ORIGINAL ARTICLE

Incidence of SARS-CoV-2 re-infection in anti-nucleocapsid IgG-positive healthcare workers: a prospective cohort study

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Abstract

Background Since the pandemic of SARS-CoV-2 began, our understanding of the pathogenesis and immune responses to this virus has continued to evolve. It has been shown that this infection produces natural detectable immune responses in many cases. However, the duration and durability of immunity and its effect on the severity of the illness are still under investigation. Moreover, the protective effects of antibodies against new SARS-CoV-2 variants still remain unclear.

Objectives To assess the incidence and associated demographic features of SARS-CoV-2 infection in anti-nucleocapsid IgG-positive and anti-nucleocapsid IgG-negative healthcare workers.

Material and methods This prospective longitudinal cohort study was conducted in Peshawar Medical College group of hospitals of Prime Foundation. Anti-nucleocapsid IgG sero-positive and anti-nucleocapsid IgG sero-negative healthcare workers were followed for a period of 6 months (from 1 Aug 2020 to 31 Jan 2021), and the incidence of SARS-CoV-2 was confirmed by RT-PCR.

Results A total number of 555 cohorts were followed for a period of 6 months; of them 365 (65.7%) were anti-nucleocapsid-negative (group A) and 190 (34.3%) were anti-nucleocapsid-positive (group B) healthcare workers. The mean age of the study cohort was 33.85 ± 9.80 (anti-N (−), 34.2 ± 10.58; anti-N (+), 33.5 ± 9.50). The median antibody level in anti-nucleocapsid-positive HCWs was 15.95 (IQR: 5.24–53.4). Male gender was the majority in both groups (group A, 246 (67%), group B, 143 (48%)) with statistically significant difference ($P < 0.05$). Majority of the HCWs were blood group B in both groups (34% each). None of the 190 anti-nucleocapsid-positive HCWs developed subsequent SARS-CoV-2 re-infection, while 17% ($n = 65$) HCWs developed infection in anti-nucleocapsid-negative group during the 6-month follow-up period.

Conclusion In conclusion, none of the anti-nucleocapsid-positive HCWs developed SARS-CoV-2 re-infection in this study, and the presence of IgG anti-nucleocapsid antibodies substantially reduce the risk of re-infection for a period of 6 months.

Keywords Anti-nucleocapsid antibody · Healthcare workers · Incidence · Re-infection · SARS-CoV-2

Introduction

The SARS-CoV-2 pandemic began in December 2019 in Wuhan, Hubei Province of China as a cluster of pneumonia-like illness [1].The infection spread rapidly throughout the world and was declared a global pandemic by WHO (World Health Organization) on 11 March 2020 [2]. Since then the number of infections continue to rise. Our understanding of the pathogenesis and immune responses to this virus continue to evolve, and it has been shown that this infection produces natural detectable immune responses in many cases. However, the duration and durability of

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immunity and its effect on the severity of the illness is still under investigation [3]. Moreover, the protective effects of antibodies against new SARS-CoV-2 variants still remain unclear [3–5].

There is limited evidence regarding the post-infection immunity; despite the emergence of new variants with faster rate of transmission and a very high global rate of infections, there is little evidence of re-infection [6]. One of the main reasons could be a lack of diagnostic facilities especially in developing countries. Although there are some reports suggesting that naturally developing antibodies maybe associated with protection up to a few months, longitudinal studies comparing infection rates in sero-positive and sero-negative individuals are still lacking [7].

Following infection, immunity may be contributed by both the innate and adaptive immune systems of the human body having different cells with specific functions. The adaptive immune system comprises B cells, CD4 + T cells, and CD8 + T cells. B cells produce antibodies. CD4 + T cells possess a range of helper and effecter functions, and the CD8 + T cells kill virus infected cells. COVID-19 virus is an enveloped, single-stranded RNA virus and is composed of 16 nonstructural (NS) proteins and 4 structural proteins named as Spike (S), Envelope (E), Membrane (M), and nucleocapsid (N) [8]. Serologic tests based on the presence of antibodies against the antigens to these structural proteins (S) and (N) have increased our understanding of the epidemiology, seroprevalence rates, and identifying potential convalescent plasma donors. However, the duration of persistence of antibodies and its neutralizing role confirming immunity against a subsequent infection is still an area of intense clinical research [9].

We performed a prospective cohort study to assess and compare the incidence and associated demographic features of subsequent SARS-CoV-2 infection confirmed by real-time polymerase chain reaction (RT-PCR) in anti-nucleocapsid-positive and anti-nucleocapsid-negative healthcare workers for a period of 6-month follow-up.

Methodology

This prospective cohort study was conducted from August 01, 2020, to January 31, 2021. All the study participants (HCWs) were employees of Peshawar Medical College (PMC) group of hospitals of Prime Foundation. The study was approved by the Institutional Review Board (IRB) of Prime Foundation, Pakistan. Participant of the study were selected using non-probability consecutive sampling technique. After written and informed consent, physical examination was performed and data regarding the co-morbidities and socio-demographic characteristics were recorded on a structured proforma. At the time of study, none of the participants was vaccinated against the virus. Standard protocols were followed in the hospitals and at home by all the participants to limit the spread of infection; this included hand hygiene, social distancing, and using of personal protective equipment (PPE).

Baseline SARS-CoV-2 antibody testing were performed using Roche Elecsys®S-CoV-2kit [9]. The assay uses a recombinant protein representing the nucleocapsid (N) antigen in a double-antigen sandwich assay format. On the basis of antibody testing, the healthcare workers (HCWs) were then divided into antibody positive and negative. Both groups were followed for a period of 6 months. Symptomatic HCWs were offered real-time polymerase chain reaction (RT-PCR) testing of nasopharyngeal swab specimen. The PCR analyses were made using Geneproof ® SARS-CoV-2 PCR kit. Immunocompromised healthcare workers and those above 60 years and below 18 years of age were excluded from the study.

Results

A total number of 555 cohorts were followed for a period of 6 months; of them, 365 (65.7%) were anti-nucleocapsid-negative (group A) and 190 (34.3%) were anti-nucleocapsid-positive (group B) healthcare workers. The mean age of the study cohort was 33.85 ± 9.80 (anti-N (–), 34.2 ± 10.58; anti-N (+), 33.5 ± 9.50). The median antibody level in anti-nucleocapsid-positive HCWs was 15.95 (IQR: 5.24–53.4). Majority of the HCWs in group A were in the age group 31–40 years (n = 128, 35%), while age group 20–30 years has the highest number of HCWs in group B (n = 92, 48%). Male gender was the majority in both groups (group A, 246 (67%); group B, 143 (48%)) with statistically significant difference when chi-square test was applied (P < 0.05). (Table 1)

Majority of the HCWs were identified with blood group B in both groups (34% each) followed by blood group O (27%), blood group A (26%), and blood group AB (13%) in anti-nucleocapsid-negative group and blood group A (32%), blood group O (23%), and blood group AB (11%) in anti-nucleocapsid-positive healthcare workers. In both groups, RH factor (negative) were the highest (group A 95%, group B 77%) compared to RH factor (positive) (group A 5%, group B 23%). When blood groups of the COVID-19 re-infected HCWs were checked, majority had blood group A (33.8%) followed by O (29.2%) and B (20%), while only 16% had blood group AB. (Table 3)

None of the 190 anti-nucleocapsid-positive healthcare workers developed subsequent COVID-19 re-infection, while 17% (n = 65) HCWs developed infection in anti-nucleocapsid-negative group during the 6-month follow-up period. (Table 2).

Table 1 Socio-demographic characteristics

	Anti-nucleocapsid negative (Group A)	Anti-nucleocapsid positive (Group B)	*P* value
Mean age in years (± SD)	33.5 ± 9.50	34.2 ± 10.58	
Median (IQR) antibody level (AU/ml)	–	15.95 (5.24–53.4)	
Age Group			
20–30	124 (34%)	92 (48%)	< 0.01
31–40	128 (35%)	58 (31%)	
41–50	62 (17%)	24 (13%)	
51–60	51 (14%)	16 (8%)	
Gender			
Male	246 (67%)	143 (75%)	< 0.05
Female	119 (33%)	47 (25%)	
Blood group			
A	95 (26%)	61 (32%)	> 0.05
B	124 (34%)	65 (34%)	
AB	47 (13%)	21 (11%)	
0	99 (27%)	44 (23%)	
RH factor			
Positive	18 (5%)	44 (23%)	< 0.01
Negative	347 (95%)	146 (77%)	
Total	**365 (100%)**	**190 (100%)**	

Discussion

Since December 2019, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been rapidly spreading across the globe. Over the past 1 year, our understanding of the pathogenesis and diagnostic possibilities of the disease has been rapidly evolving.

Serology can play a major role in the true estimation of prevalence of SARS-CoV-2 infection as it detects the asymptomatic or subclinical cases too. These assays have a high sensitivity, and antibodies can be detected in 1 up to 2 weeks following infection [10]. The serologic tests developed so far are limited to the detection of antibodies against one or two antigens, and they may cross-react with antibodies to other human corona viruses. The serology for detection of emerging coronaviruses is largely focused on antibodies against the spike (S) protein, particularly the S1 domain, and the nucleocapsid protein (N) but how to optimally combine antigens to most accurately detect strain-specific coronavirus antibodies still remains a debatable issue [11]. There is some data available regarding the sero-prevalence in various population subsets and the degree to which they are at risk of developing infection with SARS- CoV-2 [12]. There is currently little knowledge available regarding the duration of persistence of the antibodies and their durability as well as their protective role in human subjects [13].

The results of our study show several interesting features. It was found that age has a positive association with the antibody titers so antibody response may differ in various age groups as shown in Table 1. Similarly, it was observed that Rh status also affects the antibody levels; however, the ABO status has no significant association with the levels of antibodies achieved (Tables 2 and 3). Gender of the participants also had a significant association with the N antibody response (Table 1).

None of the antibody-positive participants developed a subsequent re-infection, and it is safe to assume that anti-nucleocapsid antibodies can confer immunity for at least 6 months irrespective of the antibody titers. Further follow-up may clarify the duration for which the antibodies persist and continue to provide protection against this deadly virus.

A study shows that the N-antibody may persist up to 8 months [14]. Lumley, Sheila F et al. also found similar results; in their study, the presence of anti-nucleocapsid

Table 2 Subsequent re-infection distribution among anti-nucleocapsid-positive and anti-nucleocapsid-negative participants

	Subsequent/ infection Developed	Subsequent re-infection Not developed	Total
Anti-nucleocapsid positive	0 (0%)	190 (100%)	190 (100%)
Anti-nucleocapsid negative	65 (17.8%)	300 (82.2%)	365 (100%)

Table 3 Blood group distribution among re-infected participants

Blood Group	COVID-19 infection (n)	(%)
A	22	33.8%
B	13	20.0%
AB	11	16.9%
O	19	29.2%
Total	**65**	**100%**

IgG antibodies was associated with a reduced risk of SARS-CoV-2 reinfection [15]. In their study, the antibody persisted for 6 months similar to our findings. In another the trajectory of the neutralizing antibodies (S and N antibodies) showed that the median neutralization potency decreased by 45% per month, and S-based serological assay best predicted the neutralization potency [16]. In contrast, our study found that the antibody titers of the N type provided immunity and all participants with positive antibodies remained infection free for six months.

In our study, the major limitation was the fact that following up the RT-PCR was done only on those who became symptomatic and the actual incidence of subsequent infections could be higher if the PCR was done on the asymptomatic participants as well. Despite this limitation, the results have provided new insights into the pathogenesis of SARS-CoV-2 infections, and further studies with longer follow-up are needed to confirm and quantify the protective role of these antibodies.

Conclusion

In this longitudinal study, none of the anti-nucleocapsid-positive HCWs developed SARS-CoV-2 re-infection, and the presence of IgG anti-nucleocapsid antibodies substantially reduces the risk of re-infection for a period 6 months. Further studies are needed to assess the risk of re-infection in sero-positive individuals beyond a 6-month period and in diverse population.

Declarations

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