



ISSN: 2617-6548

URL: www.ijirss.com



Post-pandemic immune profiles: Antibody patterns in vaccinated individuals with and without Prior SARS-CoV-2 infection

Manar Alzahrani^{1,2}, Afnan Alsultan¹, Aisha Alamri¹, Rawabi Aldossary^{1,3}, Lamya Yamani¹, Elfadil Abass^{1*}

¹Department of Clinical Laboratory Science, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia.

²Department of Laboratory and Blood Banking, King Fahad Specialist Hospital, Tabuk, Saudi Arabia.

³Department of Pathology and Laboratory Medicine, King Fahad Specialist Hospital, Dammam, Saudi Arabia.

Corresponding author: Elfadil Abass (Email: emabass@iau.edu.sa)

Abstract

This study aimed to investigate the serological responses to COVID-19 vaccines in the Saudi population post pandemic, a period characterized by presumed hybrid immunity, and to assess demographic and clinical factors influencing antibody responses. IgG antibody responses to the SARS-CoV-2 spike protein were assessed using chemiluminescent immunoassay (CLIA) in 401 individuals from the Saudi population. Of the 401 participants, 395 were vaccinated and 397 (99%) were positive for anti-SARS-CoV spike protein antibodies, including five of the six unvaccinated individuals. Antibody positivity peaked in ages 20-29 and was lowest in children (0-9 years) and adults over 80 years. IgG levels differed between vaccinated and unvaccinated participants ($P < 0.0001$) and between sexes ($P = 0.0072$), but not by vaccine type, dose, or comorbidities. Unexpectedly, vaccinated individuals without prior infection had higher IgG than those with prior infection, challenging the presumed advantage of hybrid immunity. In this Saudi cohort, age and sex significantly influenced spike IgG responses, while vaccine type, dosage, and comorbidities had no effect. Unexpectedly, hybrid immunity showed no advantage, indicating altered immune dynamics at the pandemic's end. These findings provide valuable insights into population-level COVID-19 immunity and can inform public health strategies and vaccine policies in settings with widespread prior infection or vaccination.

Keywords: CLIA, SARS-CoV-2, Saudi population, Serological response, Vaccine immunogenicity.

DOI: 10.53894/ijirss.v8i12.11026

Funding: This work was supported by the Deanship of Scientific Research, Imam Abdulrahman Bin Faisal University (Grant Number: 2022-048-CAMS).

History: Received: 23 October 2025 / **Revised:** 18 November 2025 / **Accepted:** 21 November 2025 / **Published:** 5 December 2025

Copyright: © 2025 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Acknowledgments: We thank Dr. Amir Msmar and Dr. Omar Soliman Elmasry for their assistance with statistical analysis.

Publisher: Innovative Research Publishing

1. Introduction

Since its onset, the COVID-19 pandemic has posed an unprecedented challenge to the global public health, driven by the virus. This situation has led to widespread morbidity, mortality, and socioeconomic disruption worldwide. The disease has exhibited diverse clinical manifestations, from silent infections to severe illness characterized by interstitial pneumonia and respiratory failure [1]. This crisis has required significant efforts in prevention, treatment, and vaccine development. Emerging variants have complicated vaccine strategies [2, 3].

Vaccination has been central to reducing COVID-19 severity and mortality, though immune responses vary [4]. Factors such as age, sex, comorbidities, and prior infection influence antibody levels. Elderly individuals often exhibit reduced responses due to immunosenescence [5] while females typically mount stronger vaccine responses, attributed to hormonal and genetic differences [6, 7].

Prior SARS-CoV-2 infection contributes to “hybrid immunity,” which may provide stronger and longer-lasting protection than vaccination or infection alone through reactivation of memory B and CD4⁺ T cells [8]. However, evidence is mixed, with some studies showing superior antibody levels and others finding no difference [9]. Vaccine regimen also matters: heterologous (mixed) vaccination has been associated with higher antibody titers and broader protection than homologous regimens, though responses to different combinations remain under investigation [10, 11].

The impact of pre-existing health conditions on immune response to COVID-19 vaccines has also attracted attention. Individuals with chronic diseases or conditions requiring immunosuppressive treatment, such as organ transplant recipients, have been found to exhibit weaker responses to vaccination [12]. Comorbidities, including obesity and diabetes, have similarly been associated with attenuated antibody production following vaccination [13, 14]. However, not all studies have found significant associations between comorbidities and antibody responses, suggesting that additional research is necessary to clarify these relationships.

In the Saudi Arabian context, assessing the serological response to COVID-19 vaccines is particularly important given the diverse demographic and health characteristics of the population [15, 16]. The present study aims to contribute to this understanding by examining spike IgG antibody levels among vaccinated and unvaccinated individuals within a Saudi cohort. This includes evaluating the influence of variables such as age, sex, vaccination status, and comorbidities on antibody response. By exploring these factors, the study seeks to provide insight into the effectiveness of vaccination and identifying vulnerable populations and shaping policies to maximize vaccine efficacy and coverage.

2. Materials and Methods

2.1. Ethics

This study was reviewed and approved by the Institutional Review Board (Approval Number: IRB-PGS-2022-03-526). All procedures followed institutional guidelines and the ethical standards of the responsible committee on human experimentation, in accordance with the Declaration of Helsinki (1975, revised 2013). Participation was voluntary, with written informed consent obtained from all adult participants and assent from children over 7 years in line with national regulations. Confidentiality was strictly maintained by anonymizing all identifying information.

2.2. Study Design and Participants

We retrospectively analyzed prospectively collected data from 401 Saudi individuals between January 15 and March 21, 2023. Clinical samples, vaccination histories, and infection status (asymptomatic/symptomatic) were obtained through structured interviews and forms during the presumed peak of hybrid immunity.

2.3. Sample Collection and Storage

Venous blood samples (4 mL) were collected from all participants using EDTA vacutainer tubes. A total of 401 samples were collected and were centrifuged at 3000 rpm for 10 minutes to separate plasma, which was then aliquoted into 2 mL microcentrifuge tubes and stored at -80°C.

2.4. Immunoassay Analysis

Detection of SARS-CoV-2 Spike IgG using Chemiluminescence Immunoassay (CLIA): A total of 401 plasma samples was tested for SARS-CoV-2 Spike IgG using the LIAISON® XL Analyzer (DiaSorin, Italy) and commercial kits, following manufacturer instructions.

2.5. Statistical Analysis

Data distribution was assessed using the D'Agostino–Pearson test with QQ plots ($\alpha = 0.05$). Group comparisons employed Mann–Whitney and Kruskal–Wallis tests. Spearman correlation was classified as weak (0–0.25), moderate (0.25–0.75), or strong (0.75–1). Categorical data were expressed as frequencies/percentages and continuous data as medians (IQR). Analyses were performed with GraphPad Prism 10 and IBM SPSS 28.

3. Results

3.1. Baseline Characteristics of the Study Population

Table 1 summarizes baseline characteristics of 401 participants: 69.8% were female, median age 35 years. Most (98.5%) received ≥ 2 vaccine doses, mainly Pfizer-BioNTech; 47.4% had prior infection, and 45.9% reported comorbidities.

Table 1.
Baseline Characteristics of the Study Population.

Characteristics (n)	Number (%)
Sex (n=401)	Female
	280 (69.8%)
	Male
	121 (30.2%)
Age (n=401)	0-9
	3 (0.7%)
	10-19
	30 (7.5%)
	20-29
	172 (42.9%)
	30-39
	51 (12.7%)
	40-49
	57 (14.2%)
	50-59
	49 (12.2%)
	60-69
	28 (7.0%)
	70-79
	9 (2.2%)
	≥80
	2 (0.5%)
SARS-Cov-2 Vaccination status (n=401)	Vaccinated
	395 (98.5%)
	Unvaccinated
	6 (1.5%)
Administered vaccine doses (n=401)	Zero
	6 (1.5%)
	Two
	28 (7.0%)
	Three
	360 (89.8%)
	Four
	7 (1.7%)
1st vaccine type (n=401)	Pfizer-BioNTech
	329 (82.0%)
	Oxford-AstraZeneca
	57 (14.2%)
	Maderna
	4 (1.0%)
	Sinopharm
	1 (0.2%)
	Janssen
	0 (0%)
	Un-known
	4 (1.0%)
	None
	6 (1.5%)
2nd vaccine type (n=401)	Pfizer-BioNTech
	340 (84.8%)
	Oxford-AstraZeneca
	41 (10.2%)
	Maderna
	8 (2.0%)
	Sinopharm
	1 (0.2%)
	Janssen
	0 (0%)
	Un-known
	5 (1.2%)
	None
	6 (1.5%)
3rd vaccine type (n=401)	Pfizer-BioNTech
	314 (78.3%)
	Oxford-AstraZeneca
	13 (3.2%)
	Maderna
	25 (6.2%)
	Sinopharm
	0 (0%)
	Janssen
	2 (0.5%)
	Un-known
	13 (3.2%)
	None
	34 (8.5%)
4th vaccine type (n=401)	Pfizer-BioNTech
	7 (1.7%)
	Oxford-AstraZeneca
	0 (0%)
	Maderna
	0 (0%)
	Sinopharm
	0 (0%)
	Janssen
	0 (0%)
	None
	394 (98.3%)
Two identical doses administered according to vaccine type (n=27)	Pfizer-BioNTech
	18 (66.6%)
	Oxford-AstraZeneca
	4 (14.8 %)
	Mix types
	5 (18.5 %)
Three identical doses administered according to vaccine type (n=347)	Pfizer-BioNTech
	259 (74.6%)
	Oxford-AstraZeneca
	5 (1.4%)
	Mix types
	83 23.9%)
History of previous COVID-19 infection (n = 401)	Yes
	189 (47.4%)
	No
	212 (52.6%)
COVID-19 symptoms (n=189)	Asymptomatic
	21 (11.1%)
	Symptomatic (hospitalized)
	6 (3.2 %)
	Symptomatic (home isolation)
	162 (85.7%)
Vaccinated individuals with a history of COVID-19 infection (n=395)	Vaccinated with previous Covid19 infection
	187 (47.3%)
	Vaccinated with no previous Covid19 infection
	208 (52.6%)
Comorbidities (n=401)	Yes
	184 (45.9%)

	No	217 (54.1)
Healthy individuals with a history of COVID-19 infection (n=298)	Healthy with no previous Covid-19	112(37.4%)
	Comorbidities with no previous Covid-19	97 (32.6%)
	Comorbidities with previous Covid-19 infection	89 (30%)

Note: Individuals aged 0-9 were not included in the vaccination schedule based on national and international guidelines.

3.2. COVID-19 Infection History and Health Status

Overall, 52.6% had no prior COVID-19, while 47.4% were infected-mostly symptomatic at home (85.7%), with 3.2% hospitalized and 11.1% asymptomatic. Nearly half of vaccinated participants had prior infection, and 45.9% had comorbidities. Combined, 37.4% were healthy/uninfected, 32.6% had comorbidities only, and 30.0% had both.

3.3. SARS-CoV-2 Spike IgG Antibody Seroprevalence

The SARS-CoV-2 Spike IgG antibody levels among the study participants ranged between 4.81 and 2080 BAU/mL, with 33.8 BAU/mL set as the positivity threshold according to the manufacturer's guidelines. Among the 401 participants, 397 (99.0%) tested positive for Spike IgG antibodies, with varying levels above the cut-off. Only 4 participants (1.0%) were seronegative.

3.4. Effect of Vaccination on Spike IgG Antibody Levels

The study included 395 vaccinated participants and 6 unvaccinated individuals as controls. The distribution of antibody levels significantly deviated from normality, as indicated by the D'Agostino-Pearson normality test ($p < 0.0001$). Nearly all vaccinated participants were seropositive, with only three testing negatives. Among the unvaccinated controls, one individual tested negative, while five tested positive with antibody levels above the 33.8 BAU/mL cutoff. Analysis revealed a significant difference in Spike IgG antibody levels between the groups ($p < 0.0001$), with the vaccinated group showing a significantly higher median antibody level (Fig. 1).

3.5. Sex- based Differences in Spike IgG Antibody Levels

The study population was divided into male and female groups. The D'Agostino and Pearson tests confirmed that Spike IgG antibody levels were not normally distributed ($P < 0.0001$) in both groups. CLIA results showed that 99.0% of participants were positive for Spike IgG antibodies, with only 4 individuals (1.0%) testing negative (2 males and 2 females). The analysis demonstrated a statistically significant difference in antibody levels between sexes ($p = 0.0072$), with females displaying higher Spike IgG levels than males (Fig. 2).

3.6. Age- Related Variations in Spike IgG Antibody Levels

Spike IgG positivity was highest in the 20–29 group (43.1%) and lowest in the 0–9 and >80 groups (0.5% each). All participants in the 10–19, 30–39, 60–69, 70–79, and ≥80 groups were positive, with varying antibody levels; only one individual in the 0–9, 20–29, 40–49, and 50–59 groups tested negative. Antibody levels differed significantly across age groups (Kruskal–Wallis, $P = 0.001$). Dunn's test showed significantly lower levels in 0–9 compared with several groups, and between 30–39 and 50–59; other comparisons were not significant (Fig. 3).

3.7. Relationship Between Number of Vaccine Doses and Spike IgG Antibody Levels

Participants were grouped by vaccine doses: none ($n=6$), two ($n=28$), three ($n=360$), and four ($n=7$). Nearly all vaccinated individuals were IgG-positive, except one in the two-dose group and two in the three-dose group. Antibody levels differed significantly across groups (Kruskal–Wallis, $P < 0.0001$). Dunn's test showed the zero-dose group had significantly lower levels than all vaccinated groups, while no significant differences were observed among the two-, three-, and four-dose groups (Fig. 4).

3.8. Comorbidities and SARS-CoV-2 Spike IgG Antibody Levels

Two hundred ninety-eight vaccinated participants were grouped based on their health status and history of COVID-19 infection: those with comorbidities but no prior COVID-19 infection ($n = 97$), healthy individuals with no prior COVID-19 infection ($n = 112$), and those with comorbidities and a history of COVID-19 infection ($n = 89$). CLIA results showed that all participants from these groups had IgG antibody levels above the cut-off of 33.8 BAU/mL against the Spike antigen, except for one participant from the comorbidities group (with a kidney transplant history) and two participants from the healthy group, all without previous COVID-19 infections. The Kruskal–Wallis test was used to compare antibody levels among the three groups, revealing no significant difference in antibody levels ($P = 0.2616$).

3.9. Effect of Vaccine Type and Dose on Spike IgG Antibody Levels

Most participants ($n=286$) received two or three doses of Pfizer-BioNTech or Oxford-AstraZeneca, while 87 received mixed regimens (Pfizer, Oxford-AstraZeneca, Moderna, Sinopharm, Janssen). They were grouped into six categories (two or three doses of Pfizer, Oxford-AstraZeneca, or mixed vaccines). All groups were above the 33.8 BAU/mL cut-off, except for three negatives in Pfizer groups (two in three-dose, one in two-dose). Antibody levels did not differ significantly across groups (Kruskal–Wallis, $P=0.1221$).

3.10. Hybrid Immunity and Spike IgG Antibody Levels

The 395 vaccinated individuals were categorized into two groups based on their history of COVID-19 infection: those who were vaccinated and had a prior COVID-19 infection (n=187) and those who were vaccinated without any prior COVID-19 infection (n=208). The CLIA findings indicated that Spike IgG antibody was positive in all subjects tested from both groups, with varying levels of positivity. However, three individuals from the group of vaccinated individuals with no history of prior COVID-19 infection showed negative results below the cut-off. The statistical analysis revealed a significant difference in Spike IgG antibody levels between the groups, with a P-value of 0.0257. Notably, the vaccinated group without a history of COVID-19 infection had significantly higher levels of Spike IgG antibodies compared to the vaccinated group with a history of COVID-19 infection. This unexpected pattern indicates that, contrary to prevailing assumptions, hybrid immunity may not always result in superior levels- particularly in populations exposed to multiple vaccine doses over time.

3.11. COVID-19 Clinical Outcome and Spike IgG Antibody Levels

Individuals previously infected with COVID-19 (n=189) were classified into two groups according to their disease outcomes: asymptomatic (n=21) and symptomatic (n=168). CLIA results showed that all 189 individuals were positive for Spike IgG antibodies, with levels above the cut-off, though the degree of positivity varied. The Mann-Whitney U test was used to compare antibody levels between the asymptomatic and symptomatic groups, revealing a significant difference in Spike IgG antibody levels, with a P-value of 0.0004. Notably, the symptomatic group exhibited markedly elevated Spike IgG antibody levels compared to the asymptomatic group (Fig. 5).

3.12. Correlation Between COVID-19 Clinical Outcome and Spike IgG Antibody Level

Among previously infected individuals, outcomes were classified as protected (asymptomatic, n=21) or susceptible (symptomatic, n=168). Based on antibody levels, four subgroups emerged: asymptomatic-moderate (n=2), asymptomatic-high (n=19), symptomatic-moderate (n=4), and symptomatic-high (n=164). Spearman's correlation showed a weak, non-significant positive association between higher antibody levels and asymptomatic outcomes ($r = 0.128$, $P = 0.078$). As antibody levels may derive from vaccination, infection, or both, results reflect total Spike IgG and should not be attributed solely to vaccination.

4. Discussion

This study evaluated Spike IgG antibodies in vaccinated and unvaccinated Saudi participants. The findings showed a high seropositivity rate (99.0%) for Spike IgG antibodies. Vaccinated participants, as expected, had significantly higher antibody levels compared to unvaccinated individuals, reinforcing the effectiveness of COVID-19 vaccines in generating protective immune responses, in line with current literature [17, 18].

Interestingly, five out of six unvaccinated participants showed varying levels of Spike IgG antibodies. This presence of antibodies in the unvaccinated group is likely due to previous virus exposure. Only one of these five participants had a known history of COVID-19, consistent with studies indicating that unvaccinated individuals with prior infections can have detectable levels of Spike IgG antibodies [19, 20]. The other four unvaccinated participants had no documented history of infection, suggesting the possibility of asymptomatic cases where individuals do not exhibit symptoms and remain unaware of their infection. Such asymptomatic carriers can still spread the virus, with viral loads comparable to those of symptomatic individuals [21, 22]. The 99.0% antibody positivity observed in this representative Saudi cohort suggests that herd immunity may have been reached at the population level.

The finding showed a significant sex difference in Spike IgG antibody levels ($p = 0.0072$), with females exhibiting higher levels than males. This finding aligns with recent research conducted in Saudi Arabia [23]. It is well-documented that adult females generally mount stronger immune responses, leading to higher vaccine efficacy compared to males. Additionally, females have shown better immune responses to vaccinations against viruses such as hepatitis and influenza [6, 7].

Sex differences in COVID-19 outcomes have also been reported, including a large US-based cohort study that indicated higher mortality in males, attributed primarily to lifestyle-related risk factors rather than direct correlations with Spike IgG antibody levels [24]. This implies that the observed differences in COVID-19 outcomes between males and females may be influenced by a range of confounding factors beyond antibody levels, such as underlying health conditions and behavioural differences.

While it is evident that females have a stronger vaccine-induced antibody response to the virus, how these differences translate into variations in immune responses to the virus and COVID-19 outcomes remains unclear. The exact mechanism behind this phenomenon is not yet fully understood [25-27].

Our study revealed age-related differences in the humoral immune responses, with a decline in immune response observed as age increased. The highest antibody positivity was noted in the 20-29 age group, making them the most immunocompetent demographic. Children aged 0-9 years showed detectable but significantly lower Spike IgG antibody levels compared to older age groups, likely due to prior virus exposure and their unvaccinated status. The oldest (≥ 70 years) age group exhibited lower positivity rates, attributed to immunosenescence in older adults [28].

During the global COVID-19 vaccination campaign, significant debate emerged over the efficacy of vaccine doses and the protective effect of different vaccine brands against the disease. The present study found that in the studied Saudi cohort, Spike IgG levels were not significantly influenced by the number of COVID-19 vaccine doses received, whether it was two, three, or four doses. This suggests that receiving at least two vaccine doses is sufficient to elicit adequate immune

protection against COVID-19. This finding contrasts with previous studies [29, 30] which reported that higher levels of Spike IgG were typically associated with an increased number of vaccine doses. Notably, individuals who received at least two doses exhibited significantly higher antibody levels compared to unvaccinated individuals, implying that two doses can induce a robust antibody response.

The debate over the effectiveness of different types of the vaccines has been longstanding. In this study, Spike IgG levels did not significantly differ among participants who received different vaccine types. While a recent study indicated that the efficacy of AstraZeneca and Pfizer vaccines after the second dose was 67% and 93%, respectively, with mRNA-based vaccines showing the highest overall efficacy [31] the present study found no significant difference in antibody levels among vaccine types. Although the Oxford-AstraZeneca vaccine elicited the lowest median Spike IgG level after two doses, its antibody response was comparable to other vaccines when three doses were administered, showing no significant difference from mRNA-based vaccines. This discrepancy may be attributed to the relatively small cohort size in the present study. Overall, the findings suggest that all vaccines were effective in inducing antibodies against the virus, regardless of the vaccine brand or number of doses received.

The mixed-vaccine approach, combining different COVID-19 vaccines, has been associated with strong immune responses. Unlike some reports, heterologous vaccines did not enhance IgG levels in our cohort [8, 9].

The COVID-19 pandemic resulted in varied clinical outcomes, from severe illness and hospitalization to asymptomatic cases, raising questions about immune response differences. This study found that symptomatic individuals had significantly higher Spike IgG antibody levels compared to asymptomatic individuals ($p = 0.0004$), suggesting a hyperactive immune response that may contribute to severe disease. Research has linked immune hyperactivation in COVID-19 to potential long-term effects, such as long COVID, marked by ongoing symptoms post-infection. This aligns with findings that asymptomatic individuals generally exhibit lower anti-spike IgG levels [32].

Additionally, the correlation between COVID-19 outcomes and antibody protection from vaccination was weakly positive ($r = 0.128$) and not significant between symptomatic and asymptomatic individuals previously infected with COVID-19 ($p = 0.078$), which is in line with findings from Feng, et al. [33]. This suggests that while higher antibody levels are associated with symptomatic cases, the relationship between antibody levels and protection from severe outcomes remains complex and influenced by multiple factors.

Patient comorbidities are significant factors influencing COVID-19 outcomes and increasing mortality risk [34]. While comorbidities can affect immune function and vaccine responses, this study found that they did not significantly impact Spike IgG antibody levels in the Saudi cohort, regardless of prior infection. However, one participant, who had undergone a kidney transplant and was on immunosuppressive therapy, had undetectable antibody levels, highlighting impaired immune response.

Although hybrid immunity has been associated with enhanced antibody responses [35] our data showed unexpectedly higher IgG levels in individuals who were vaccinated without prior infection. This may be due to variability in the interval between natural infection and vaccination, which was not uniformly controlled in our cohort. It is possible that some individuals with hybrid immunity were infected earlier during the pandemic resulting in waning antibody levels by the time of sampling. Additionally, repeated antigen exposure may, in some cases, lead to immune tolerance or altered memory responses. These factors could explain the unexpected inverse trend observed in our study. This observation, taken from a Saudi cohort sampled at the end of the pandemic, suggests potential alteration in immune priming or waning that merit further investigation. It challenges the notion of hybrid immunity as universally superior and underscores the need for time-contextualized immune profiling.

5. Conclusion

Vaccinated individuals without prior infection showed higher IgG than those with hybrid immunity, suggesting altered immune dynamics. Age and sex affected responses, while vaccine type, dose, and comorbidities did not. These end-pandemic findings highlight the need for time-sensitive immune profiling and challenge the presumed superiority of hybrid immunity.

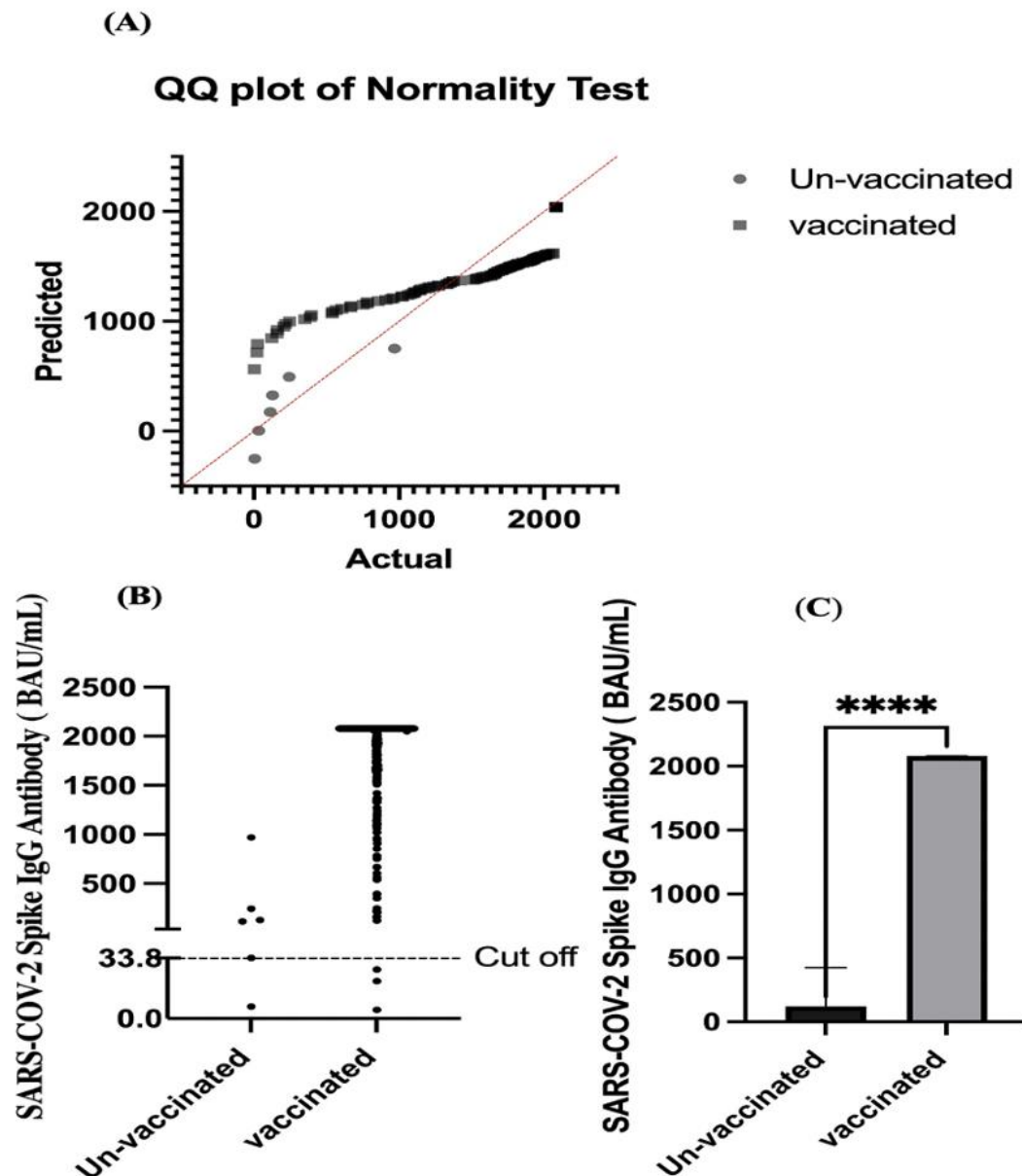


Figure 1. SARS-CoV-2 Spike IgG Antibody Levels Among Vaccinated and Unvaccinated Groups: (A) QQ normality test shows a significant deviation from the reference normal distribution line, indicating that the data is not normally distributed. (B) Individual Spike IgG antibody levels are plotted on the y-axis in BAU/mL, with a seropositivity threshold is indicated by a horizontal dotted line. (C) Median and interquartile range (IQR) of antibody levels are represented, with statistical comparison performed using the Mann–Whitney test. **** $P < 0.0001$.

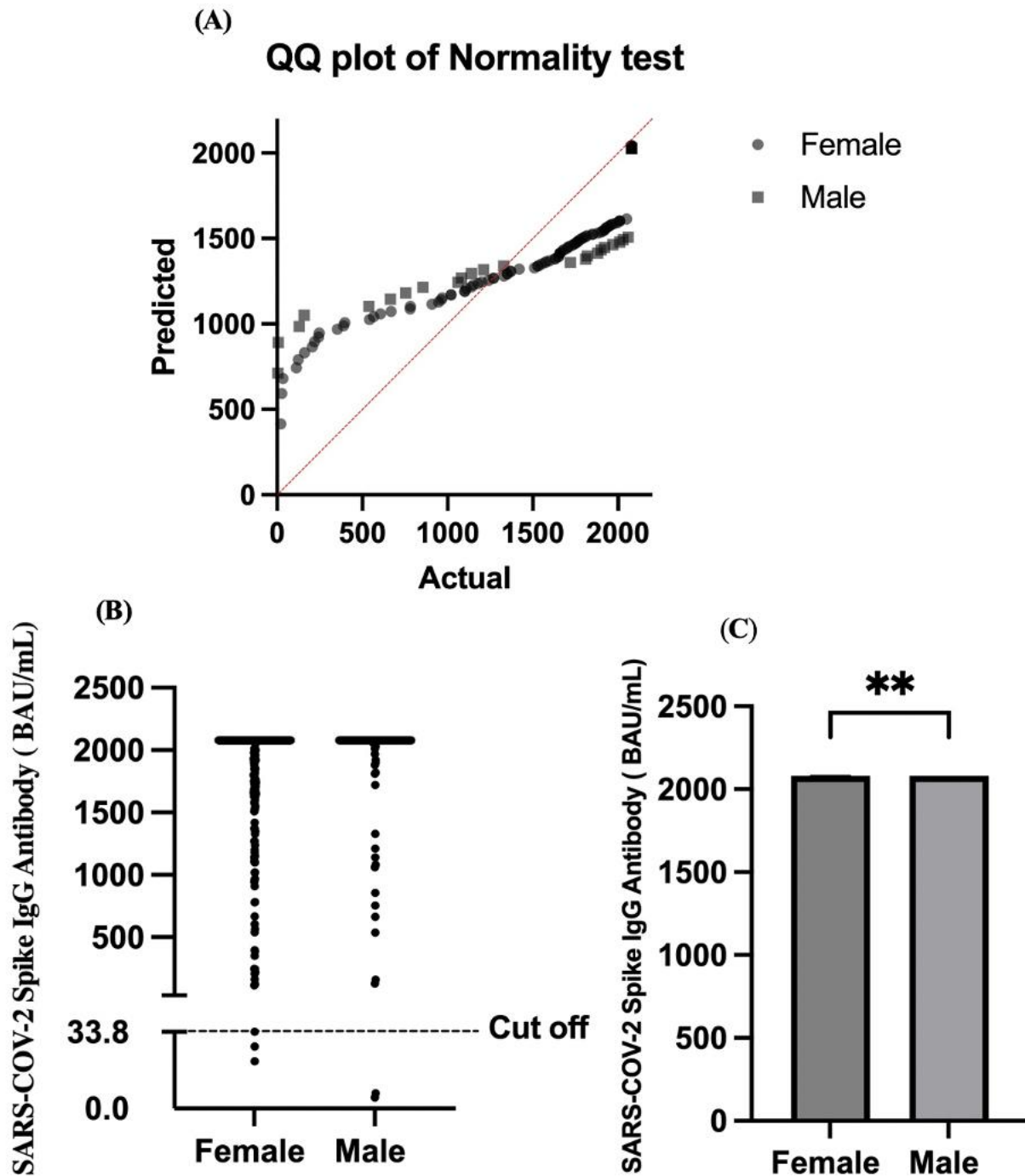


Figure 2. SARS-CoV-2 Spike IgG Antibody Levels by Sex: (A). plot of the normality test shows a significant deviation from the reference normal distribution line, indicating non-normally distributed data. (B) Individual Spike IgG antibody levels are shown as dots for both female and male groups. Antibody levels are plotted on the y-axis in BAU/mL, with a cutoff value of 33.8 BAU/mL (the horizontal dotted line). (C) Comparison of median Spike IgG antibody levels between female and male groups using the Mann–Whitney U test, demonstrated a significant P value of 0.0072.

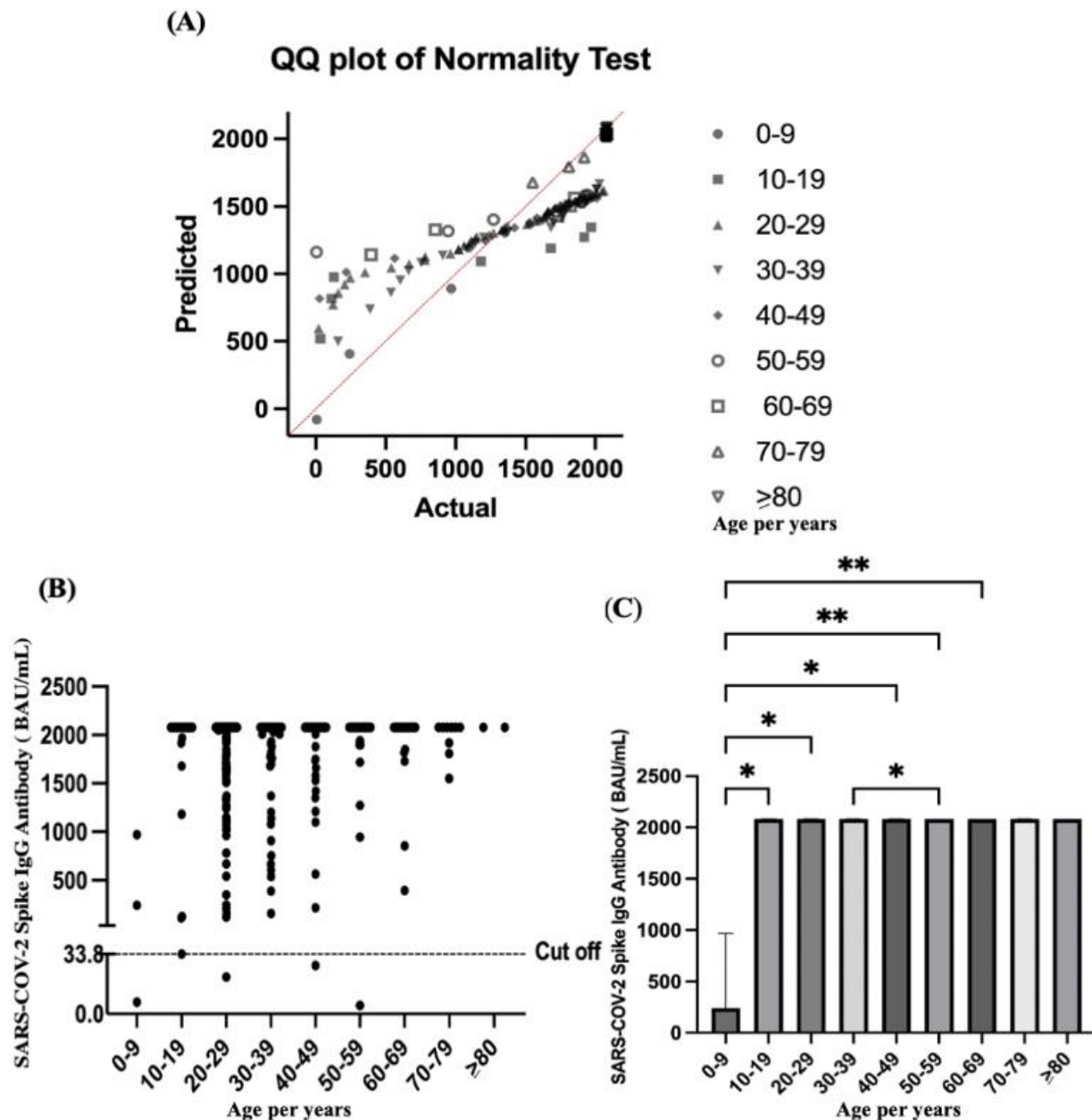


Figure 3.

Spike IgG Antibody Levels by Age Group: (A).QQ plot of the normality test shows a significant deviation from the reference normal distribution line, indicating non-normally distributed data. (B) Individual antibody levels for each age group are displayed as dots. Antibody levels are shown on the y-axis in BAU/mL. (C) Median antibody levels across age groups, analyzed using the Kruskal-Wallis test. Multiple age group comparisons were performed using Dunn's Test. Significance levels indicated are as follows: * $P = 0.0332$, ** $P = 0.0021$.

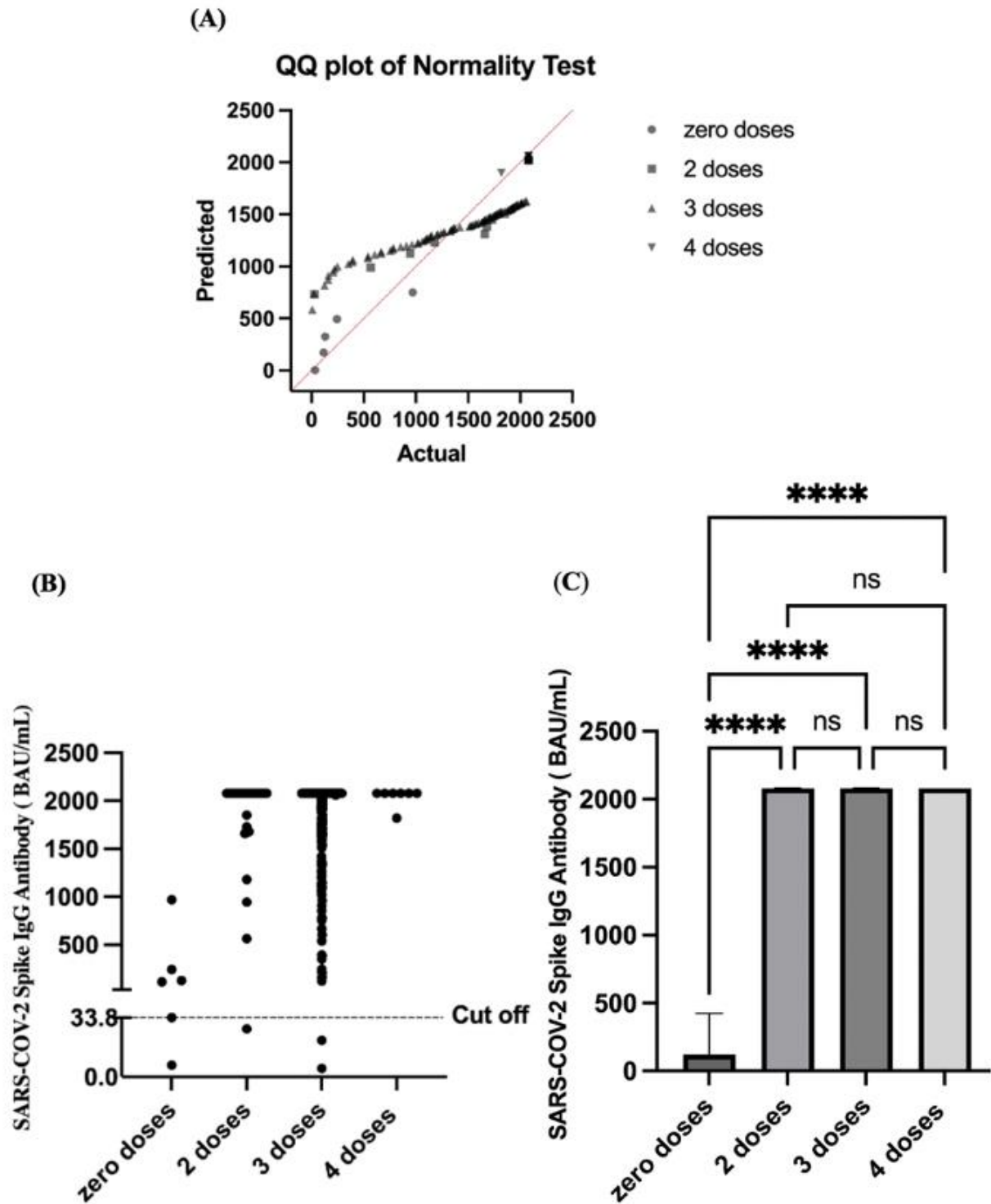


Figure 4.

Influence of Vaccine Doses on Spike IgG Antibody Levels.(A) QQ plot of the normality test shows a significant deviation from the reference normal distribution line, indicating non-normally distributed data. (B) Individual antibody levels are shown as dots across groups based on the number of vaccine doses received. Antibody levels are plotted on the y-axis in BAU/mL, with a cutoff value marked by a horizontal dotted line. (C) Median Spike IgG antibody levels with interquartile range (IQR) for each dose group, analysed using the Kruskal-Wallis test. Significance between dose groups were determined by Dunn's Test: **** $P < 0.0001$; ns, not significant.

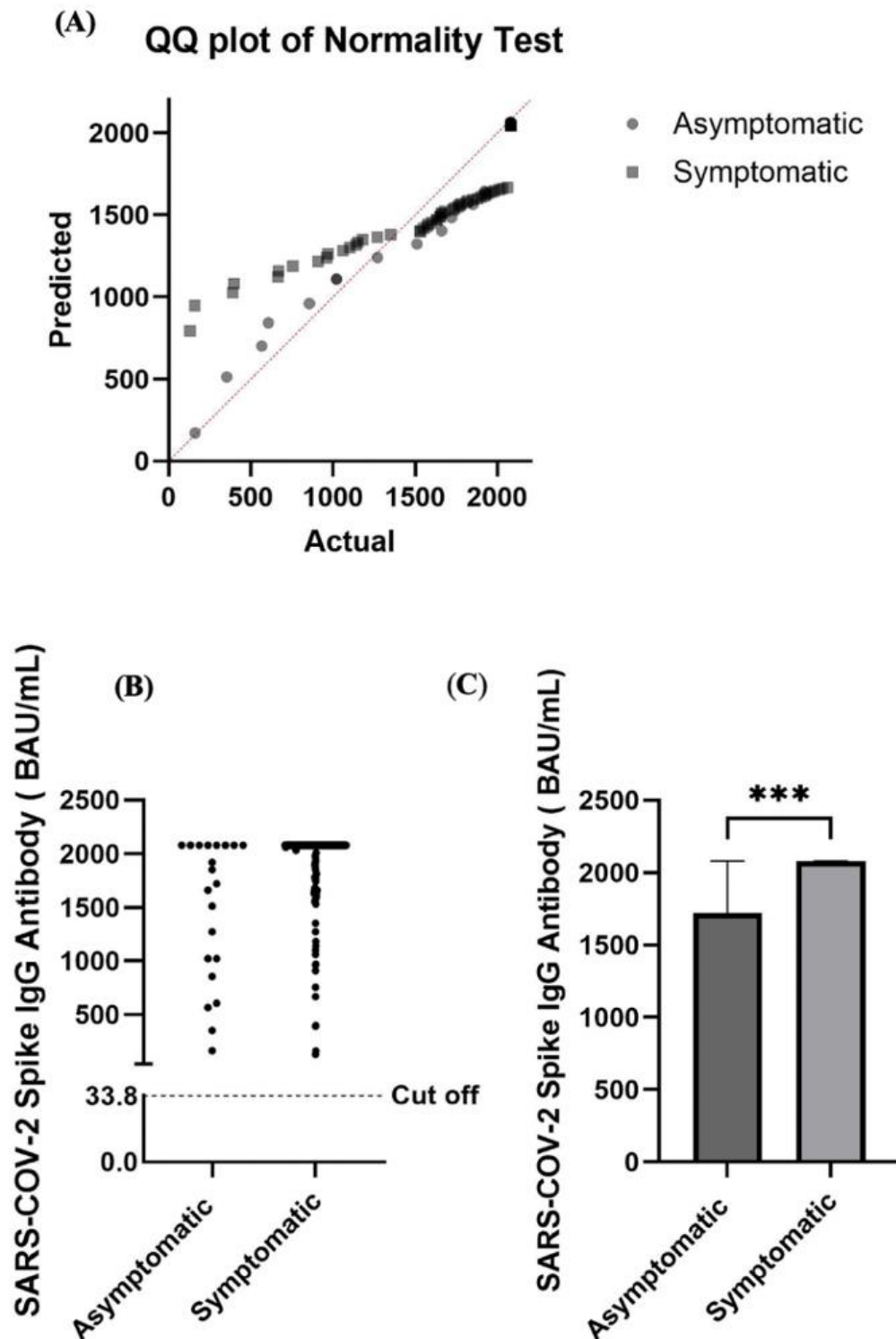


Figure 5.

COVID-19 Outcome and Spike IgG Antibody Levels. (A) QQ plot of the normality test showing a significant deviation from the reference normal distribution line, indicating non-normally distributed data. (B) Individual values (dots) of Spike IgG antibody levels among asymptomatic and symptomatic groups are presented. Antibody levels are displayed on the y-axis in BAU/mL, with the cutoff value marked by the dotted horizontal line. (C) Median Spike IgG antibody levels for asymptomatic and symptomatic groups. The results are presented as medians with interquartile ranges (IQR) and analysed using the Mann–Whitney U test. ***P-value = 0.0004.

References

- [1] R. da Rosa Mesquita *et al.*, "Clinical manifestations of COVID-19 in the general population: Systematic review," *Wiener Klinische Wochenschrift*, vol. 133, no. 7, pp. 377-382, 2021. <https://doi.org/10.1007/s00508-020-01760-4>
- [2] A. M. Carabelli *et al.*, "SARS-CoV-2 variant biology: Immune escape, transmission and fitness," *Nature Reviews Microbiology*, vol. 21, no. 3, pp. 162-177, 2023. <https://doi.org/10.1038/s41579-022-00841-7>
- [3] Z. Zhao, S. Bashiri, Z. M. Ziora, I. Toth, and M. Skwarczynski, "COVID-19 variants and vaccine development," *Viruses*, vol. 16, no. 5, p. 757, 2024. <https://doi.org/10.3390/v16050757>

- [4] S. Jiang, C. Hillyer, and L. Du, "Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses," *Trends in Immunology*, vol. 41, no. 5, pp. 355-359, 2020. <https://doi.org/10.1016/j.it.2020.03.007>
- [5] J. S. Kim *et al.*, "Demographic and clinical factors associated with SARS-CoV-2 Spike 1 antibody response among vaccinated US adults: the C4R study," *Nature Communications*, vol. 15, no. 1, p. 1492, 2024. <https://doi.org/10.1038/s41467-024-45468-9>
- [6] S. L. Klein, I. Marriott, and E. N. Fish, "Sex-based differences in immune function and responses to vaccination," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 109, no. 1, pp. 9-15, 2015. <https://doi.org/10.1093/trstmh/tru167>
- [7] K. L. Flanagan, A. L. Fink, M. Plebanski, and S. L. Klein, "Sex and gender differences in the outcomes of vaccination over the life course," *Annual Review of Cell and Developmental Biology*, vol. 33, pp. 577-599, 2017. <https://doi.org/10.1146/annurev-cellbio-100616-060718>
- [8] J. R. Spinardi and A. Srivastava, "Hybrid immunity to SARS-CoV-2 from infection and vaccination—evidence synthesis and implications for new COVID-19 vaccines," *Biomedicines*, vol. 11, no. 2, p. 370, 2023. <https://doi.org/10.3390/biomedicines11020370>
- [9] S. Pilz and J. P. Ioannidis, "Does natural and hybrid immunity obviate the need for frequent vaccine boosters against SARS-CoV-2 in the endemic phase?," *European Journal of Clinical Investigation*, vol. 53, no. 2, p. e13906, 2023. <https://doi.org/10.1111/eci.13906>
- [10] R. L. Atmar *et al.*, "Homologous and heterologous Covid-19 booster vaccinations," *New England Journal of Medicine*, vol. 386, no. 11, pp. 1046-1057, 2022. <https://doi.org/10.1056/NEJMoa2116414>
- [11] S. C. Moore *et al.*, "Evolution of long-term vaccine-induced and hybrid immunity in healthcare workers after different COVID-19 vaccine regimens," *Med*, vol. 4, no. 3, pp. 191-215, 2023. <https://doi.org/10.1016/j.medj.2023.02.004>
- [12] N. Chavarot *et al.*, "Weak antibody response to three doses of mRNA vaccine in kidney transplant recipients treated with belatacept," *American Journal of Transplantation*, vol. 21, no. 12, pp. 4043-4051, 2021. <https://doi.org/10.1111/ajt.16814>
- [13] C. Fu, N. Lin, J. Zhu, and Q. Ye, "Association between overweight/obesity and the safety and efficacy of COVID-19 vaccination: A systematic review," *Vaccines*, vol. 11, no. 5, p. 996, 2023. <https://doi.org/10.3390/vaccines11050996>
- [14] Y.-F. He *et al.*, "Correlation between COVID-19 vaccination and diabetes mellitus: A systematic review," *World Journal of Diabetes*, vol. 14, no. 6, p. 892, 2023. <https://doi.org/10.4239/wjd.v14.i6.892>
- [15] N. K. Alharbi *et al.*, "Nationwide seroprevalence of SARS-CoV-2 in Saudi Arabia," *Journal of Infection and Public Health*, vol. 14, no. 7, pp. 832-838, 2021. <https://doi.org/10.1016/j.jiph.2021.04.006>
- [16] F. Alasmari *et al.*, "Seroprevalence and longevity of SARS-CoV-2 nucleocapsid antigen-IgG among health care workers in a large COVID-19 public hospital in Saudi Arabia: A prospective cohort study," *PLoS One*, vol. 17, no. 8, p. e0272818, 2022. <https://doi.org/10.1371/journal.pone.0272818>
- [17] A. Marchant *et al.*, "Enabling the evaluation of COVID-19 vaccines with correlates of protection," *Biologicals*, vol. 85, p. 101723, 2024. <https://doi.org/10.1016/j.biologicals.2023.101723>
- [18] E. Barnes *et al.*, "SARS-CoV-2-specific immune responses and clinical outcomes after COVID-19 vaccination in patients with immune-suppressive disease," *Nature Medicine*, vol. 29, no. 7, pp. 1760-1774, 2023. <https://doi.org/10.1038/s41591-023-02414-4>
- [19] G. Joseph *et al.*, "Humoral immunity of unvaccinated COVID-19 recovered vs. Naïve BNT162b2 vaccinated individuals: A prospective longitudinal study," *Microorganisms*, vol. 11, no. 7, p. 1628, 2023. <https://doi.org/10.3390/microorganisms11071628>
- [20] L. N. Yamani *et al.*, "SARS-CoV-2 IgG antibody status in unvaccinated and 2-dose vaccinated Indonesians by AstraZeneca," *Journal of Public Health in Africa*, vol. 14, no. 12, p. 2697, 2023. <https://doi.org/10.4081/jphia.2023.2697>
- [21] S. H. Ra, J. S. Lim, G.-u. Kim, M. J. Kim, J. Jung, and S.-H. Kim, "Upper respiratory viral load in asymptomatic individuals and mildly symptomatic patients with SARS-CoV-2 infection," *Thorax*, vol. 76, no. 1, pp. 61-63, 2021. <https://doi.org/10.1136/thoraxjnl-2020-215042>
- [22] S. Panaro and M. Cattalini, "The spectrum of manifestations of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV2) infection in children: what we can learn from multisystem inflammatory syndrome in children (MIS-C)," *Frontiers in Medicine*, vol. 8, p. 747190, 2021. <https://doi.org/10.3389/fmed.2021.747190>
- [23] K. Zaher, F. Basingab, J. Alrahimi, K. Basahel, and A. Aldahlawi, "Gender differences in response to COVID-19 infection and vaccination," *Biomedicines*, vol. 11, no. 6, p. 1677, 2023. <https://doi.org/10.3390/biomedicines11061677>
- [24] S. A. Kharroubi and M. Diab-El-Harake, "Sex-differences in COVID-19 diagnosis, risk factors and disease comorbidities: A large US-based cohort study," *Frontiers in public health*, vol. 10, p. 1029190, 2022. <https://doi.org/10.3389/fpubh.2022.1029190>
- [25] F. X. Heinz and K. Stiasny, "Distinguishing features of current COVID-19 vaccines: knowns and unknowns of antigen presentation and modes of action," *npj Vaccines*, vol. 6, p. 104, 2021. <https://doi.org/10.1038/s41541-021-00369-6>
- [26] E. C. Abebe and T. A. Dejenie, "Protective roles and protective mechanisms of neutralizing antibodies against SARS-CoV-2 infection and their potential clinical implications," *Frontiers in immunology*, vol. 14, p. 1055457, 2023. <https://doi.org/10.3389/fimmu.2023.1055457>
- [27] Q. Li *et al.*, "Immune response in COVID-19: what is next?," *Cell Death & Differentiation*, vol. 29, no. 6, pp. 1107-1122, 2022. <https://doi.org/10.1038/s41418-022-01015-x>
- [28] A. M. Tizazu, H. M. Mengist, and G. Demeke, "Aging, inflammaging and immunosenescence as risk factors of severe COVID-19," *Immunity & Ageing*, vol. 19, no. 1, p. 53, 2022. <https://doi.org/10.1186/s12979-022-00309-5>
- [29] J. P. Farias *et al.*, "The fourth COVID-19 vaccine dose increased the neutralizing antibody response against the SARS-CoV-2 Omicron (B. 1.1. 529) variant in a diverse Brazilian population," *Microbiology Spectrum*, vol. 11, no. 6, pp. e02857-23, 2023. <https://doi.org/10.1128/spectrum.02857-23>
- [30] T. Kadowaki, A. Sasaki, N. Matsumoto, T. Mitsushashi, S. Takao, and T. Yorifuji, "Antibody titers after a third and fourth SARS-CoV-2 vaccine dose in Bizen City, Japan," *Journal of epidemiology*, vol. 33, no. 9, pp. 484-486, 2023. <https://doi.org/10.2188/jea.JE20230034>
- [31] M. Soheili *et al.*, "The efficacy and effectiveness of COVID-19 vaccines around the world: a mini-review and meta-analysis," *Annals of clinical microbiology and antimicrobials*, vol. 22, p. 42, 2023. <https://doi.org/10.1186/s12941-023-00594-y>

- [32] J. Dufloo *et al.*, "Asymptomatic and symptomatic SARS-CoV-2 infections elicit polyfunctional antibodies," *Cell Reports Medicine*, vol. 2, no. 5, p. 100275, 2021. <https://doi.org/10.1016/j.xcrm.2021.100275>
- [33] S. Feng *et al.*, "Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection," *Nature medicine*, vol. 27, pp. 2032-2040, 2021. <https://doi.org/10.1038/s41591-021-01540-1>
- [34] C. D. Russell, N. I. Lone, and J. K. Baillie, "Comorbidities, multimorbidity and COVID-19," *Nature medicine*, vol. 29, pp. 334-343, 2023. <https://doi.org/10.1038/s41591-022-02156-9>
- [35] E. Andreano *et al.*, "Hybrid immunity improves B cells and antibodies against SARS-CoV-2 variants," *Nature*, vol. 600, no. 7889, pp. 530-535, 2021. <https://doi.org/10.1038/s41586-021-04117-7>