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ORIGINAL ARTICLE

Seroprevalence of SARS-COV-2 antibodies among health-care workers exposed to COVID-19 patients in a large reference hospital, Iran

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ABSTRACT

Background and Objectives: Health care workers (HCWs) are a high-risk group for acquiring and transmitting severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Aim of the study was the evaluation of sero-prevalence of SARS-CoV-2 in a random sample of HCWs at a large acute care hospital in Iran.
Materials and Methods: We collected blood samples of 180 medical staffs from September 22, 2020 to January 26, 2021. The enzyme linked immunosorbent assays (ELISA) tests were used for evaluation of the presence of IgG antibodies. Participants completed a self-report questionnaire, comprising demographics, occupational, the work area, and personal protection data.
Results: Of the 180 HCWs who participated in this study, 44 (24.4%) were seropositive for anti-SARS-CoV-2 IgG. The percentage of IgG positivity was higher in males than females ($P<0.05$). Also, there was statistically significant difference between presence of the antibodies and the occupation, location, and infecting family members with Covid -1 ($P<0.05$). Other factors did not associate significantly to antibody presence against SARS-CoV-2 ($P>0.05$).
Conclusion: According to this point that the number of COVID-19 cases is still growing rapidly among HCWs. So, the epidemiological estimate of SARS-CoV-2 infection remains a major challenge that is needed to prevent the spread of infection in the hospitals.

Keywords: Health care workers; SARS-CoV-2; COVID-19; Sero-epidemiological study; Iran

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as the 2019 novel coronavirus (2019-nCoV) (1). In 2019, it rapidly started to spread all over the world from Wuhan city. SARS-COV-2 is one of the enveloped viruses with a large plus-strand RNA genome. The virus is part of the *Coronaviridae* family, which is commonly known as the cause of seasonal colds (2). But in recent years, the *Coronaviridae* family has become life-threateningsuch as severe acute respiratory syndrome coro-

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navirus (SARS-CoV) in 2003, Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, and 2019 novel coronavirus (2019-nCoV, later officially named SARS-CoV-2) in late December 2019. SARS-CoV-2 has a genetic similarity of about 70 percent to SARS-CoV-1. The virus infected about 800 people between 2003 and 2003, with an estimated mortality rate of 4-7 percent (3).

COVID-19 can be caused by SARS-CoV-2 infection. COVID-19 has a wide range of clinical symptoms. Suffering ranges from asymptomatic to acute respiratory syndrome and multi-organ dysfunction involvement. Symptoms of the COVID-19 are similar to the symptoms of flu-like symptoms, which include fever, cough, sore throat, headache, fatigue, headache, myalgia and breathlessness (4-5). On the 11th of March 2020, COVID-19 was declared a pandemic disease by the World Health Organization. In total 112,456,453 confirmed COVID-19 infections and 2,497,514 deaths (fatality rate 7.1%) were reported worldwide (2/26/2021) by the WHO. It seems that both humoral and cellular immune systems are involved in the immunization of the individual against the SARS-CoV-2 virus. IgM and IgG antibodies can be detected 1-2 weeks after infection (6). However, no link has yet been found between neutralizing antibodies, However, the association between neutralizing antibodies and T-cell, disease severity and clinical outcomes has not been found (7), but convalescent plasma as a treatment option (8), and studies have shown that the level of antibody titer is directly related to the severity of the disease (9). It is noteworthy that the level of antibodies in individuals decreases over time and the severity of this decrease is much higher in asymptomatic individuals (9-10). Major neutralizing antibodies target the S1 of the spike (S) protein and prevent virus fusion to the host cell (11-12). High levels of antibodies against N protein are also seen in people infected with the SARS-CoV-2 (13). Enzyme-Linked Immunosorbent Assay (ELISA) is a highly efficient as well as high-speed and safe method for analyzing antibody titers against N protein (14-16). According to the studies, IgM reaction to the S and N proteins in SARS-COV-2 peaks four weeks after the appearance of symptoms and is no longer detectable three months later, although IgG was identified about the fourteenth days following the occurrence of symptoms and was detectable for up to 36 months (17-18). However, there is still a discrepancy between the stability of the antibody titer aginst of SARS-COV-2 in individuals.

Health-care workers (HCWs) working in the department of hospital-related to COVID-19 disease, in addition to playing a vital role in combating the pandemic and providing health services, are known as high-risk persons to outbreaks of the disease (19-20). Therefore, knowledge of the health and safety of these HCWs is very important. According to the above, the purpose of this study was to investigate the seroepidemiology of SARS-COV-2 virus in HCWs in the department of hospital-related to COVID-19 of Valiasr Hospital in Birjand city.

MATERIALS AND METHODS

Study design. This study was approved by the Birjand University of Medical Sciences (BUMS). HCWs work in the department of hospital-related to COVID-19. The proposal was approved by the ethics committee of BUMS (The ethic cod number: IR.BUMS.REC.1399.108). After written, informed consent, we enrolled 180 employees of the Valiasr Hospital. The study was carried out between September 22, 2020 to January 26, 2021, blood samples were collected by nursing staff and the presence of SARS-CoV-2 antibodies (in particular of the IgG class) in serum was analyzed. In addition, each subject completed a questionnaire. All data was collected in strictly pseudonymous form according to the study protocol. Epidemiological, clinical, and laboratory data were collected in all HCWs. This questionnaire contained information about age group, gender, profession, location, underlying disease, family member with COVID-19, area of care, occupational stress, and safety equipment. Serum samples were collected in clinical laboratories in the hospital. All samples were stored at -20°C before testing. Detection of anti-N (nucleocapsid) SARS-CoV-2 antibodies was performed using SARS-CoV-2 IgG ELISA kits (Pishtaz Teb, Iran; Catalogue No. PT-SARS-CoV-2. IgG-96) according to the manufacturer's protocol. In brief, a volume of 100 µL of diluted specimens (1:101) was applied to a 96-well microplate (coated with N protein) and incubated at 37°C for 30 minutes. The microtiter were washed five times and shaken. Then, the wells were filled with 100 µL of anti-human IgG -HRP conjugate and incubated at 37°C for 30 minutes. Following the secondary wash cycle, 100 µL of chromogen- substrate solution was added to the wells

and incubated at 37°C for 15 minutes. Finally, the reaction was arrested by applying 100 µl of stop solution to the wells. Within 30 minutes, each well's optical density (OD) was measured using a microplate reader set to 450 nm (Using 630 nm filter as the reference filter). The antibody concentration was calculated as the ratio of OD to the cut-off value. Accordingly, the samples higher than 1.1 considered as positive, and those less than 0.9 assigns as negative, and those between these two values, 0.9-1.1, were considered as suspicious or equivocal, and tested again with fresh serum/plasma sample after a while.

Statistical analysis was processed with SPSS statistical package (SPSS Inc., Chicago, IL, USA). Statistical analyses were based on population, defined as subjects who gave a blood probe and completed the questionnaire. The results are presented as frequencies. Differences between groups were analyzed by $\chi 2$ test. P-values <0.05 were interpreted as statistically significant.

RESULTS

Characteristics of study participants. The study period was 18 weeks and it began on September 22, 2020. We tested 180 HCWs in a large COVID-19 reference hospital in Birjand city, Iran. Of whom, 61.7% (111) were female, and 38.3% (69) were male. The ages of those tested ranged from 20 to 58 years; the mean age was 34.47 (±8.99). The majority of study participants were working in infectious diseases (27.2%; n=49), followed by laboratory (21.7%; n=39), and Hospital emergency (17.8%; n=32) wards. Of note, 67.7% of HCWs had close contact with SARS-CoV-2- positive patients. Table 1 provides an overview of the characteristics of the participants in this study.

Anti-SARS-CoV-2 IgG antibodies. Overall, 44 (24.4%) of participants were seropositive for anti-SARS-CoV-2 IgG. The percentage of IgG positivity was higher in males than females, and there were a significance differences between in these two groups (P= 0.03). Among seropositive HCWs, there were no statistically significant associations between age and the presence of anti-SARS-CoV-2 IgG antibodies (P=0.24).

The data demonstrate that there were a statistically significant associations between presence of antibodies and the occupation (P<0.05). Among the HCWs occupational groups, nurse and Hospital service workers had the highest seroprevalence rates, followed by physicians and Laboratory technician. Based on work area,the seroprevalence was highest in those working in the infectious diseases (10%; n=18) and Hospital emergency (5.6%; n=10) wards and lowest in participants working in pediatric ward and radiology (0.6%; n=1). Furthermore, the logistic regression analysis showed that the chances of getting infected with Covid-19 in HCWs working in infectious diseases, emergency medicine and internal wards were 4/93, 3/86, and 4/86 times higher from the administrative staff, respectively. The other characteristics studied, including marital status, education,working hours,occupational stress, work experience, and night work, did not show any statistically significant association with presence of antibodies to SARS-CoV-2 (Table 1). On the contrary, there were a significant difference between the seroprevalence of antibodies with location and infecting family members with Covid -19 (P<0.05).

Although one of the important factor which could be associated to the infection rate is the use of personal protective equipment such as hand washing, masks, gloves, coveralls, and protective shield. The surgical mask was the most accessible instrument for health care workers. But, in the current study, no statistically significant differences were found between the use of personal protective equipment and the IgG prevalence (Table 2).

DISCUSSION

In the present study, the number of people who worked in Corona Health Center and had a history of COVID-19 was reported to be 22.9%, of which the highest prevalence was observed among women under 30 years of age. However there was no significant difference between sex and COVID-19 was detected in this research. On the other hand a study in Sotgiu showed that the prevelance of IgM is higher in men than women (24% vs 9.1%) (21) wheras the other finding suggested that the seroprevalence of SARS-CoV-2 IgG antibody is higher in female than men (22).

In general, COVID-19 has strong focus for investment of nurses and health personnel to achieve global health (23). According to this point that transmission

Table 1. Seroprevalence of SARS-CoV-2–specific IgG antibodies in HCWs in the Valiasr Hospital of Birjand, Iran

		No. (%) of participants	Seroprevalence (95% CI), %	P value
Overall		180	24.4% (18.2-30.7)	P=0.03
Sex	Male	69 (38.3)	12.8 (7.9-17.7)	
	Female	111 (61.7)	11.7 (7-16.4)	
Age, y	≤30	72 (40)	9.1 (4.8-13.3)	P=0.24
	31-40	61 (33.9)	6.3 (2.7-9.8)	
	≥41	47 (26.1)	8.6 (4.5-12.7)	
Location	Village	177 (98.3)	22.5 (16.4-28.6)	P=0.002
	Town	3 (1.7)	1.7 (0-3.6)	
Underlying disease	Yes	15 (8.3)	1.7 (0-3.6)	P=0.68
	No	165 (91.7)	22.8 (16.7-28.9)	
Family member with	Yes	62 (34.4)	13.2 (8.2-18.1)	P=0.002
COVID-19	No	118 (65.6)	10.9 (6.3-15.4)	
Occupation	Physician	32 (17.8)	3.9 (1.1-6.7)	P=0.004
	Nurse	60 (33.3)	9.4 (5.1-13.7)	
	Laboratory technician	34 (18.9)	2.2 (0-4.3)	
	Hospital service workers	38 (21.1)	8.9 (4.7-13)	
	Administrative staff	16 (8.9)	0 (0-0)	
Area of care	Infectious diseases	49 (27.2)	10 (5.6-14.4)	P=0.09
	Hospital emergency	32 (17.8)	5.6 (2.2-8.9)	
	Intensive care unit	13 (7.2)	1.1 (0-2.6)	
	Internal ward	11 (6.1)	2.2 (0-4.3)	
	Pediatric ward	14 (7.8)	0.6 (0-1.7)	
	Radiology	3 (1.7)	0.6 (0-1.7)	
	Laboratory	39 (21.7)	3.3 (0.6-5.9)	
	Administrative department	19 (10.5)	1.1 (0-2.6)	
Working hours (week)	≤45	96 (53.3)	11.3 (6.7-15.9)	P=0.58
	≥45	84 (46.7)	12.8 (7.9-17.7)	
Night shift (month)	≤ 2	93 (51.7)	13.3 (8.3-18.3)	P=0.19
	3-6	77 (42.8)	10.9 (6.3-15.4)	
	≥7	10 (5.5)	3.1 (0-5.6)	
Work experience	≤2	35 (19.4)	4.1 (1.2-7)	P=0.98
	3-10	67 (37.2)	8.9 (4.7-13)	
	11-20	55 (30.6)	7.5 (3.6-11.3)	
	≥21	23 (12.8)	2.7 (0.3-5.1)	
Occupational stress	Yes	104 (57.8)	13.2 (8.2-18.1)	P=0.62
	No	76 (42.2)	10.9 (6.3-15.4)	

of SARS-CoV-2 through hospitals is common, and COVID-19 is a huge threat to HCP and global public health systems, the prevalence and associated factors of SARS-CoV-2 infection are very important among HCWs caring for COVID-19 patients (24). Considering the importance of preventing the transmission of biological agents such as corona virus among HCP in the workplace and hospitals, and according to that these employees have contact more with suspects or infected patients, and particularly the risk of disease is high during caring and transporting. So, effective strategies have been developed to minimize SARS-CoV-2 in healthcare settings including the use of protective equipment, appropriate clothing, COVID-19 pre-task training and quarantine for those working in COVID-19 departments (22).

Many national and regional studies have estimated the prevalence of IgG SARS-CoV-2 antibodies in

Table 2. The use of personal protective equipment and the IgG prevalence antibodies in HCWs in the Valiasr Hospital of Birjand, Iran

Personal protection	Always	Mostly	Sometimes	Rarely	Never	Mean ± sd	X^2	P-value
Mask M3	10.3%	3%	4.2%	36.1%	56.4%	1.85 ± 1.28	X^2=1.91	P=0.7
N95 Mask	51.1%	15.9%	14.2%	8.5%	10.2%	3.89 ± 1.38	X^2=9.81	P=0.04
Surgical mask	72.4%	14.1%	4.7%	5.3%	3.5%	4.46 ± 1.04	X^2=3.26	P=0.51
without mask	5.5%	1.2%	0.6%	27.9%	64.8%	1.54 ± 0.99	X^2=1.73	P=0.82
Gloves	65%	16.4%	10.2%	5.1%	3.4%	4.34 ± 1.07	X^2=3.19	P=0.55
Shield	41.7%	20%	14.9%	10.9%	12.6%	3.67 ± 1.43	X^2=5.55	P=0.24
Gun	43.8%	14.2%	11.9%	15.9%	14.2%	3.57 ± 1.52	X^2=5.02	P=0.28
Hand washing	87.5%	11.4%	0.6%	0	0.6%	4.85 ± 0.45	X^2=5.14	P=0.18

the population. Diagnosis and serological tests for detection of SARS-COV-2 virus in throat and nasal are used in clinical studies of HCWs (25). However prevalence rates of serum IgG antibodies against SARS-CoV-2 among HCWs vary widely. For example, in Europe, Germany, Greece, Croatia and Austria reported lower prevalence of seroprevalence in the HCW group (1%, 1.07%, 2%, 3.2%, respectively), While Belgium and Spain had the highest seroprevalence of SARS-CoV-2 (6.4%, 9.3%, respectively) (26). In a meta-analysis study, the prevalence of specific seroprevalence of SARS-CoV-2 was varied from 0.37 to 22.1% in health care population (27). However the other study with 2.3 million people from 50 countries showed that the seroprevalence of SARS-CoV-2 in the population was low (3.2%) (28). On the other hand, another study suggested that the level of IgG or IgM against SARS-CoV-2 in the hospital was 2.5% (6919/170), which was higher than in the community (0.8%, 81/10,449) (29). Differences in future actions against the virus could be related to regional variations of seroprevalence of SARS-CoV-2 IgG antibodies. Analysis shows that seroprevalence of SARS-CoV-2 is higher in the United States compared to Europe and East Asia (30).

In the UK, the seroprevalence of SARS-CoV-2 was reported 10.6% among 405 asymptomatic HCWs and 44.7% among 1,299 asymptomatic HCWs. In addition the highest rate of seroprevalence of SARS-CoV-2 was found among staff in a clinical setting who had direct contact with COVID-19 patients (34.7%) and the lowest rate was belong to those working in non-clinical setting with no patient contact (22.6) (31). In contrast, a study in the United States demonstrated that the seroprevalence of SARS-CoV-2 in employees who exposed to COVID-19 was similar to that of non-exposed individuals, suggesting that personal protective equipment (PPE) was effective in preventing of COVID-19 infection in HCWs. Another study with similar findings show that the prevalence of serum was lower among staff who used face masks when caring for patients (6%) than among those who did not (9%) (32). In a review study, no association was detected between the incidence of COVID-19 and seroprevalence (33). These findings may be due to differences in seroprevalence of anti-SARS-CoV-2 antibodies in different countries, time, age, sex, methods used for serological testing and duration of COVID-19. Therefore, the reported seroprevalence can only reflect the time and place where the research was performed using a specific test method.

CONCLUSION

According to this point that the number of COVID-19 cases is still growing rapidly and significantly high prevalence of IgG among HCWs indicates a high rate of past infection. So, the epidemiological estimate of SARS-CoV-2 infection remains a major challenge that is needed to prevent the spread of infection in the hospital. In addition HCW routine testing for SARS-CoV-2 should be considered after vaccination to identify areas of increased transmission.

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