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Seroprevalence of SARS-CoV-2 antibodies among Jordanian citizens: A cross-sectional study of the demographic and clinical factors that ameliorate serum IgG concentration

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ABSTRACT

The emergence of a confound virus in China has caused a new pandemic spread worldwide, and the disease was entitled "coronavirus disease-19 (COVID-19)." High incidence rates combined with an exponential increase in mortality numbers among infected persons have been reported by the ministries of health all over the world. Screening of IgG immunoglobulin against severe acute respiratory syndrome coronavirus (SARS-CoV-2) was implemented for the determination of the prevalence and durability of the disease among citizens throughout the pandemic. The objectives of this study were to determine the levels of IgG antibodies against COVID-19 in the serum of Jordanian citizens after the third corona wave and to highlight the correlation between both demographic and clinical factors and the levels of IgG immunoglobulin. A cross-sectional study was conducted between September 2021 and January 2022 including 412 Jordanian citizens. Total IgG antibodies were qualitatively assayed against SARS-CoV-2 using the enzyme-linked immunosorbent assay technique. The median age was 34 years, 59% were male, and 41% were female. The seroprevalence was 81.8% for the study population with a mean of 15.17 IU/ml. 45.4% of the positive participants reported a previous COVID-19 infection, whereas the rest of the study population were subjected to vaccine shots. There was no significant difference in IgG levels when the participants were grouped by gender. IgG antibody levels were significantly lower among smokers and those with O blood groups, whereas levels were higher among participants with the B blood groups or negative Rh groups. Demographic factors including gender, age, education, residence, vaccination, and vaccine type did not influence the immunoglobulin level, whereas smoking and blood group affected the levels negatively.

INTRODUCTION

In October 2019, China announced the emergence of a new severe acute respiratory syndrome coronavirus (SARS-CoV-2) in Wuhan city, Hubei province. A contagious outbreak of the viral disease aroused a new pandemic spread worldwide, and the disease was named "coronavirus disease-19 (COVID-19)" (Chen *et al*., 2020). Simultaneously, exponential increases in incidence and mortality rates have been reported by the ministries of health all over the world (Abbas *et al*., 2021; Al-Domi *et al*., 2021; Bendavid *et al*., 2021; Brune *et al*., 2021). The clinical manifestations of the infection varied from mild pharyngitis to severe respiratory distress syndrome. Governments gathered their forces to contain the outbreak and to hamper the ubiquitous dissemination by implementing a bundle of imperative rules. Concomitantly, scientists applied the utmost efforts to endow conceivable explanations about the notorious virus. Researchers sought different aspects such as the structure of the virus, route of transition, the clinical manifestation of infected patients, and prognostic vaccines for the disease (Tai *et al*., 2020). The real-time reverse transcriptase-polymerase chain reaction (RT-PCR) technique is considered the golden standard for sensitive and precise identification of COVID-19 (Alandijany and Faizo, 2021). Additionally, RT-PCR was implemented to monitor the incidence

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rates of the disease and to control the spread of the virus abroad through epidemiological investigation teams (Rakotonanahary *et al*., 2021). On the other hand, screening of immunoglobulins IgM and IgG against SARS-CoV-2 was implemented to determine the prevalence and incidence rates during the disease outbreak in different countries (Faller *et al*., 2021; Zaidi *et al*., 2021). Epidemiological studies have acquired valuable information about the prevalence of COVID-19 and offered infrastructure for politicians and decision-makers to confront the virus spread (Dick *et al*., 2021).

Early in March 2020, the Jordanian Ministry of Health announced the incidence of the first case of COVID-19 in a citizen who arrived from Italy (Sughayer *et al*., 2020). Afterward, an exponential increase was demonstrated in the number of incidences of COVID-19 until the Jordanian government confronted the outbreak with a comprehensive lockdown (Basheti *et al*., 2021).

Early in 2021, the Jordanian Ministry of Health approved the vaccination program against COVID-19 for citizens. The immunization plan prioritized vaccination of risk groups such as healthcare workers, military forces, and the elderly above 60 years (Ministry of Health, 2020). Later, the plan was expanded to include the remaining categories of citizens according to two criteria: age and career. Consequently, a large number of citizens underwent a serum screening test for IgG antibodies in medical laboratories against COVID-19 to determine antibodies level before exposure to vaccination shots, whereas other citizens underwent the screening test to evaluate the immune response to COVID-19 after acquiring one or two vaccine shots. Data deduced from COVID-19 antibodies in the serum of citizens combined with the demographic and clinical factors could highlight the prevalence of infection in different communities. The concomitant variables such as gender, age, health status, education, and residence play a crucial role in the biological systems development and the immune response at a certain stage (Walid, 2016). These confounders could contribute to a hyperactivity of the immune response or even on the contrary hinder the immune activity (Al-Majali *et al*., 2017). In addition, this could emphasize the factors that contribute to the durability of COVID-19 antibodies acquired during the infection period or through vaccination.

The objectives of this study were to determine the prevalence of COVID-19 antibodies among Jordanian citizens and to highlight the correlation between demographic and clinical factors and serum IgG levels.

MATERIALS AND METHODS

This cross-sectional study was conducted on 412 Jordanian citizens in the period from January 2021 to December 2021 in cooperation with Al-Safwa Medical Laboratories and Al-Hakeem Medical Laboratories. Participation in the current study was voluntary, the procedure was described to the participants in detail before conducting the study, and a consent form was filled out by the participants. The form included information about demographic data of age, gender, level of education, residence, smoking, previous infection with COVID-19, number of vaccine shots, and the type of vaccine. Blood phlebotomy was conducted by a certified research assistant from the cubital vein in a blood phlebotomy room at the medical laboratories. Participant information, such as the name and phlebotomy date, was documented on the blood collection tubes. 5 ml of venous blood was collected using a 5 cc syringe. 2 ml of the collected blood was dispensed in an ethylenediaminetetraacetic acid (EDTA) tube, and 3 ml was dispensed in a plain tube. The EDTA tube was used for the determination of the ABO system and Rh group, whereas the plain tube was used for serum separation and IgG determination. The serum was separated by centrifugation at 4,000 rpm for 10 minutes and then collected and stored in a −20°C freezer for further investigation. The study was approved by the IRB Committee in the University of Al-Balqa Applied University No. IRB 26/3/1525/2022.

Using the enzyme-linked immunosorbent assay (ELISA) technique, the S1 domain of the COVID-19 spike protein was utilized for the quantitative determination of IgG against SARS-CoV-2. ELISA kits were purchased from EUROIMMUN AG (Lübeck, Germany). The 96-well plates were coated with a COVID-19 antigen of spike protein, and the procedure was performed according to the company instructions. Later, the 96-well plates were measured using a Mindray reader (Shenzhen, China) at the Biochemistry Department. For the determination of the blood group, both forward and reverse blood grouping were performed for each participant using the ABO antibodies, whereas anti-D antibody was used for the determination of the Rh group.

The data generated were subjected to a statistical analysis using SPSS software version 25. The chi-squared test was used to study the correlation between the categorical variables and IgG levels. The multinomial regression model was used to study the effect of the demographic and clinical covariants on IgG levels of SARS-CoV-2. A p value of <0.05 was considered statistically significant.

RESULTS

The demographic and clinical data of 412 participants are summarized in Table 1. There was a higher participation of males in the study as there were 242 (59%) males and 169 (41%) females. The seropositive ratio against COVID-19 was 81.8% among all participants. The mean seropositive level for the study population was 15.17 IU/ml. The total smoking percentage was 24.6% in the participant group, and the smoking habit was higher among males than females (62% versus 38%, respectively) (Fig. 1). Smokers showed significantly lower levels of IgG levels, as presented in Table 1. IgG serum levels were significantly higher in participants with the B blood group or negative Rh group. There was no statistical significance between the seropositive levels when appended to the participants' gender, age, education, residence, vaccination, and vaccine type.

Pearson's correlation showed a lower seroprevalence of COVID-19 antibodies among smokers and participants in the O blood group, whereas a higher seroprevalence of COVID-19 antibodies was demonstrated among participants in the B blood group or negative Rh group. Multinomial regression analysis for statistically significant variables showed a nonsignificant correlation of blood groups and Rh, whereas smoking sustained the solo significant negative covariant affecting IgG levels with an odds ratio of 0.661 (Table 2).

DISCUSSION

Jordan witnessed the earliest reported cases of COVID-19 infection in March 2020. At that time, Sughayer *et al.* (2020) reported a seroprevalence of zero for SARS-CoV-2 antibodies at the beginning of the pandemic in a study conducted in Amman, the capital of Jordan (Sughayer *et al*., 2020). Then, after 4 months of the comprehensive lockdown imposed in March 2020, the seroprevalence for COVID-19 antibodies was 14.5% for blood donors in Irbid city, a northern city in Jordan (Elnasser

Table 1. Demographic and clinical data of the study population.

		Frequency	Percent	Mean	Standard deviation	Sig.
Seroprotective	Positive	337	81.8	18.0	15.9	0.01*
	Negative	75	18.2	0.24	0.23	
Gender	Male	243	59.0	15.3	15.8	0.756
	Female	169	41.0	14.8	15.4	
Age	<20	21	5.1	14.1	15.2	0.369
	21–30	267	64.8	15.7	17.7	
	31–40	52	12.6	17.4	14.9	
	41–50	34	8.3	20.8	18.2	
	51–60	26	6.3	16.1	14.9	
	>60	12	2.9	15.1	15.7	
Blood group	A	144	35.0	15.0	16.7	0.05*
	B	77	18.7	18.6	15.9	
	AB	50	12.1	16.7	15.4	
	O	140	34.0	12.7	14.2	
RH group	Positive	319	77.4	13.9	15.3	0.01*
	Negative	92	22.3	20.4	16.6	
Smoking	Yes	101	24.5	13.3	15.1	0.05*
	No	310	75.5	18.4	17.0	
Vaccinated	Yes	367	89.1	15.3	16.0	0.376
	No	44	10.7	13.1	12.8	
Vaccine type	Pfizer	212	51.5	16.3	16.0	0.402
	Sinopharm	138	33.5	14.4	16.3	
	AstraZeneca	14	3.4	11.9	11.3	

*p-value < 0.05 is considered statistically significant.

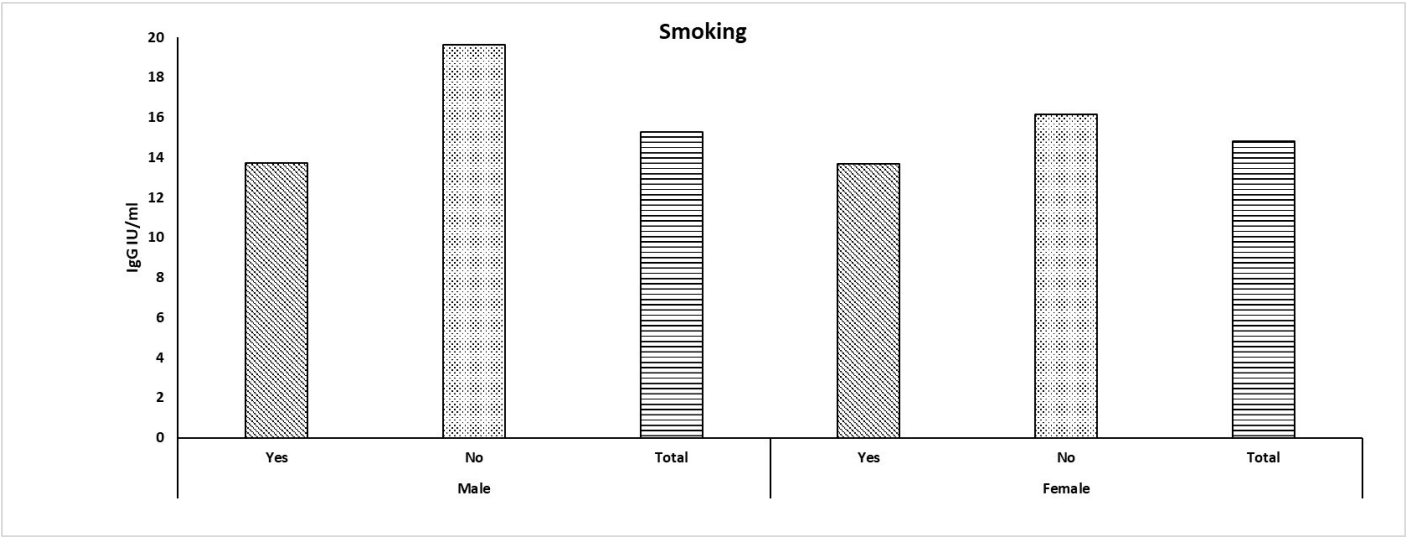

Figure 1. Immunoglobulin level according to smoking habit among males and females. The IgG levels were classified according to smokers, nonsmokers, and the total level of both smokers and nonsmokers.

Table 2. Multinomial regression analysis for the demographic and clinical factors.

Risk factor	OR	95% confidence interval		*p*-value
RH system	1.252	0.75	2.091	0.18
Smoking	0.661	0.523	1.767	0.044*
ABO system	1.15	0.404	1.294	0.146

OR: Odds ratio.
*p-value < 0.05 is considered statistically significant.

et al., 2021). Later, the percentage of seroprevalence showed ascertainment up to 27.4% in early 2021 among healthy blood donors in Amman (Sughayer *et al*., 2021a). Following that, a study conducted by Sughayer *et al*. (2021b) demonstrated a high prevalence of 74.4% for COVID-19 antibodies in healthy blood donors in Amman (Sughayer *et al*., 2021a). Several studies all over the world declared an escalation in the percentage of positive seroprevalence of COVID-19 (Alsuwaidi *et al*., 2021; Balou *et al*., 2021; Soeorg *et al*., 2022; Tunheim *et al*., 2022). Additionally, a study conducted on 1,443,519 blood donation specimens in the United States of America showed a seroprevalence of 83.3% for participants for combined infection- and vaccine-induced antibodies in May 2021 (Jones *et al*., 2021). In the current study, there was a high seroprevalence of positive COVID-19 antibodies of 81.8% with a mean IgG level of 15.17 IU/ml for the combined subcategories of vaccinated and infected participants. This high ratio of positive prevalence could be attributed to one of two factors: either the increase in the number of citizens subjected to vaccination shots or the acquisition of a COVID-19 infection before the study conduction. Specifically, 89.9% of the study population reported having one or two shots of vaccine. Additionally, we could not attribute the high seroprevalence of COVID-19 antibodies to the subjection to a prior infection since 61% of the study population reported they do not know if they were infected with COVID-19 or have not been infected ever. This level of seroprevalence is concomitant with the results of a multicenter study of antibody seroprevalence of COVID-19 antibodies in Iran that showed a conversion of seroprevalence percentage of 79.5% when measured for combined vaccinated and COVID-19-infected participants (Javadinia *et al*., 2022). On the other hand, the type of vaccine did not significantly affect the IgG levels in the current study, and all vaccines conferred relevant levels of immunoglobulin. The vaccines reported by the participants were Pfizer, Sinopharm, and AstraZeneca. Likewise, Wei *et al*. (2021) reported a nonsignificant difference in immunoglobulin levels among the study groups when subjected to different vaccine kinds. Specifically, they concluded that the major difference in IgG levels is attributed to the durability of IgG immunoglobulin and the time required for reaching the highest immune response (Wei *et al*., 2021).

The current study showed a higher incidence of smoking among males than females. Smoking habits showed a paradox for the level of IgG of COVID-19, regardless of gender. There was a significant decrease in seroprotective levels of smoking COVID-19 patients. This point has been noticed by several scientists who reported a decrease in the levels of seroprotective levels in smokers' blood (Kahlert *et al*., 2021; Maraqa *et al*., 2021; Michos *et al*., 2021). Congruently, Warszawski *et al*. (2022) reported a statistically significantly lower likelihood of IgG antibodies against COVID-19 in daily smokers versus nonsmokers. Similarly, a study conducted in Iraq on diabetics and smokers as comorbidities for corona infection reported smoking as a less significant virulence cofactor than diabetes which conferred lower immunoglobulins in patients' serum (Abbas *et al*., 2021). The plausible explanation for such levels is the anti-inflammatory effect of nicotine found in cigarettes which suppresses the adaptive immune activity and hinders antibody formation (Usman *et al*., 2021).

The average smoking rate of the participant population was lower than in the neighboring countries; for instance, the rates were higher in both Lebanon and Turkey at 42.3% and 29.3%, respectively (Abdulrahim and Jawad, 2018; Özer *et al*., 2018), whereas the smoking rate in the current study is consistent with rates reported in the United States and Italy at 25.1% and 23.40%, respectively (Gorini *et al*., 2020; Jeong *et al*., 2021).

In the current study, the male smoking rate was extremely higher than the female smoking rate. This note was congruent in other countries as males showed higher smoking rates than females in Turkey, Iraq, and Lebanon (Abdulrahim and Jawad, 2018; Ibrahim *et al*., 2018; Özer *et al*., 2018; Walid, 2016).

Remarkably, there was no significant difference in IgG levels between males and females or in the different age groups in the current study. Zeng *et al*. (2020) reported in a study conducted on 331 patients, with confirmed SARS-CoV-2 infection, a nonsignificant correlation of the IgG immunoglobulin levels among males and females and between young and elderly patients. They concluded that the major difference they noted in IgG levels is attributed to the state of disease severity (Zeng *et al*., 2020).

Several research groups clarified the relationship between the blood group and the COVID-19 seroprevalence; for instance, Valenti *et al*. (2021) reported in a study conducted in Italy on blood donors the highest prevalence for seroprotective IgG was demonstrated in donors of the O and A blood groups. Likewise, Sughayer *et al*. (2021a) showed a high prevalence of seroprotective immunoglobulins in the O blood groups, whereas the same author reported in a previous study the seroprevalence of IgG in the A blood group donor. In the current study, there was a significant prevalence for the B blood group with a higher seroprevalence for negative Rh participants.

In the current study, there was no significant correlation between the different blood and Rh groups on the IgG levels when the data was subjected to a multinomial analysis. Statistical analysis confirmed the dominancy of smoking over other covariates as a blood group on IgG results. These findings are consistent with a study from Saudi predictors of SARS-COV-2 infection among blood donors in Saudi Arabia (Banjar *et al*., 2021) and UAE (Alsuwaidi *et al*., 2021).

One of the limitations of this study is that these studies are subjected to variation in the population type and size. For instance, several studies from the Middle East, Saudi Arabia, Pakistan, China, Italy, Brazil, US, England, Scotland, and Spain have concluded a variation in the seroprevalence rates that were directly or proportionally correlated to numerous factors such as the demographic data, sample size, gender, study period, lockdown procedures, and the onset of the outbreak wave (Abouzid *et al*., 2021; Alserehi *et al*., 2021; Davis *et al*., 2021; Naiyar *et al*., 2021; Qin *et al*., 2021).

CONCLUSION

Based on the sample of participants in Jordan from January 2021 to December 2021, demographic factors including gender, age, education, and residence did not influence the immunoglobulin level, whereas smoking and blood group affected the levels negatively.

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CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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ETHICAL APPROVAL

This study was approved by the Ethical Committee of the Al-Balqa Applied University, Al-Salt, Jordan (Ethical Approval No. 26/3/1/525/2022).

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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