

SARS-Cov-2 Seroprevalence among Non-Vaccinated Healthcare Workers In Saudi Arabia

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ABSTRACT

Background: This cross-sectional study among healthcare workers (HCWs) in Abha, Saudi Arabia, assessed the prevalence of SARS-CoV-2 antibodies to understand exposure and potential immunity during the COVID-19 pandemic.

Methods: Between January 2021 and February 2021, 489 asymptomatic, unvaccinated HCWs from hospitals and clinics participated. Serological testing for SARS-CoV-2 antibodies was performed using ELISA.

Results: The overall seropositivity rate was 18.6% (95% CI: 15.2–22.0%). Nurses showed the highest adjusted seropositivity at 22.8%, followed by laboratory staff (20.2%) and physicians (14.9%). Multivariate analysis revealed nurses (OR=6.7) and laboratory staff (OR=6.1) had significantly higher odds of seropositivity. No significant differences were found based on age, gender, obesity, contact with COVID-19 patients, or adherence to PPE.

Conclusions: The findings suggest that PPE alone is insufficient to prevent virus transmission. A comprehensive strategy including vaccination, regular testing, symptom monitoring, ongoing training, and institutional support is essential for reducing infection risk and ensuring a safer healthcare environment.

Keywords: COVID-19, Serosurvey, Unvaccinated, Healthcare Workers

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INTRODUCTION

The COVID-19 pandemic has indeed placed unprecedented stress on healthcare systems globally, revealing vulnerabilities that were often downplayed in pre-pandemic assessments. The acute shortage of healthcare workers (HCWs) was exacerbated by several factors such as increased morbidity and mortality, burnout and mental health strain, inadequate healthcare resources, changing patient needs and global disparities.¹

HCWs face a heightened risk of contracting COVID-19 due to their close proximity to infected patients, particularly those with severe symptoms who are more likely to be hospitalized.²

A multi-center study highlights important findings about the impact of COVID-19 on healthcare workers during the first wave of the pandemic in Saudi Arabia. With healthcare professionals being at a higher risk of exposure to the virus, the statistic indicating that they represented 12.5% of all laboratory-confirmed positive cases is significant. Additionally, the fact that 9.3% of these cases were asymptomatic underscores the importance of robust testing and monitoring protocols, as asymptomatic individuals can still spread the virus to patients and colleagues.³

Asymptomatic workers can indeed contribute significantly to the transmission of infectious diseases, including respiratory viruses and other pathogens. Robust testing programs that encompass both symptomatic and asymptomatic healthcare workers are essential for effective disease control and the safety of healthcare environments.^{3,4} A review conducted in China indicated that asymptomatic individuals may represent around 40% of SARS-CoV-2 cases and have the potential to spread the virus to others over an extended duration, possibly exceeding 14 days.⁵ A cross-sectional study assessed the prevalence of IgG antibodies among HCWs in a second-level teaching hospital in Spain.⁶ Nearly 61.6% of the Spanish healthcare workers tested positive for IgG antibodies, indicating prior exposure to the virus (presumably SARS-CoV-2).

There were no significant differences in IgG positivity rates based on age, sex, or history of previous diseases among the workers. Notably, 48.5% of the Spanish workers who tested positive for IgG antibodies reported not having had any previous symptoms related to the virus.

The standard diagnostic test for identifying COVID-19 infection is the reverse transcription-polymerase chain reaction (RT-PCR). However, WHO recommended that antibodies testing for COVID-19 is essential for understanding the extent and prevalence of COVID-19 infections.⁷

The prevalence of infections among healthcare workers (HCWs) as determined by antibody tests varies widely in different regions.⁸⁻¹⁰ The implementation of safety measures, such as vaccination pro-

grams and training on infection prevention, can vary widely between regions, further contributing to the differences in prevalence rates. By understanding these factors, healthcare facilities can better strategize their approach to protecting HCWs and managing infection control effectively.^{1,11}

Seroprevalence uncovered a high rate of infection previously unnoticed among HCWs. Non suspected COVID-19 patients and asymptomatic HCWs may be relevant sources for nosocomial SARS-CoV-2 transmission. Also, identifying the HCWs' seropositivity of SARS-CoV-2 antibodies is vital for understanding the extent of the spread of COVID-19 among HCWs and assessing the success of infection alleviation measures in healthcare settings.¹ A 2022-2023 study in Yemen found a high SARS-CoV-2 antibody prevalence among HCWs, with 67.7% (268/396) seropositive, reflecting limited vaccination and PPE access. No significant sex difference ($P=0.29$) was noted, but seropositivity varied by occupation and workplace factors.¹² In Lebanon, among 92 HCWs, 72.3% received PPE training, over 70% adhered to PPE, and 80% were vaccinated; nurses experienced higher exposure, with 28.6% infected.¹³ Vaccinated HCWs showed higher anti-S IgG titers than unvaccinated, previously infected ones ($P=0.0043$). In Saudi Arabia, seropositivity was 26.5%, higher among non-Saudi HCWs and those with longer COVID-19 contact (both $P < 0.01$).¹⁴

This study aimed to assess the seroprevalence of COVID-19 among healthcare workers (HCWs) in southwestern Saudi Arabia, focusing on asymptomatic, non-vaccinated individuals. By measuring exposure rates, the research sought to identify factors influencing susceptibility to SARS-CoV-2 within this group. Understanding these determinants can inform targeted interventions and preventive strategies to protect HCWs and their patients. Additionally, evaluating seroprevalence provides insights into the extent of natural infection, emphasizing the importance of ongoing surveillance, infection control measures, and vaccination efforts. The study specifically excluded vaccinated HCWs to avoid confounding seropositivity from vaccine-induced antibodies, ensuring that the detected antibodies accurately reflected prior or natural infection rather than immunization. The findings underscore the need for comprehensive approaches including regular testing, personal protective measures, and vaccination to mitigate virus transmission in healthcare settings and ensure a safer environment for both staff and patients.

METHODOLOGY

Design: A cross-sectional study was conducted in the Aseer region, Southwestern Saudi Arabia.

Description of the study area: Aseer region is located in the southwest of Saudi Arabia, bordering the northwestern part of Yemen. The area extends from high mountains chain called Sarawat down to the

eastern coast of the Red Sea. In most of these areas, scattered are several inhabited cities, towns, and villages. Health care facilities in the study area include 23 hospitals and 247 PHCCs.

Target Population and sampling method: A sample of 489 health care workers (HCWs) was included. The sample size was estimated based on the average prevalence of COVID-19 antibodies of 70%¹⁵, with a precision of 6% at a 95% confidence level and design effect of 2. A precision of 6% was chosen to balance feasibility with statistical power, given the expected high prevalence (70%) and resource constraints in recruiting HCWs, unlike standard 5% which would require a larger sample.

The sample size was calculated using Epi-Info 7 software. The sample was collected from Abha metropolitan areas, including Abha, Khamis Mushait and Ahad Rufeida. The sample included hospitals (Aseer Central Hospital, Khamis Mushait General and Pediatric hospitals, Ahad Rufeida Hospital, and King Khalid University outpatients' clinics) and primary health care centers in the chosen areas. A consecutive sampling technique was used of accessible HCWs until the required sample size was fulfilled. HCWs included physicians, dentists, nurses, laboratory technicians, and pharmacists. The sample was selected using weighted distribution according to each health facility's included staff.

Inclusion criteria: All healthcare workers in the selected study areas.

Exclusion criteria: History of confirmed COVID-19 infections or vaccination and any symptoms suggestive of current covid-19 infection (fever, chills, myalgia, ageusia, fatigue, anosmia, cough, and shortness of breath).¹⁶

Data collection: The study field teams arranged scheduled visits to the selected centers.

All individuals enrolled in the study were requested to fill out a questionnaire that collected details on their demographic background, clinical history, and exposure-related information.

The questionnaire included 15 items covering various domains: demographics (age, gender, body mass index), occupational exposure (job title, department, contact with known COVID-19 patients), and adherence to personal protective equipment (PPE). Adherence to PPE was assessed through self-reported frequency on a scale: always, often, sometimes, never.

Serological Assay Details: Commercially available indirect ELISA kits (Diapro, Milano, Italy) were used for the detection of COVID-19 specific IgG, IgM and IgA antibodies. The ELISA testing was performed using the Diapro COVID-19 IgG/IgM/IgA ELISA kit (Catalog Number: DP-CO19-01), with a sensitivity and specificity of 98%.

Definition of Confirmed COVID-19 Cases: Participants with confirmed COVID-19 infections were

those with a positive reverse transcription polymerase chain reaction (RT-PCR) test result for SARS-CoV-2, conducted according to standard diagnostic criteria. Individuals with prior RT-PCR confirmed infection were excluded from the seroprevalence analysis to focus on undiagnosed or asymptomatic cases.

Measurement of Protective Measures Adherence: Non-adherence was quantified based on self-reported responses, with participants categorized as non-adherent if they reported 'sometimes' or 'never' using masks, gloves, or practicing hand hygiene during patient interactions. This stratification facilitated analysis of the relationship between PPE compliance and seropositivity.

Laboratory Quality Control: To ensure reliability of serological results, all ELISA tests were performed in duplicate, and inter-assay variability was monitored by including positive and negative controls in each run. The laboratory staff adhered to strict standard operating procedures, and any discrepancies between duplicates were resolved by retesting. The laboratory participated in external quality assurance programs to maintain assay accuracy.

Laboratory evaluations: Laboratory and biosafety guidance for COVID-19 was followed. Five ml of venous blood was aseptically collected from each participant into tubes with gel and clot activator (Improve, Hamburg, Germany). Blood samples were left for 30 minutes at room temperature to clot and centrifuged at 1000g for 10 minutes. Serum samples were aliquoted and kept at -80°C. Before the test, aliquots were thawed and put on ice until the test time.

Serum samples were tested for COVID-19-specific antibodies (IgG, IgM, IgA) using ELISA. Diluted sera (1:20 for IgM, 1:40 for IgG and IgA) were added to microtiter plates with neutralizing or DILAS solutions, along with negative and positive controls in triplicate, and a blank well for substrate only. Plates were sealed and incubated at 37°C for 45 minutes, then washed five times with an automated washer. Subsequently, 100 µl of HRP-conjugated anti-IgG, IgM, or IgA was added, and plates incubated again at 37°C for 45 minutes. After washing, 100 µl of substrate was added and incubated for 15 minutes at room temperature, followed by 100 µl of stop solution. Optical densities were measured at 450/620 nm with an ELISA reader, and results were expressed as antibody ratios relative to the cutoff value.

Data Analysis: After data were extracted, it was revised, coded, and fed to statistical software IBM SPSS version 24 (SPSS, Inc. Chicago, IL). All statistical analysis was done using two-tailed tests. P-value less than 0.05 was statistically significant. The frequency and percent distribution of descriptive analysis was done for all variables, including HCWs socio-demographic data, infection control measures, job title, screening results, and Sero-prevalence. Asymptomatic SARS-CoV-2 infection was defined as evident seroconversion in the absence of reported symptoms

suggestive of COVID-19.¹⁶ Adjusted seropositive prevalence among health care workers was calculated besides crude prevalence to account for screening test sensitivity and specificity as the test validity measures are not 100% with some probability for false-positive and false-negative results.¹⁷ crosstabulation was done to test some relations with serological findings among HCWs, such as Sero-positivity with screening results. The significance of relations was tested using an exact probability test for small frequency distribution. To identify the most significant predictors for being Sero-positive, with crude multivariate relation using crude odds ratio with its 95% CI. A multiple logistic regression model was applied to detect the adjusted odds ratio for Sero-positivity among HCWs. Model calibration and fit were tested based on the Sero-positivity classification accuracy of HCWs and Hosmer-Lemshow Test for model goodness of fit. The model demonstrated acceptable goodness of fit, with a Hosmer-Lemeshow p-value <0.05, indicating significant difference between observed and predicted outcomes and vice versa.

Ethical Consideration: Ethical approval was obtained from King Khalid Research Ethics Committee (ECM#2020-1206). Acceptance of the relevant health authorities was attained before the study (REC-15-11-2020). Informed consent was obtained from all individuals willing to participate in the study before any procedure.

RESULTS

Description of the study sample: During the study period which lasted for two months in the early outbreak period in Saudi Arabia (from the first of January 2021 to the end of February 2021) a total of 489 asymptomatic non-vaccinated health care workers (HCWs) were included in the present study. Table 1 shows their biodemographic data. Their ages ranged from 22 to 60 years with an average of 35.5 ± 9.1 years. The sample included 270 (55.2%) females. Saudi HCWs were 57.3% (280). As for job title, 254 (51.9%) were nurses, 143 (29.2%) were physicians, 39 (8%) were dentists, 37 (7.6%) work at labs, and 16 HCWs (3.3%) were pharmacists. A total of 236 (48.3%) HCWs worked in direct contact with covid patients, and only 59 (12.1%) had contact with confirmed covid case. Ninety-four (19.2%) HCWs reported travelling outside their residence areas in the previous seven months. Wearing mask was reported by 467 (95.5%) HCWs, 454 (92.8%) adhere to social distancing at public areas and work, and 465 (95.1%) sterilize hands with soap/disinfectant at public areas.

Sero-positivity of Covid-19 antibodies among asymptomatic non-vaccinated HCWs: Table 2 shows sero-positivity of Covid-19 antibodies among asymptomatic non-vaccinated HCWs in Aseer region, Saudi Arabia. A total of 404 (82.6%) HCWs were seronegative for all antibodies.

Table 1: Bio-demographic data of asymptomatic non-vaccinated Health Care Workers in Aseer region, Saudi Arabia (n=489)

Bio-demographic data	Participants(%)
Age in years	
20-29	123 (25.2)
30-39	242 (49.5)
40-49	86 (17.6)
50+	38 (7.8)
Gender	
Male	219 (44.8)
Female	270 (55.2)
Nationality	
Saudi	280 (57.3)
No-Saudi	209 (42.7)
Job title	
Dentist	39 (8)
Physician	143 (29.2)
Pharmacist	16 (3.3)
Nursing	254 (51.9)
Laboratory	37 (7.6)
Work in direct contact with covid patients?	
Yes	236 (48.3)
No	253 (51.7)
Had contact with confirmed covid case?	
Yes	59 (12.1)
No	430 (87.9)
Travelled outside your residence area last 7 months	
Yes	94 (19.2)
No	395 (80.8)
Body mass index	
Underweight	12 (2.5)
Normal weight	209 (42.7)
Overweight	161 (32.9)
Obese	107 (21.9)
Wearing mask	
Yes	467 (95.5)
No	22 (4.5)
Adhere to social distancing at public areas and work	
Yes	454 (92.8)
No	35 (7.2)
Sterilize hands with soap / disinfectant at public areas	
Yes	465 (95.1)
No	24 (4.9)
Co-morbidities	
DM	16 (3.3)
Respiratory disease	16 (3.3)
Cardiac diseases	7 (1.4)
Hypertension	20 (4.1)

Table 2: Sero-positivity of Covid-19 antibodies among asymptomatic non-vaccinated Health Care Workers in Aseer region, Saudi Arabia (n=489)

Sero-positivity	Participants (%)
Seronegative#	404 (82.6)
Exclusive IgM positive	5 (1)
Exclusive IgG positive	24 (4.9)
Exclusive IgA positive	12 (2.5)
IgM & IgG positive	21 (4.3)
IgM & IgA positive	2 (0.4)
IgG & IgA positive	9 (1.8)
IgM & IgG & IgA positive	12 (2.5)
#Negative for all antibodies	

Table 3: Crude and adjusted Sero-positivity of Covid-19 antibodies among asymptomatic non-vaccinated Health Care Workers in Aseer region, Saudi Arabia

Sero-positivity	Positive cases	Crude prevalence (95% CI)	Adjusted prevalence (95% CI) *
IgG +ve	66	13.5% (10.4-16.5%)	14.4% (11.3-17.5%)
IgM +ve	40	8.2% (5.7-10.6%)	8.7% (6.1-11.2%)
IgA +ve	35	7.2% (4.9-9.5%)	7.6% (5.3-9.9%)
Sero-positive #	85	17.4% (14.0-20.8%)	18.6% (15.2-22.0%)

#Positive for any antibody (IgM, IgG, or IgA)

*Adjusted for screening test sensitivity and specificity

Table 4: Crude and adjusted Seroprevalence of Covid-19 antibodies among asymptomatic non-vaccinated Health Care Workers by their job title in Aseer region, Saudi Arabia

Job title	Positive cases #	Crude prevalence (95% CI)	Adjusted prevalence * (95% CI)
Physician	20	14.0% (8.2-19.7%)	14.9% (9.1-20.7%)
Dentist	2	5.1% (0.0-12.4%)	5.4% (0.0-12.5%)
Pharmacist	2	12.5% (0-30.7%)	13.3% (0.0-29.9%)
Nursing	54	21.3% (16.2-26.3%)	22.8% (17.6-28.0%)
Laboratory	7	18.9% (5.6-32.2%)	20.2% (7.3-33.1%)

#Positive for any antibody (IgM, IgG, or IgA)

*Adjusted for screening test sensitivity and specificity

As for Sero-positive HCWs; IgM was exclusively positive among 5 (1%) HCWs, IgG was exclusively positive among 24 (4.9%) HCWs, IgA was exclusively positive among 12 (2.5%) HCWs, 21 (4.3%) had IgM & IgG positive results, 9 (1.8%) had IgG & IgA positive results, 2 (0.4%) had IgM & IgA positive, and 12 (2.5%) had IgM & IgG & IgA positive results.

Table 3 shows the crude sero-positivity and adjusted sero-positivity of Covid-19 antibodies among asymptomatic non-vaccinated HCWs in Aseer region, Saudi Arabia. The adjusted prevalence for IgG antibodies among HCWs was 14.4% (95% CI: 11.3-17.5%), IgM antibodies was 8.7% (95% CI: 6.1-11.2%), IgA antibodies prevalence was 7.6% (95% CI: 5.3-9.9%). Totally, Sero-positivity was found among 18.6% (95% CI: 15.2-22.0%) of asymptomatic non-vaccinated HCWs.

Table 4 the Crude and adjusted Seroprevalence of Covid-19 antibodies among asymptomatic non-vaccinated HCWs by their job title in Aseer region, Saudi Arabia. The highest adjusted seropositivity was found among nurses (22.8%; 95% CI: 17.6-28.0%), followed by lab staff (20.2%; 95% CI: 7.3-33.1%), and Physicians (14.9%; 95% CI: 9.1-20.7%). The lowest adjusted seropositivity was found among pharmacists (13.3%; 95% CI: 0.0-29.9%) and Dentists (5.4%; 95% CI: 0.0-12.5%).

Determinants of Sero-positivity of Covid-19 antibodies among asymptomatic non-vaccinated HCWs: Table 5 shows Multivariate analysis of different risk factors of Sero-positivity among HCWs in Aseer region, Saudi Arabia. Among all included predictors, only job title showed significant relation with Sero-positivity among HCWs. Nurses showed about 7 times more likelihood for covid-19 Sero-positivity than dentists (OR=6.7; 95% CI: 1.3-34.4). Similarly, laboratory HCWs staff showed 7 times more likelihood for Sero-positivity compared to dentists (OR=6.1; 95% CI: 1.1-36.5). Other potential determinants including age, gender, nationality, BMI, work in

direct relation with Covid 19 patients and non-adherence to protective measure were found to be non-significant. The model calibration was more than satisfactory where classification accuracy was 82.6% and Hosmer-Lemshow test showed statistical insignificance which mean acceptable model goodness of fit.

DISCUSSION

Following the identification of Saudi Arabia's initial COVID-19 case on March 2, 2020, the country saw a rise in confirmed infections and their contacts across various regions. By August 23, 2020, the total confirmed cases had reached 307,479, with 3,649 reported fatalities. Concurrently, the government expanded testing efforts, implemented preventive strategies, and enhanced preparedness measures to curb the transmission of the virus, leading to a total of 280,143 recoveries.¹⁸ The Saudi Food and Drug Authority approved the first COVID-19 Vaccine on September 1, 2020 and the first vaccine distribution for adults was launched on December 10, 2020.^{19,20}

The present study evaluated the prevalence of sero-positivity to SARS-CoV-2 infection among asymptomatic non-vaccinated HCWs in southwest Saudi Arabia. The study was conducted during the COVID-19 pandemic between January 1, 2021 and the end of February 2021. It showed seropositivity of 18.6% (95% CI: 15.2-22.0%). This result indicates that at least one in every six non-vaccinated healthcare workers has acquired asymptomatic COVID-19 infection within one year of the pandemic, therefore, demonstrating positive antibodies.

The asymptomatic infection rate for COVID-19 helps conclude the actual infection rate, particularly since PCR-based diagnostic testing is only indicated for HCWs with COVID-19 suggestive symptoms.²¹ Based on the results of other studies, the asymptomatic infection rate ranges between 18%-to- 45% in different populations.^{5,22-24}

Table 5: Multivariate analysis of factors associated with Covid-19 Sero-positivity among asymptomatic non-vaccinated Health Care Workers in Aseer region, Saudi Arabia

Category	Sero-positive N (%)	Sero-negative N (%)	COR (95% CI)	AOR (95% CI)	P-value
Age in years					
20–29	22 (17.9)	101 (82.1)	1	1	0.608
30–39	44 (18.2)	198 (81.8)	1.1 (0.58–1.79)	1.1 (0.57–1.85)	
40–49	11 (12.8)	75 (87.2)	0.67 (0.31–1.47)	0.66 (0.27–1.64)	
50+	8 (21.1)	30 (78.9)	1.2 (0.49–3.01)	1.1 (0.36–2.95)	
Gender					
Male	34 (15.5)	185 (84.5)	1	1	0.340
Female	51 (18.9)	219 (81.1)	1.3 (0.78–2.04)	1.2 (0.68–2.03)	
Nationality					
Saudi	46 (16.4)	234 (83.6)	1	1	0.548
Non-Saudi	39 (18.7)	170 (81.3)	1.2 (0.73–1.86)	1.3 (0.75–2.12)	
Body Mass Index					
Non-obese	38 (17.2)	183 (82.8)	1	1	0.938
Obese	47 (17.5)	221 (82.5)	1.02 (0.64–1.64)	1.12 (0.68–1.84)	
Direct COVID Contact					
Yes	43 (18.2)	193 (81.8)	1.11 (0.70–1.78)	1.2 (0.66–1.92)	0.720
No	42 (16.6)	211 (83.4)	1	1	
Contact with Confirmed Case					
Yes	12 (20.3)	47 (79.7)	1.3 (0.63–2.47)	1.2 (0.68–2.51)	0.582
No	73 (17.0)	357 (83.0)	1	1	
Travel History					
Yes	13 (13.8)	81 (86.2)	0.72 (0.38–1.36)	0.80 (0.40–1.59)	0.365
No	72 (18.2)	323 (81.8)	1	1	
Wearing Mask					
Yes	82 (17.6)	385 (82.4)	1.3 (0.39–4.66)	1.18 (0.25–5.46)	0.780
No	3 (13.6)	19 (86.4)	1	1	
Social Distancing					
Yes	80 (17.6)	374 (82.4)	1.3 (0.48–3.41)	0.97 (0.28–3.41)	0.817
No	5 (14.3)	30 (85.7)	1	1	
Hand Sterilization					
Yes	82 (17.6)	383 (82.4)	1.5 (0.44–5.14)	1.3 (0.31–5.57)	0.782
No	3 (12.5)	21 (87.5)	1	1	
Job Title					
Dentist	2 (5.1)	37 (94.9)	1	1	0.052
Physician	20 (14.0)	123 (86.0)	3.0 (0.67–13.41)	3.6 (0.71–17.84)	
Pharmacist	2 (12.5)	14 (87.5)	2.6 (0.34–20.61)	4.0 (0.47–33.80)	
Nursing	54 (21.3)	200 (78.7)	5.0 (1.17–21.38) *	6.7 (1.3–34.4) *	
Laboratory	7 (18.9)	30 (81.1)	4.3 (0.83–22.33)	6.1 (1.1–36.5) *	

COR: Crude Odds Ratio; AOR: Adjusted Odds Ratio; CI: Confidence interval

HCWs with asymptomatic COVID-19 infection are an essential source of nosocomial infection and could disseminate infection to their families and communities.²⁵ Studies suggest that infected persons without symptoms, comprising pre-symptomatic and asymptomatic subjects, account for more than 40% of all SARS-CoV-2 transmission.²⁶ Therefore, it suggested that HCWs exposed to COVID-19 infection undertake regular serological testing and symptom checking for proper infection control.^{27,28}

When comparing our study results with the broad national study that evaluated the SARS-CoV-2 seroprevalence in Saudi Arabia, the overall seroprevalence of antibodies in HCWs in our study is greater than the 11% prevalence in the general population of Saudi Arabia.²⁹ This indicates a significant occupational risk of exposure to SARS-CoV-2 in healthcare settings.³⁰

Our result of 18.6 % SARS-CoV-2 seropositivity among asymptomatic non-vaccinated HCWs is within

the range of prevalence reported in other similar studies which shows a high diversity; 11.9%³¹, 12.1%²⁸, 14.0%³², 17.1%³³, 27.2%³⁴ and 45.1%³⁵. Our 18.6% seropositivity is lower than 45.1% in Nigeria³² and 27.2% in Italy but higher than 11.9% in Italy³¹, 12.1% in Netherlands²⁸, 14.0% in Brazil³², 17.1% in United Kingdom³³ likely due to differences in PPE availability and community transmission rates.

This variation may be explained by the fact that seropositivity is markedly affected by various factors. These factors include; variation in the study periods and settings, sociodemographic criteria of the study participants, exposure risk, working conditions, working load, and degree of compliance with infection control policies and PPE.^{36–38} Also, the high prevalence of asymptomatic cases in the present study and the other similar studies could partially be related to the possibility that HCWs might have underestimated mild symptoms or attributed the symptoms to job fatigue, stress, and tiredness.²³

The study examined some demographics and occupational factors associated with seropositivity. Although being male, advanced age, and obese are risk factors for COVID-19 disease severity³⁹, our study did not find a risk difference of seropositivity by age, gender, and obesity. Similarly, other studies observed no risk difference by age and sex³¹ and BMI⁴⁰.

Interestingly, the present study demonstrated no difference in the risk of seropositivity between HCWs at direct and indirect contact with COVID-19 patients. The reasons for this remain unclear, but a possible explanation is that SARS-CoV-2 transmission might occur in pre-symptomatic or asymptomatic patients and colleagues.²⁶ Additionally, several employees in low contact risk might be infected in their community.⁴¹ A study in the United States examined the seroprevalence in HCWs with different risks of exposure to COVID-19 cases observed a no-risk difference with a greater level of exposure to COVID-19 patients. This finding possibly suggests that adherence to PPE use effectively prevented transmission to HCWs.⁴²

Early in the pandemic, Saudi Arabia has imposed several strict measures to minimize the risk of COVID-19 nosocomial infection, including; intensified awareness about the proper use of PPE, hand hygiene compliance, and decreasing the possibility of patient care exposures. These actions might be a cumulative experience from MERS epidemics that helped Saudi Arabia have good hospital infection control policies and measures.¹⁸ In the present study, the majority of HCWs always wear a face mask (95.5%) and adherence to social distancing at public areas and work (92.8%), and hand hygiene (95.1%).

Our results of seropositivity prevalence of SARS-CoV-2 according to job title revealed that the most affected group was nurses (22.8%), followed by laboratory staff (20.2%), and physicians (14.9%). By multivariate logistic regression, nurses were independently associated with the highest odds of seropositivity. Other studies indicate that nurses are among the most common HCWs infected with COVID-19.^{23,43} These results may suggest that the risk of acquiring SARS-CoV-2 infection in the workplace increased by the duration of exposure and close contact with COVID-19 patients. Compared to physicians, nurses spend prolonged time in patients' rooms and often have more immediate and direct contact.⁴⁴ No association between PPE adherence and the outcomes was observed. This could suggest underreporting of non-adherence, as self-reported data may be subject to social desirability bias, or it may reflect uniformly high compliance levels (estimated at 95%), which could mask potential associations (a phenomenon known as a ceiling effect). To obtain more accurate and objective assessments of PPE adherence, future studies should incorporate observational audits or direct monitoring methods. These approaches can help accurately capture adherence behaviors and better elucidate their relationship with outcomes. In the present study and unlike the other studies^{35,45}, the laboratory technicians were at risk of COVID-19

infection greater than the physicians. The exact reason for this remains unclear. However, a possible explanation is the role of laboratory staff, who were recruited in the present, in phlebotomy and blood sampling services of COVID-19 patients. Such a role makes them at a decreased distance from the patients. Besides the use of only surgical masks for protection, this close contact put them at a higher risk of exposure to SARS-CoV-2 transmission.⁴⁶

Although the proximity of individuals during dental procedures and the generation of aerosols, dentists are at high risk of transmission of COVID-19, our findings showed that the recruited dentists had the slightest chance of seropositivity. The possible explanation is that from March 20, 2020, and throughout the study period, the Saudi Ministry of Health restricted the governmental and private dental facilities to manage only emergency/urgent dental care.⁴⁷

Our study is limited by its inherited cross-sectional study design. This design potentially includes recall and reporting of COVID-19 symptoms from weeks or even months earlier. Another limitation is our rate of asymptomatic infections is not confirmed with PCR testing. So, it should be interpreted with caution. Transitioning from consecutive sampling to random sampling methods in future research will strengthen the robustness of findings, reduce selection bias, and enhance the overall quality and applicability of the evidence generated. Limitations include the lack of PCR confirmation for asymptomatic individuals, which may lead to misclassification of infection status, and potential cross-reactivity in ELISA assays, which could result in inflated seropositivity rates.

The strengths of the present study include the screening of SARS-CoV-2 antibodies in a large sample of asymptomatic HCWs of different types of healthcare establishments across a large geographic area in southwestern Saudi Arabia after one year of the pandemic before introducing the national comprehensive vaccination program in Saudi Arabia. This uncovers the hidden infection rate among HCWs and restricts the other confounders of seropositivity. Additionally, the study provided insight into the risk of seropositivity among HCWs.

CONCLUSION

This study highlights the substantial prevalence of asymptomatic COVID-19 infections among non-vaccinated healthcare workers, underscoring significant job-specific risks that require targeted intervention. The findings emphasize the importance of regular serological testing and diligent symptom monitoring to identify asymptomatic carriers and prevent transmission within healthcare settings. To advance understanding and inform effective strategies, there is a need for longitudinal follow-up studies to assess post-vaccination seroprevalence and immunity over time. Policy recommendations include targeted training for nurses and laboratory staff on aerosol-

generating procedures to reduce transmission and enhance safety protocols.

Supplementary Data: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request via email.

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