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RESEARCH **ORIGINAL ARTICLE**

Seroprevalence of SARS-CoV-2 Antibodies among healthcare workers at a Tertiary Cancer Center

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ABSTRACT

BACKGROUND - Implementation of strict measures to ensure the safety of cancer patients during the coronavirus disease (COVID-19) pandemic includes modification of treatment plans, strict physical distancing measures, and early detection of suspected cases. Serological testing can identify immunological responses, i.e., seroconversion, in HCWs presenting with subclinical symptoms. The detection of immunoglobulin (Ig) M specific antibodies demonstrates active disease, while (Ig) G specific antibodies indicate previous exposure to SARS-CoV-2.

METHODS - A cross-sectional study was conducted in a tertiary cancer center in Jordan to detect HCWs who had a positive serology albeit previous negative diagnosis with COVID-19. We sent an internal invitation e-mail to all HCWs in direct contact with cancer patients. After consenting, blood microsampling was done via a lancet for COVID-19 immunoglobulin analysis.

RESULTS - We recruited 583 asymptomatic participants, who had a previously negative COVID-19 polymerase chain reaction (PCR) testing, between December 2020 and January 2021, with an unplanned equal distribution between genders and a mean age of 34.0 ±9.3 years. The majority of participants were from the nursing department (n=390, 66.9%). A history of an upper respiratory tract infection was reported by 144 individuals (24.7%) with varying symptoms. Positive exposure was reported in 441 participants (75.6%). IgG seroconversion was detected in 41 participants (7.0%), while IgM seroconversion was only detected in three (0.5%). There was no correlation between positive IgG seroconversion and history of upper respiratory tract infection (URTI) (p-value = 0.51), exposure to infected patients (p-value = 0.57), or profession (p-value = 0.46).

CONCLUSION - In a tertiary cancer center, we found the rates of SARS-CoA-2 IgG or IgM seroconversion amongst HCW to be relatively low during the COVID-19 pandemic. There was no correlation between IgG seroconversion and history of URTI or prior exposure to infected patients.

KEYWORDS - COVID-19, seroconversion, pandemic, healthcare workers, IgG, IgM, immunoglobulins

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List of Abbreviations:

COVID-19: coronavirus disease
HCW: healthcare worker
Ig: immunoglobulin
RT-PCR: reverse transcription-polymerase chain reaction
URTI: upper respiratory tract infection
WHO: World Health Organization
SARS: severe acute respiratory syndrome
MERS: Middle East respiratory syndrome
RBD-S: receptor-binding domain of S protein
KHCC: King Hussein Cancer Center

BACKGROUND

Coronaviruses constitute a large family of enveloped viruses with a positive-stranded RNA genome [1] that can induce multi-system disease in infected individuals [2]. It all started in Wuhan, China, when multiple cases of pneumonia were reported, which shared the same history of exposure, for which the causing pathogen was unknown [3]. The World Health Organization (WHO) designated the novel disease caused by the coronavirus "SARS-CoV-2" outbreak; coronavirus disease 2019 (COVID-19), which spread across the globe and in March 2020 was declared a pandemic[4].

Jordan reported its first confirmed case of COVID-19 in March 2020 [5], followed by the declaration of a state of emergency then the institution of a country-wide lockdown [5], although the actual number of cases did not justify the strict measures, a total of 84 confirmed cases by the 20th of March [5], delaying the first wave of infection which spanned from August 2020 until late January 2021. The peak of reported cases was 7,933 cases in a single day reported in November 2020. Soon afterward, the second wave started in February 2021. Jordan reported its highest peak in March 2021, with the largest number of reported cases in a single day (9,535). The WHO continued to encourage increasing testing capacity in order to identify silent carriers [6]. However, at that time and due to financial constraints, Jordan was only able to perform tests for individuals with direct contact of confirmed COVID-19 cases and random tests in areas where an infection was highly suspected [5].

Currently, polymerase chain reaction with reverse transcription (RT-PCR) remains the most commonly used test for COVID-19 diagnosis. However, reports of false-negative results arise due to inappropriate specimen-collection timing – outside of active disease phase and deficiency in sampling techniques [8].

Serological testing is essential to identify individuals who demonstrate seroconversion with IgG/IgM-specific antibodies and are potentially immune to SARS-CoV-2 (8). Based on several studies, during the severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) outbreaks, it was evident that specific viral antibodies were detectable in 80-100% of exposed individuals 14 days after the onset of symptoms [9 – 13].

A study by Burbelo et al. found that nucleocapsid proteins antibodies emerge early in infection, thus are a more sensitive target in serological testing when compared to spike protein antibodies [7]. The receptor-binding domain of the S (RBD-S) protein is the host attachment protein, which, if detected, can increase sensitivity as well [14]. It should be clarified that cross-reactivity is possible with other coronaviruses [15]. Lastly, recent commercial rapid point-of-care tests for the detection of antibodies have been widely developed and marketed. They are, however, of variable quality, are qualitative, without specification for the viral-antigens targeted, or the detected antibodies' class. A plaque reduction neutralization test can only confirm the presence of neutralizing antibodies, which can be positively correlated with high titers of IgG [14] . Whether detected antibodies confer, long-lasting immunity is still unclear.

In this study, we aim to identify asymptomatic healthcare workers (HCW) at a tertiary cancer center who are in direct contact with patients, never tested positive for the novel coronavirus, and who have a SARS-CoV-2 specific IgG/IgM seroconversion.

METHODS

The study utilized a cross-sectional design to detect a sample of frontline HCWs with evidence of seroconversion. The study was conducted at King Hussein Cancer Center (KHCC) after obtaining approval from the Institutional Review Board (20 KHCC 92). An e-mail summarizing the protocol of the study was sent to all KHCC HCWs. Briefly, HCWs who meet the inclusion criteria were provided contact numbers to schedule an appointment to perform a an SARSCoV-2 IgG/IgM antibodies testing. The candidates were interviewed in a private, designated room to obtain informed consent and answer an online questionnaire (SurveyMonkey, USA). Minimal amount of personal data was collected throughout the survey to serve the purpose of the study. Subject confidentiality was protected by de-identifying the final set of data. The survey consisted of four subsections and nine items. The first section was on demographic data, including age, gender, comorbidities, degree of education, occupation, hospital department, and history of upper respiratory symptoms. The second section included a question about previous seasonal flu vaccine administration and the date. The third section investigated previous exposure to SARS-CoV-2 . The final section included questions regarding the frequency of patient contact, interaction with people outside the hospital vicinity, and the date of previous SARS-CoV-19 testing and results. The data was only accessible to the research team approved by the Institutional Review Board.

A sterile single-use lancing device (Accu-Check, Safe-T-Pro Uno, Roche, Germany) was used on the second- or fourth-hand finger after wiping with an alcohol swab (Alcohol Prep Pad, Lights Medical Manufacture Co. Ltd., China), followed by a 30 µl blood sample collection using a specialized C-tip. This was analyzed using the AFIAS COVID-19 Ab kit, a fluorescent immunoassay for the automatic qualitative detection of SARS-CoV-2 specific IgG and IgM antibodies, using recombinant nucleocapsid protein as an antigen, processed with an AFIAS-6 machine (US FDA 510(K) cleared). The reported clinical sensitivity and specificity of the AFIAS COVID-19 Ab kit are 95.8% and 96.7%, respectively (Boditech, Korea).

The test initiates with the blood sample moving automatically from the sample well into a 'dilution buffer chamber.' The diluted sample automatically travels into a 'detection buffer chamber' initiating a binding reaction with the fluorochrome-antigen conjugates from the buffer to the anti-SARS-CoV-2 IgG/IgM antibodies present in the sample. The mixture is then automatically delivered into a 'test mixture well' to travel through the test strip of the inserted cartridge. Fluorochrome-antigen complexes combined with anti-SARSCoV-2 IgG/IgM antibodies are then captured by the immobilized anti-human IgG and anti-human IgM at the 'IgG test line' and the 'IgM test line' of the test cartridge. A laser source is responsible for detecting fluorescence from the fluorochrome-labeled complexes accumulated at the test lines. A test was deemed positive if the Cut-Off Index (COI) value was ≥ 1.1, negative if it was ≤ 0.9, and undetermined if it was between 0.9 – 1.1, thus requiring retesting. (Boditech Med Inc., AFIAS COVID-19 Ab Insert Paper, Rev. 01. April 8, 2020.). In IgM positive results, the HCW was referred to a SARS-CoV-2 PCR test, and the corresponding department was informed as this anti-SARSCoV-2 IgG/IgM antibodies didn't consider a diagnostic test for -SARSCoV-2.

We used descriptive statistics for participants' demographics, disease history, and symptoms. Counts and percentages were used for categorical variables, while mean, median, and standard deviation (STD) were used for continuous variables. We used the Chi-square test or t-test to compare symptomatic/asymptomatic patients' groups and serological conversion according to the type of correlated variables. A significance value of $p \leq 0.05$ was used in the analysis. All analyses were be performed using SAS version 9.4 (SAS Institute Inc, Cary, NC).

RESULTS

A total of 583 participants were recruited between December 2020 and January 2021, with an unplanned equal distribution between males (n=286, 49.1%) and females (n=297, 50.9%), and a mean age of 34.0 years (±9.3). The most common comorbidities included autoimmune disease (n=36, 6.2%), hypertension (n=32, 5.5%), asthma (n=26, 4.5%), and diabetes mellitus (n=17, 2.9%). The majority of participants were from the nursing department (n=295, 50.6%) followed by physicians (n=184, 31.6%). The majority of participants were responsible for primary household shopping (n= 419, 71.9%), continued to socialize (n= 95, 16.3%), and only a minority adhered to strict social isolation measures (n=68, 11.7%). History of an upper respiratory tract infection (URTI) three months prior to enrollment was reported by 216 (37.0%) participants with varying symptoms including rhinorrhea (n= 144, 24.7%), generalized fatigue (n= 130, 22.3%), nasal congestion (n= 124, 21.3%), odynophagia (n= 121, 20.8%), cough (n= 118, 20.2%), headache (n= 111, 19.0%), fever (n= 54, 9.3%), and wheezing (n= 29, 5%). Most of the participants confirmed receiving the seasonal influenza vaccine few months prior to the date of the study (n= 479, 82.2%).

Table 1. Characteristics of the participating HCWs

Variable		Total n=583(100%)
Gender	Male	286 (49.1%)
	Female	297 (50.9%)
Age (years)	Min	22
	Max	70
	Mean	34
	SD	9
Comorbidities	Hypertension	32 (5.5%)
	Diabetes Mellitus	17 (2.9%)
	Cardiac Disease	15 (2.6%)
	Pulmonary Disease	26 (4.5%)
	Autoimmune Disease	36 (6.2%)
Occupation	Nurse	295 (50.6%)
	Physician	184 (31.6%)
	Pharmacist	30 (5.14%)
	Others	74 (12.7%)

Adherence to Social isolation	Household Shopping	419 (71.9%)
	Socialize	95 (16.3%)
	Strict Social isolation	68 (11.7%)
History of URTI b	Cough	118 (20.2%)
	Fever	54 (9.3%)
	Nasal Congestion	124 (21.3%)
	Fatigue	130 (22.3%)
	Headache	111 (19%)
	Wheezing	29 (5%)
	Pain during Swallowing	121 (20.8%)
	Runny Nose	144 (24.7%)
History of taking flu-vaccine in the current year	Yes	479 (82.2%)
	No	104 (17.8%)
History of Exposure to Positive Personnel	Yes	441 (75.6%)
	No	142 (24.4%)
IgG result	Negative	542 (93%)
	Positive	41 (7%)
IgM result	Negative	580 (99.5%)
	Positive	3 (0.5%)

a Anesthesia/radiology/laboratory technician, psychology/psychotherapy, child life specialist, social worker, respiratory therapist, physiotherapist, nutritionist, occupational health therapist, dentist, dental hygienist, and clinical research coordinator.
b Upper respiratory tract infection

Exposure, defined as contact with a PCR-positive patient for more than 15 minutes in a confined place, was identified in 441 (75.6%) participants. IgG seroconversion was detected in 41 (7.0%) participants. IgM seroconversion was detected in only 3 (0.5%), two of which underwent PCR testing, with one positive and one negative result. The third positive case refused to be further tested (Table 1). The highest level of seroconversion was found in the nursing subcategory (n=22, 7.5%), followed by physicians (n=11, 6%). No correlation was found between the IgG test and occupation (P-value = 0.464) (Table 2).

There was no correlation between IgG seroconversion and URTI symptoms. More than half of all positive seroconversions and negative seroconversions were asymptomatic (n = 28, 68.3% and n = 339, 62.5% respectively), while more than 30% of HCWs with positive and negative seroconversion had positive URTI symptoms (n = 13, 31.7% and n = 203, 37.5% respectively), without any statistical significance of each category (P-value = 0.507) (Table 2). No association was found between the level of IgG positive COI and time of patient contact (P-value = 0.916) (Table 3). Most HCWs (n=408, 75.3%) who had a positive history of exposure to a COVID-19 positive patient had a negative IgG seroconversion test with no evidence of a correlation between both variables (p-value = 0.572) (Table 2). Receiving the annual flu vaccine did not show any effect on the frequency of seroconversion (P-value = 0.119), nor any effect on URTI symptoms (P-value = 0.148) (Table 2 and 4). No statistical significance was found between IgG positive HCWs and adherence to strict social isolation (n = 7, 17.0%) versus those who did not (n = 34, 82.9%) (P-value = 0.809).

Table 2. Comparison between SARS-CoV-2 immunoglobulin G (IgG) result and various points of interest.

		IgG Result		P-value
		Positive	Negative	
Occupation	Physician (n=184)	11 (6%)	173 (94%)	0.464
	Nurse (n=295)	22 (7.5%)	273 (92.5%)	
	Pharmacist (n=30)	1 (3.3%)	29 (96.7%)	
	Other (n=74)	7 (9.5%)	67 (90.5%)	
History of URTI†	Positive (n=216)	13 (6%)	203 (93.9%)	0.507
	Negative (n=367)	28 (7.6%)	339 (92.3%)	
History of exposure to COVID-19‡ positive personnel	Positive (n=441)	33 (%)	408 (%)	0.572
	Negative (n=142)	8 (%)	134 (%)	
History of taking the current annual flu shot	Positive (n=479)	30 (%)	449 (%)	0.119
	Negative (n=104)	11 (%)	93 (%)	

† Upper respiratory tract infection
‡ Coronavirus disease of 2019

Table 3. Comparison between COVID-19 IgG cut of index and time of contact with positive patients.

		Positive IgG Cut of Index (COI)	P-value
Time of Contact	≤ 8 hours	0.94 (±4.9)	**0.916**
	> 8 hours	0.99 (± 4.7)	

Table 4. Comparison between history of taking the annual flu vaccine and having upper respiratory tract infection.

		URTI Symptom		P-value
		Positive	Negative	
History of taking the current annual flu shot	Positive	184 (38.4%)	295 (61.6%)	0.148
	Negative	32 (30.8%)	72 (69.2%)	

DISCUSSION

In this cross-sectional single-center study, we found the seroconversion rates for SARS-CoV-2 specific IgG/IgM antibodies amongst HCM at a tertiary cancer center to be relatively low at 7.0% (41 of 583 tested HCWs). Except one patient with IgM seroconversion, all patients with IgG/IgM seroconversion tested negative for the novel virus via RT-PCR.

There was no correlation between seroconversion with previous URTI, seasonal flu-vaccination, increased exposure to infected patients, activities that potentially increase the exposure like shopping, and the lack of strict social distancing measures. There was no correlation between seroconversion and history of chronic diseases, including autoimmune diseases, diabetes mellitus, and hypertension.
The most accurate method for detecting SARS-CoV-2 is the RT-PCR from a respiratory secretions / nasal swab sample [16]. However, such testing method fails to provide an accurate timeline for a humoral response to SARS-CoV-2, which necessitated further support by serological analysis [17]. The humoral-specific response has been detected as early as one-day post-onset of symptoms [18].

KHCC developed an internal guideline for the preparedness and management of COVID-19 pandemic. This guideline was prepared and regularly updated by the internal infection control and prevention committee. Entry to the cancer center was limited to two points with screening checkpoints at each. Informative posters and signs were placed in strategic places and educational brochures were provided for patients. Outpatient clinic appointments were categorized based on urgency and those who did not require an in-person visit, or who had a recent positive COVID-19 test were called via telephone. Also, strict measures were implemented to ensure the safety of cancer patients, including modification of treatment plans, strict physical distancing measures, early detection of suspected cases among staff or patients, and isolation measures. Emergency cases, with a positive COVID-19 test, that require immediate surgical intervention were carried out in a specially designated negative-pressure operation room, in which the surgical team adheres to the strict adherence to wearing personal protective equipment [19]. Due to the nature of their work, HCWs are at an increased risk of contracting the novel virus due to the higher rate of exposure, especially with the existence of a subclinical form of COVID-19, which necessitated dual testing for early detection [20]. Guo et al. suggested combining a PCR test of a nasopharyngeal swab and serological tests to accurately quantify the disease burden and detect individuals with active disease [18] or those considered protected from the disease [21]. However, a recent study by Patel et al. showed that 58% of individuals who had positive antibodies to SARS-CoV-2 converted back to negative results within 60 days, a finding that undermines seroprevalence studies which might miss the two months post-infection, making the immunity evaluation of any population, including HCWs, challenging to accomplish [22].

Oran et al. reported that 40-45% of individuals have subclinical COVID-19 presentation. We only could detect 13 out of 41 (31.7 %) HCWs, who reported a URTI, with varying symptoms of cough, fever, congestion, fatigue, headache, rhinorrhea, odynophagia, or wheezing, within the last three months prior to testing, associated with positive IgG seroconversion. Assuming that these were actual cases of COVID-19, those individuals can potentially spread the novel virus silently over more than 14 days [23]. Rivett et al. reported that 4.8% of HCWs had a positive RT-PCR result upon random screening regardless of symptoms. Only 3% from the asymptomatic subcategory were positive, whereas 15.4% from the symptomatic subcategory were positive. Contrary to our study, they included symptoms only up to seven days prior to testing [24]. Due to the overlapping of symptoms between seasonal influenza and COVID-19, Maltezou et al. emphasized the importance of administering the flu vaccine during the pandemic, which has a zero effect on the novel virus but will prevent overwhelming the medical service and save medical resources [25].

Scientific evidences and international reports underline how the evaluation of antibody titers cannot be considered as a diagnostic tool, both for the presence of possible cross-reactivity reactions with other coronaviruses, and for the considerable inter and intra-individual variability in the antibody response elicitation [26, 27].

Study limitation included; 1) using the AFIAS COVID-19 Ab test. Compared to other serological tests, the AFIAS COVID-19 Ab test is not the most sensitive, and it can yield false-negative results [28], increasing the chances of underreporting in the number of HCWs with seroconversions. 2) Difficulty in obtaining other antibody testing kits due to strict regulations by the Jordanian Food and Drug Administration. 3) Participants were self-recruited, which might have introduced bias in the cohort. However, the unplanned equal gender representation and adequate representation from the nurses and physicians is an accepted reflection of KHCC's HCWs. 4) Questions related to previous exposure and URTI impose recall bias. 5) A larger cohort is recommended before generalizing the results.

CONCLUSION

In a tertiary cancer center, we found the rates of SARS-CoA-2 IgG or IgM seroconversion amongst HCW to be relatively low during the COVID-19 pandemic. There was no correlation between IgG seroconversion and history of URTI or prior exposure to infected patients. "Silent carriers" are hard to identify. Amid the endless waves of COVID-19, combined with the slow pace of vaccination rate, combining RT-PCR testing and serological analysis for the novel virus might be the key to slow the transmission of the novel virus, especially within hospital settings.

AUTHORS' CONTRIBUTIONS

Mahmoud Al-Masri: conceptualization, funding acquisition, methodology, project administration, supervision, visualization, and writing – review & editing.

Maysa Al-Hussaini: conceptualization, funding acquisition, methodology, project administration, and writing – review & editing.

Mohamad K. Abou Chaar: data curation, funding acquisition, investigation, methodology, and writing – original draft.

Hani Al-Najjar and Khawlah Ammar: data curation and formal analysis.

All authors reviewed the manuscript and accepted the final version. All authors had full access to the data, and the corresponding author takes final responsibility for the decision to submit for publication.

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DATA SHARING

Data will beavailable once all planned primary and secondary outcomes have been published upon written request and a detailed statistical analysis plan is provided to the authors.

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DISCLOSURES

We declare no conflict of interest.

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