [4, 5]. It consists of 15 to 16 nonstructural proteins and four structural proteins that include spike (S), envelope (E), membrane (M), and nucleocapsid (N) [4, 6]. Of the four structural proteins, S and N proteins are the primary immunogens [7, 8]. The virus has the ability to infect any organ that has an angiotensin-converting enzyme 2 (ACE2) receptor on human cells [5]. The host ACE2 receptor is recognized by the SARS-CoV-2 S on the surface of the virion envelope. After recognition, the spike proteins attaches to ACE2 and fuses with its membrane [4]. Following infection, key viral antigens including N and S proteins, are produced and typically peak 14–25 days after the onset of symptoms [9].

Vaccination against SARS-CoV-2 also stimulates the immune system to produce antibodies against the virus. Antibodies produced after the COVID-19 vaccination are very crucial in producing herd immunity [10–12]. Currently available SARS-CoV-2 vaccines work by introducing the whole virus, a small piece of the virus (spike protein), or a spike protein transcript into the body. Following vaccination, antibodies are synthesized that identify and bind to the virus, preventing it from penetrating and infecting human cells [11, 12]. Although disease severity can be reduced following vaccination, breakthrough infections (infection despite receiving the entire recommended vaccination schedule) can occur and may lead to death [13].

Previous studies show that infected individuals can develop antibodies within 7 days after the onset of symptoms [14], and that these antibodies persist for about a year. Another study from the Democratic Republic of Congo showed that 70.5% of naturally infected people had substantial antibodies for up to 6 months [11], while a study from China demonstrated that 91.9% of COVID-19 patients retained detectable antibodies for 10–11 months [15]. In addition, Gudina et al. [16] found a high seroprevalence (63.7%) of SARS-CoV-2 antibodies in Addis Ababa. Moreover, a study reported by Ward et al. [17] indicates that individuals with comorbidities such as diabetes, stroke, renal, liver, lung, or neurological disorders, cancer, and depression exhibited lower levels of antibody production.

According to a study reported from Greece among BNT162b2 vaccinated healthcare workers, the prevalence of SARS-CoV-2 anti-S is 99.7%, and anti-N is 3.7% [18]. Karachaliou et al. [19]. have also reported that 96.5% of fully vaccinated individuals are seropositive for anti-S. However, antibody levels developed by vaccination were found to decrease over time [20]. A study conducted in Ethiopia on healthcare workers revealed, 64.7% of those who had never been exposed to the disease before and 87.5% of those who had a prior infection have detectable anti-RBD antibodies after 8 and 12 weeks of vaccination [21].

Although previous studies from different settings have indicated antibody development following natural infection or vaccination as shown above, there is limited information on the level or concentration of antibodies produced. In addition, data regarding the direct comparison of antibody levels produced by vaccinated individuals versus those naturally infected are also limited. Thus, we aimed to detect and compare SARS-CoV-2 antibody levels produced by naturally infected individuals to those who received the COVID-19 vaccine in Addis Ababa, Ethiopia.

Materials and methods

Study setting and design

We conducted a multicenter cross-sectional study at 10 selected healthcare facilities (HCF) in Addis Ababa, Ethiopia, from November 2022 to April 2023, to enroll participants for both the naturally infected and vaccinated groups. The selected HCF were those mandated

to administer the vaccine in the city. Addis Ababa is the capital city of Ethiopia, where a 50% COVID-19 positivity rate was reported nationwide on a daily basis at the height of the pandemic [22].

Inclusion and exclusion criteria

For the naturally infected group, we included individuals who were older than 18 years, reported a history of previous SARS-CoV-2 infection and had not received a SARS-CoV-2 vaccine. Of the five types of vaccines approved and administered in Ethiopia [23], we included in the vaccinated group those who had received Janssen, AstraZeneca, Pfizer, or Covishield, and had obtained a vaccine certificate following vaccination. We also included in the Vaccinated group individuals who reported no history of previous SARS-CoV-2 infection. Participants who were vaccinated and had not been infected naturally would not have anti-N, since neither the mRNA vaccine nor the non-replicating viral vector vaccine would induce anti-N. Therefore, to confirm that vaccinated individuals had not been previously infected, anti-N testing was conducted among participants who self-reported no history of natural infection. Individuals who had received the Sinopharm vaccine were excluded from the study, as it contains an inactivated form of the SARS-CoV-2 virus, which could be positive for both spike protein and nucleocapsid protein, thus making it difficult to differentiate this group from naturally infected individuals because of anti-N positivity.

Sample size and sampling technique

A total of 355 vaccinated individuals and 355 naturally infected individuals were required based on a sample-size calculation using a double population proportion, including a 95% confidence level, 80% power, and true population proportions of seropositivity of 45.9% for the vaccinated group [24] and 63.7% for the naturally infected group [16]. We allocated sample size to each of the 10 HCFs proportionally based on daily patient flow, and utilized a simple random sampling technique. Eligible participants were consecutively enrolled upon their arrival at the selected HCF for outpatient care, until the required sample size was achieved.

Data and sample collection

Data and sample collectors in each HCF were trained on how to conduct interviews to collect socio-demographic and clinical information, as well as blood samples. We obtained written informed consent from each participant after providing thorough information on the objective, procedure, and benefits of the study before sample and data collection. We used a structured questionnaire that was customized from a literature review to collect information on sociodemographic and clinical variables. We tested the standardized questionnaire on 40 study participants (20 vaccinated and 20 naturally infected) for validity and reliability. We also extracted data on vaccination status such as vaccination date, vaccine type, and number of doses administered from vaccination certificate. A trained laboratory technologist collected 3-5 ml blood samples into serum separator test tubes from a total of 355 individuals who had never been vaccinated but had a history of COVID-19 infection, and 355 individuals who were vaccinated for COVID-19. The collected blood samples were labeled with a unique identification number, similar to the interview identification number. All collected samples were transported in triple packaging and sent to the National Clinical Chemistry Reference Laboratory (NCCRL) of Ethiopian Public Health Institution (EPHI) for laboratory testing. The NCCRL is a nationally accredited referral laboratory that has fulfilled the criteria of International Standard ISO 15,189 to report quality results.

Laboratory test

We tested the samples using the Cobas 6000 automated system (Roche Diagnostics GmbH in Mannheim, Germany). This machine is approved to detect antibodies produced against SARS-CoV-2, and has 99.91% specificity and 98.8% sensitivity for the anti-S and 99.69% specificity and 97% sensitivity for the anti-N. Cobas 6000 uses the electrochemiluminescence immunoassay (ECLIA) principle to detect the presence of SARS-CoV-2 antibodies in human serum [25, 26].

The quantitative anti-S test was used to detect and measure the concentration of antibodies produced in both vaccinated and naturally infected individuals. Titers $\geq$ 0.8 U/ml were considered as positive, whereas samples with concentrations $<$ 0.8 U/ml as negative [25]. For the anti-N test, a cutoff index (COI) value below 1.0 was regarded as nonreactive/negative, while COI $\geq$ 1.0 was reported as reactive/positive, according to the manufacturer result interpretation [26]. The anti-N test is a qualitative test; therefore, we used it only to confirm the presence of a previous natural infection.

Definitions

Natural infection Individuals who developed antibodies as a result of being infected by SARS-CoV-2 but did not take the vaccine.

Vaccinated individuals An individual who has received a single or combination of Pfizer, AstraZeneca, Covishield, and Janssen vaccines.

Asymptomatic Individuals who are positive for the anti-nucleocapsid test but reported not being previously infected by the virus.

Mild Individuals who experienced only mild indications of COVID-19, such as fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and loss of smell.

Moderate patients who experienced evidence of lower respiratory disease during clinical assessment with a low level of shortness of breath.

Table 1 Sociodemographic characteristics and chronic disease distribution among participants

Variable	Category	Naturally infected group	Vaccinated group
		n (%) (*n* = 355)	n (%) (*n* = 355)
Sex	Male	154 (43.4)	154 (43.4)
	Female	201 (56.6)	201 (56.6)
Age	18–29	66 (18.6)	66 (18.6)
	30–39	82 (23.1)	82 (23.1)
	40–49	107 (30.1)	107 (30.1)
	50–59	58 (16.3)	58 (16.3)
	60–69	38 (8.7)	38 (8.7)
	> 70	11 (3.1)	11 (3.1)
Presence of previous SARS-CoV-2 infection	Yes	355 (100)	
	No*	-	355 (100)
Severity of infection	Mild	166 (46.8)	-
	Moderate	174 (49)	-
	Severe	15 (4.2)	-
Vaccination status	Vaccinated	-	355 (100)
	Not Vaccinated	355 (100)	-
Vaccine dose taken	First dose	-	154 (43.4)
	Second dose	-	131 (36.9)
	Third dose	-	70 (19.7)
Duration since vaccination	≤ 3 months	-	48 (13.5)
	3–6 months	-	64 (18)
	6–9 months	-	66 (18.6)
	9–12 months	-	28 (7.9)
	> 12 months	-	149 (42)
Hypertension	Present	52 (14.6)	64 (18)
	Absent	303 (85.4)	291 (82)
Diabetes	Present	49 (13.8)	54 (15.2)
	Absent	306 (86.2)	301 (84.8)
Asthma	Present	12 (3.4)	6 (1.7)
	Absent	343 (96.6)	349 (98.3)
Kidney disease	Present	14 (3.9)	20 (5.6)
	Absent	341 (96.1)	335 (94.4)
Liver disease	Present	9 (2.5)	15 (4.2)
	Absent	346 (97.5)	340 (95.8)
Thyroid disease	Present	17 (4.8)	21 (5.9)
	Absent	338 (95.2)	334 (94.1)

*self-responded negative for SARS-CoV-2 infection

Severe Individuals who were admitted to the hospital for COVID-19 disease and experienced symptoms such as difficulty breathing (dyspnea) and hypoxia.

Data quality control and statistical analysis

We performed data quality checks throughout the data-gathering procedure to obtain quality and reliable data. To ensure data safety, laboratory tests were performed using unique identification labels on the samples. Full data access is restricted to the principal investigator. We double-entered the data into a pre-prepared Microsoft Excel database to assure data quality. We cleaned the data for errors and cross-checked both the softcopy and hard copy data before proceeding with the main data analysis. We used a statistical package for social science (SPSS) version 26 to analyze the data. We used Mann-Whitney U test to compare the concentration of the anti-S antibody median difference between the vaccinated and naturally infected groups. We also used generalized linear regression models to assess the factors associated with anti-S antibody concentration in both groups. We utilized log-likelihood ratio statistics to evaluate model fitness and interpreted the level and direction of association by the regression coefficient (β), with a 95% confidence interval. Level of significance was set at a Pvalue < 0.05.

Results

Sociodemographic characteristics and chronic disease distribution among participants

A total of 710 participants were included in this study, comprising 355 naturally infected and 355 vaccinated individuals. Among the naturally infected participants, the majority (56.6%) were female. The median age of the naturally infected participants was 40 years, with an interquartile range (IQR) of 32–50 years. The most frequently reported comorbidities were hypertension (14.6%) and diabetes mellitus (13.8%) [Table: 1].

In the vaccinated group, 60.2% were female, and the median age was 50 years, with an IQR of 40–61 years. The median duration after vaccination was 10 months, with an IQR of 6–16 months. The most common comorbidities in the vaccinated group were hypertension (18%) and diabetes mellitus (15.2%) [Table: 1]. Individuals included in the vaccinated group reported no prior SARS-CoV-2 infection; however, laboratory investigation confirmed that 323 (91.6%) of them tested positive for the anti-N test.

Distribution of COVID-19 vaccine types administered

Figure 1 depicts the distribution of vaccine type received by vaccinated participants. Overall, Janssen was the most administered vaccine, either as part of a homologous schedule or a heterologous booster regimen. Of the participants who received the Janssen vaccine, 118 (71.5)

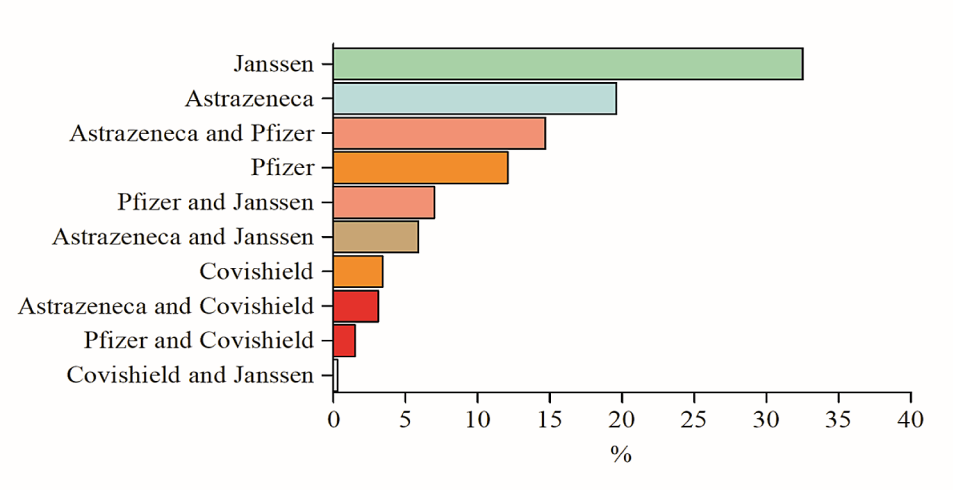

Fig. 1 Distribution of study participants in the vaccinated group according to the types of vaccines administered

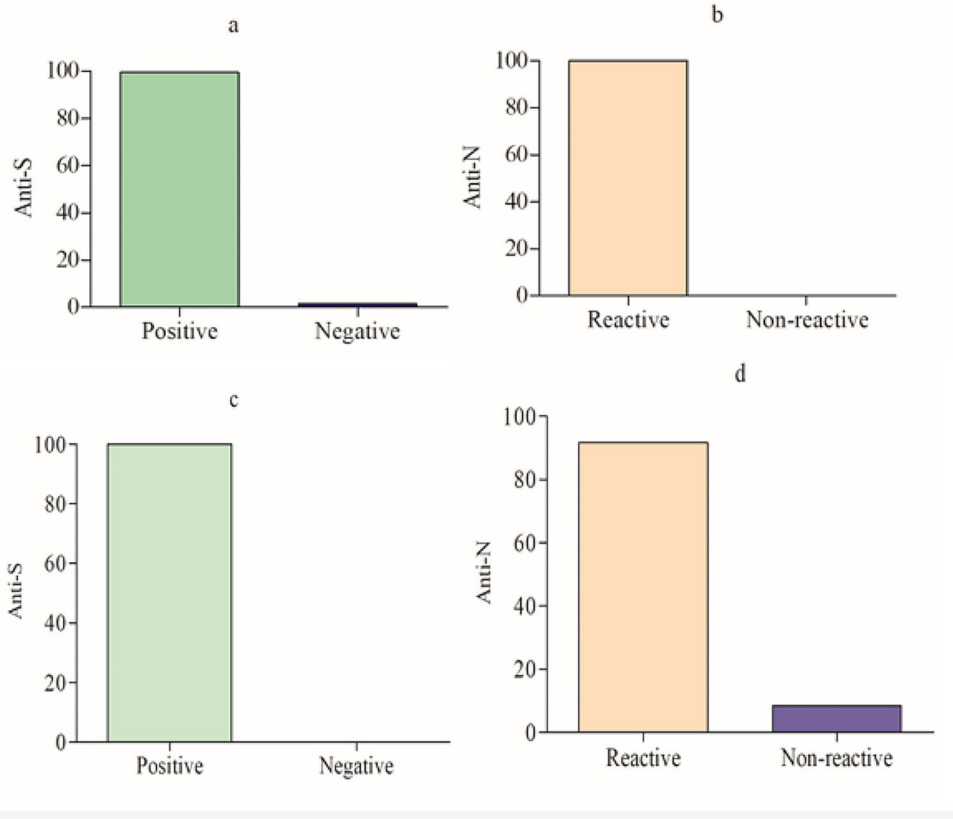

Fig. 2 (**a**) The proportion of naturally infected participants- who were seropositive for anti-S antibodies. (**b**) The proportion of naturally infected participants who were seropositive for anti-N antibodies produced. (**c**) The proportion of vaccinated participants who were seropositive for anti-S antibodies. (**d**) The proportion of vaccinated participants who were seropositive for anti-N antibodies

received a Janssen homologous schedule, while 47 (28.5%) received heterologous booster schedules: Pfizer+Janssen (25 participants, 53.2%), AstraZeneca+Janssen (21 participants, 44.68%), and Covishield+Janssen (1 participant, 2.12%) [Fig. 1].

Detection of antibody production

Of the total of 355 naturally infected participants, 352 (99.5%) had anti-S, while all (100%) produced anti-N (Fig. 2a, b). Of the total of 355 vaccinated participants, all (100%) were developed anti-S antibodies, while 323 (91.6%) developed anti-N antibodies (Fig. 2c, d).

Positive=those participants who show a positive result of anti-S antibody. Negative=participants who were negative for anti-S antibody. Reactive=those participants who were reactive (positive) for the anti-N antibody test. Non-reactive=participants who were non-reactive (negative) for the anti-N antibody test.

Comparison of anti-S concentration median between vaccinated and naturally infected participants

We used the Mann-Whitney Test U test to assess the median difference on anti-S concentration in vaccinated and naturally infected groups. The median anti-S concentration in vaccinated individuals was 6971 U/ml with an IQR of 3379 U/ml–13,448 U/ml, while in naturally infected individuals it was 2365 U/ml with an IQR of 866 U/ml–5682 U/ml. This median difference was statistically significant ($P<0.001$) [Fig. 3].

Factors associated with antibody concentration in vaccinated participants

We assessed the association between independent variables and anti-S antibody concentration separately using generalized linear regression models. As age increases, the concentration of anti-S significantly increases (β=30.4, 95% CI (27.3, 146.3); $P=0.004$) (Table 2). Hypertensive (β = -6191.6, 95% CI (-8381.8, -4001.5); $P<0.001$) and diabetic (β = -4581.2, 95% CI (-6867.9, -2294.5); $P<0.001$) patients had significantly lower concentrations of anti-S antibodies compared to those who did not have hypertension and diabetics [Table 2]. Participants who received only one dose (β = -7692.5, 95% CI (-10268.8, -5116.2); $P<0.001$) or two doses (β = -6032.9, 95% CI (-8678.9, -3386.9); $P<0.001$) (Table 2) of the vaccine were showed significantly lower concentrations of anti-S antibody compared to those who received a third (booster) dose [Fig. 4].

Durations of less than or equal to 3 months (β=13570.3, 95% CI (10855.6, 16284.9); $P<0.001$), 3–6 months (β=7315.3, 95% CI (4870.7, 9759.9); $P<0.001$), and 6–9 months (β=5551.2, 95% CI (3132.7, 7969.8); $P<0.001$) [Table 2] since vaccinated had significantly higher levels of anti-S antibodies compared to those who vaccinated before 12 months [Fig. 5].

Factors associated with COVID-19 antibody concentration in naturally infected participants

Individuals who had moderate (β = -3625, 95% CI (5411, -1838); $P=<0.001$) and mild COVID 19 symptoms (β = -6663, 95% CI (-8458, -4868); $P=<0.001$) had significantly lower concentrations of anti S antibodies than those had severe symptoms. Moreover, individuals with pre-existing diabetes (β = -1443, 95% CI (-2657.7, -228.9); $P=0.020$) had significantly lower anti-S antibody concentrations than those without pre-existing diabetes [Table 3].

Discussion

Data comparing levels of antibodies produced following natural SARS-CoV-2 infection to vaccine-induced antibodies are lacking. To the best knowledge of the authors, there is no evidence on direct comparisons between antibody produced due to vaccination to natural infections in Ethiopia. This study aimed to detect and compare SARS-CoV-2 antibodies produced in naturally infected and vaccinated individuals in Addis Ababa, Ethiopia. We found that 99.5% and 100% of naturally infected participants

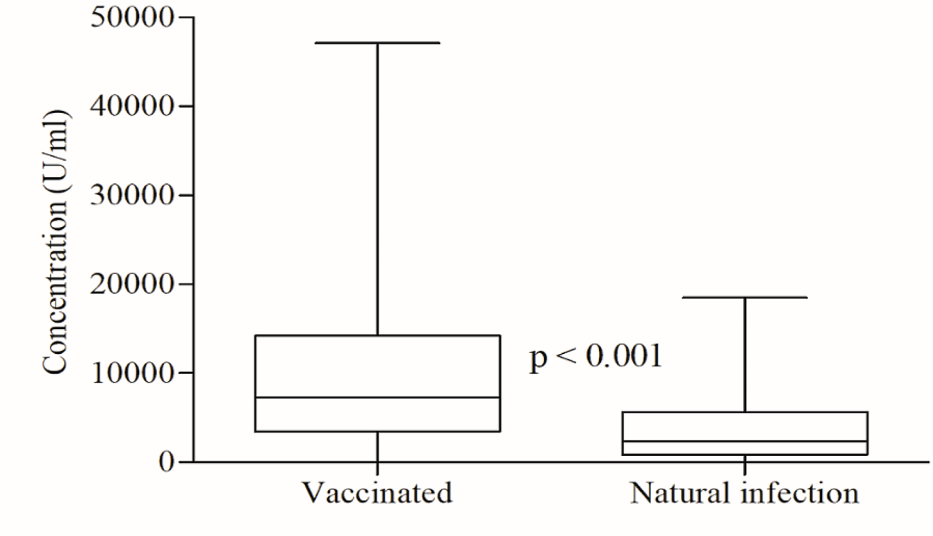

Fig. 3 Median difference of anti-S antibody concentration between vaccinated and naturally infected participants

Table 2 Factors associated with the concentration of anti-S in vaccinated individuals

Variables		Frequency n(%) (*n* = 355)	Un adjusted model		Adjusted model	
			β(CI,95%)	*p*-value	β(CI,95%)	*p*-value
Age			45.4 (-23.5–114.3)	0.197	30.4 (27.3–146.3)	0.004
Sex	Female	216 (60.2)	ref		ref	
	Male	139 (39.8)	557.5 (-1478.05–2593.1)	0.591	-34.8 (-1678.930–1609.4)	0.967
Hypertension	Absent	291 (82)	ref		ref	
	Present	64 (18)	-7227.9(-9701.9– (-4754.1))	< 0.001	-6191.6(-8381.8– (-4001.5))	< 0.001
Diabetes	Absent	301 (84.8)	ref		ref	
	Present	54 (15.2)	-7573.3 (-10226.5– (-4920.1))	< 0.001	-4581.2 (-6867.9– (-2294.5))	< 0.001
Kidney disease	Absent	335 (94.8)	ref		ref	
	Present	20 (5.6)	-3272.5(-7569.9–1024.8)	0.136	-853.4(-4508.19–2801.3)	0.647
Thyroid disease	Absent	334 (94.1)	ref		ref	
	Present	21 (5.9)	-3365.5 (-7564.2–833.1)	0.116	-1811.3 (-5143.1–1520.5)	0.287
Duration since vaccination	> 12 months	149 (42)	ref		ref	
	9–12 months	28 (7.9)	224.4 (-2914.9–3363.6)	0.889	2207.6 (-1161.4–5576.8)	0.199
	6–9 months	66 (18.6)	1125.2 (2398.8–6809.4)	< 0.001	5551.2 (3132.7–7969.8)	< 0.001
	3–6 months	64 (18)	4734.8 (2468.3–7001.3)	< 0.001	7315.3 (4870.7–9759.9)	< 0.001
	≤ 3 months	48 (13.5)	11921.3 (9435.7–14406.9)	< 0.001	13570.3(10855.6–16284.9)	< 0.001
Dose of Vaccine	Three doses(booster)	70 (19.7)	ref		ref	
	Two doses	131 (36.9)	-5334.4 (-7562.1– (-684.03)	< 0.001	-6032.9(-8678.9– (-3386.9)	< 0.001
	Single doses	154 (43.4)	-6032.9(-10268.8– (-3106.7)	< 0.001	-7692.5(-10268.8– (-5116.2)	< 0.001

ref: Reference variable

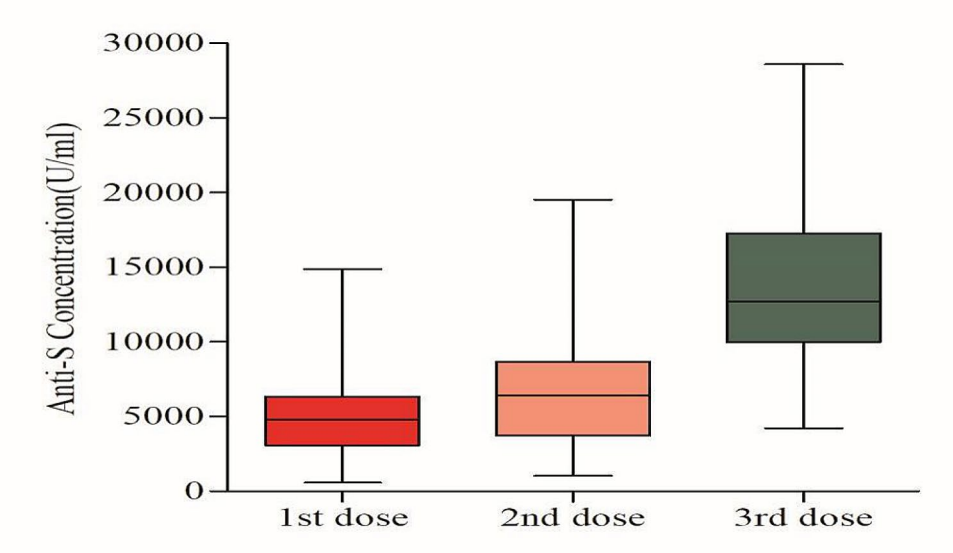

Fig. 4 Descriptive analysis of the relationship between vaccine doses and anti-S antibody concentration

were produced anti-S and anti-N antibodies respectively. Among vaccinated participants, 100% produced anti-S antibodies, and 91.6% produced anti-N antibodies. Nevertheless, the concentration of anti-S antibodies produced by vaccinated individuals were significantly greater than that of naturally infected individuals.

In the naturally infected group, the prevalence of anti-S antibodies was found to be 99.5%. This finding is consistent with the study conducted by Briggs et al. [27], which reported a 95% seroprevalence in the unvaccinated population. Moreover, our findings are similar to the study reported by Yan et al. [15], which showed a 91.9% prevalence of anti-S antibodies.

Existing evidence indicates that COVID-19 vaccines can generate antibodies that are important for developing herd immunity [11, 12]. A study conducted by Li et al. [28] showed that 100% of vaccinated individuals were seropositive for anti-S antibody. These findings are in agreement with the present study findings in which all vaccinated participants tested positive for anti-S

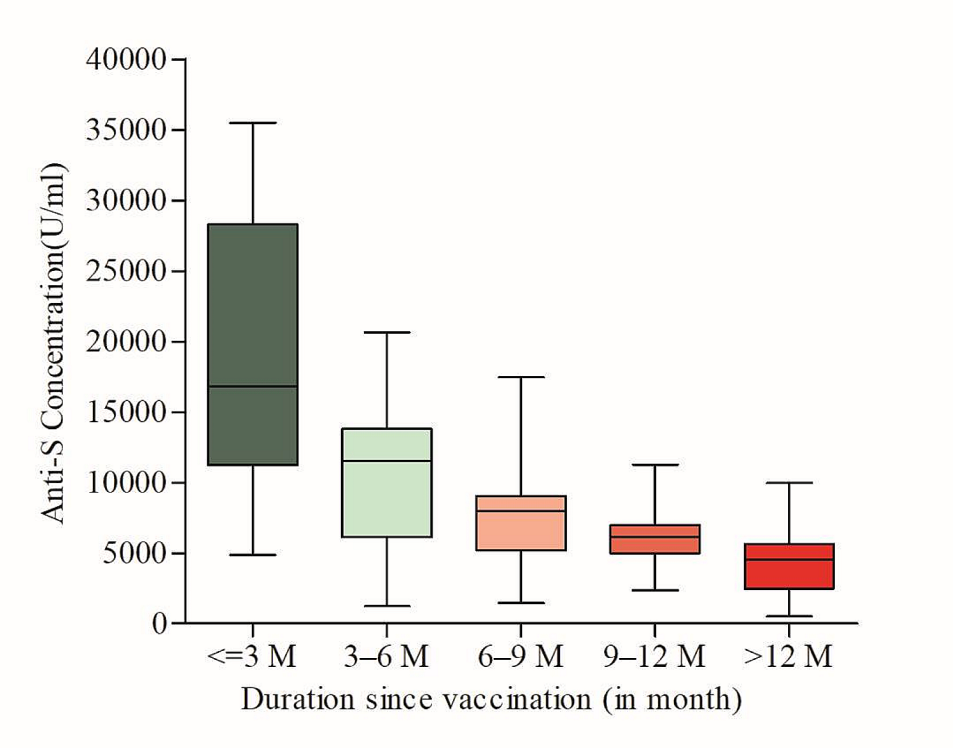

Fig. 5 Descriptive analysis of the relationship between duration since vaccination and anti-S antibody concentration
≤ 3 M = Median of anti-S less than or equal to three months since vaccinated, 3–6 M = Median of anti-S (3.1–6) months since vaccinated, 6–9 M = Median of anti-S (6.1–9) months since vaccinated, 9–12 M = Median of anti-S (9.1–12) months since vaccinated, > 12 M = Median of anti-S greater than 12 months since vaccinated

Table 3 Factors associated with the concentration of anti-S in naturally infected individuals based on a simple generalized linear regression model

Variables		Frequency n (%) (*n* = 355)	Un adjusted model		Adjusted model	
			β(CI,95%)	*p*-value	β(CI,95%)	*p*-value
Age		355(100%)	-23.3 (-55-8.6)	0.153	-5.7 (-35.5-24.05)	0.706
Sex	Female	216 (60.2)	ref		ref	
	Male	139 (39.8)	-123.8 (-953.8-706.2)	0.770	182.4(-531.1-896.01)	0.616
Hypertension	Absent	303 (85.4)	ref		ref	
	Present	52 (14.6)	-2628.2(-3759- (-1497))	< 0.001	-1172.4 (-2377.3- 32.5)	0.057
Diabetes	Absent	306 (86.2)	ref		ref	
	Present	49 (13.8)	-2751.9 (-3909 - (-1594))	< 0.001	-1443(-2657.7- (-228.9))	0.020
Level of infection	Severe	15 (4.2)	ref		ref	
	Moderate	174 (49)	-3647(-5480.5- (-1813.7))	< 0.001	-3625(-5411- (-1838))	< 0.001
	Mild	166 (46.8)	-6851(-8688.4-(-5014.6))	< 0.001	-6663 (-8458-(-4868))	< 0.001

antibodies. This suggests that the vaccine has successfully stimulated the immune systems of vaccinated individuals in the production of antibodies against the virus. The antibodies produced in response to the vaccination can assist in safeguarding against future infections by identifying and neutralizing the virus [11, 12]. In contrast to our finding, a study reported from India shows lower (66.7%) seropositivity rate among vaccinated adults [29]. This difference is probably due to the participants included in our study have received different types and doses of the vaccine.

The present study results showed that over 90% of the vaccinated participants were seropositive for anti-N antibodies. Similar findings have been reported in another study [18]. Since the vaccines received by the study participants in this study target only spike protein, individuals who tested positive for anti-N antibodies may have hybrid immunity, which comprises of immunity induced by the vaccine and natural infection [19, 25]. However, since there is no conclusive evidence on when the antibodies generated by vaccines are depleted, we were unable to identify a method for distinguishing

hybrid immunity from immunity following natural infection. In addition, all vaccinated individuals in this study who tested positive for anti-N antibodies reported that they had not experienced any noticeable signs or symptoms of SARS-CoV-2 infection in the past. This suggests that these individuals are likely asymptomatic carriers of the virus, which is consistent with the WHO's report in which approximately 80% of SARS-CoV-2 infections are either asymptomatic or mild [30]. Moreover, vaccination does not guarantee complete protection against transmission, thus breakthrough infections are possible [13, 31]. Since the symptoms of breakthrough infections are often mild and moderate [13, 32], individuals may not be aware that they have been infected with the virus, and thus not seek healthcare.

In this study, the concentration of anti-S antibody was about three-fold higher in vaccinated individuals than in naturally infected individuals. These results suggest that the vaccinated population has generated more antibodies than those who are only naturally infected, or the higher antibody amongst the vaccinated participants may be due to the repeated exposure with natural infection. Studies comparing antibody levels acquired from natural infection versus vaccination have shown controversial results, indicating either similar or higher concentrations from vaccination [33, 34]. Our finding is consistent with previous study results, in which antibody production is higher in vaccinated individuals compared to those naturally infected [11, 18, 29, 35]. In contrast, a previous studies have reported higher antibody levels in individuals who were naturally infected with SARS-CoV-2 compared to those who were vaccinated [21, 36, 37]. This disagreement between the results of previous studies and the present study's results might be due to the fact that different vaccine types were used in this study, as the combination of different vaccine types (heterologous boosters) could increase the level of antibody production [38, 39].

In this study, older age in vaccinated individuals significantly increased the concentration of anti-S antibodies. This result aligns with previous findings, which observed higher antibody production in older individuals compared to younger ones [40]. In contrast, some earlier studies reported a negative association between young age and SARS-CoV-2 antibody concentration [20]. The discrepancy between the current study and previous studies may be due to the fact that the majority of participants in this study who received more than two doses were older.

Previous study [20, 28] have reported similar findings to the present study, showing that the concentration of anti-S antibodies significantly decreases with the increasing number of months post-vaccination. This finding strongly supports the fact that produced humoral immunity or antibody gradually declines/decay over time [20]. The extent of declining humoral immunity might vary depending on several factors, such as type of vaccine, age, and underlying medical conditions. Therefore, the gradual decline of vaccine-produced antibodies observed in the present study could be attributed to one or more of these factors. This highlights the importance of regular monitoring of seroprevalence of vaccine induced antibody and considering booster doses to provide continued protection against COVID-19.

We also assessed whether the number of vaccine doses or booster vaccinations impacted antibody concentration. We found that participants who received only one or two doses of the vaccine had significantly lower concentrations of anti-S antibodies as compared to those who received the third dose. This suggests that a booster dose of the vaccine is necessary to maintain high antibody levels. This finding is supported by a previous study reported from Catalonia, which found that the second dose of the vaccine produced more antibodies than the first dose alone [19]. Therefore, administering a booster dose of the vaccine is a wise strategy to provide high antibody level.

In this study, the presence of diabetes in naturally infected individuals was negatively associated with anti-S antibody concentration. This finding is consistent with previous studies findings, which suggested that patients with diabetes produced low levels of SARS-CoV-2 antibodies [41, 42]. In contrast, another study reported that anti-S antibodies produced by diabetic patients are comparable to those produced by non-diabetic patients [43]. The possible reason for the discrepancy could be that diabetic patients may have other complications or be on other medications that can negatively affect the immune system. For instance, the use of corticosteroids could contribute to a reduced antibody response [44].

Limitation of the study

Since both natural infection and hybrid immunity result in positive anti-S and anti-N responses, one limitation of this study was the inability to distinguish hybrid immunity, as there are currently no available criteria to identify it. We also did not conduct statistical tests to compare the antibody levels produced by the vaccine alone with those produced from natural infection because of our sample size for the solely vaccine-induced group was small ($n=32$), which makes it difficult to compare with the larger sample size of natural infection ($n=355$).

Conclusion and recommendation

Our study shows a high seropositivity rate in both naturally infected and vaccinated individuals. The vaccinated individuals had a higher concentration of SARS-CoV-2 antibody than that of naturally infected. However, the majority of vaccinated individuals had repeated exposure

to natural infection. We recommend using a sufficient sample size to compare antibodies generated purely from vaccination with those from natural infection. Our study also demonstrates that the concentration of antibodies increases with the number of vaccine doses, underscoring the importance of a series of vaccinations and booster vaccinations in order to achieve high antibody level. Moreover, we found that antibody levels tend to decline over time after vaccination, which indicates the requirement of booster dose.

Abbreviations

ACE2	Angiotensin-Converting Enzyme 2
Anti-N	Anti-Nucleocapsid
Anti-S	Anti-Spike
ARBs	Angiotensin receptor blockers
COI	Cutoff Index
COVID-19	Coronavirus Disease 19
ECLIA	Electrochemiluminescence immunoassay
EPHI	Ethiopian Public Health Institute
NCCRL	National Clinical Chemistry Reference Laboratory
RBD	Receptor Binding Domain
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SPHMMC	Saint Paul's Hospital Millennium Medical College
WHO	World Health Organization

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Author contributions
CB: conceptualized the study. CB, HHT, TL, AG, WT, and GBH: involved in methodology and validation of the study. CB, DB, BZ, KG, and AG: involved in sample collection, visualization, supervision and data acquisition. CB, WT, AG, GBH, DB, FC, and BZ: involved in data curation. CB, HHT and TL: involved in data analysis and interpretation. DM, SA, and FC: provided resources for the study. CB: writing—original draft preparation. All authors contributed to writing—review and editing of the manuscript. CB, AG and WT: overall administered the project. All authors critically read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from St. Paul's Hospital Millennium Medical College Institutional Review Board (SPHMMC-IRB: PM23/253) and the Addis Ababa City Administration Health Bureau (AAHB/4938/227). Confidentiality of the collected information was assured by limiting data access to data collectors, data managers, and the principal investigator. Sensitive information that exposes the identity of the participants was not collected. All collected data were saved exclusively on the principal investigator's computer.

Consent for publication
The consent was obtained from the study participants that the result of the study would be published.

Competing interests
The authors declare no competing interests.

Author details
[1]National Laboratory Capacity Building Directorate, Ethiopian Public Health Institute, Addis Ababa, Ethiopia
[2]Department of Microbiology, Immunology and Parasitology, St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia
[3]National TB Reference Laboratory, Ethiopian Public Health Institute, Addis Ababa, Ethiopia
[4]Department of Public Health, College of Health Sciences, Salale University, Fitche, Ethiopia

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