

## Comparison of SARS-COV-2 Infection Between Sero Positive and Sero Negative Healthcare Workers After Six Months in a Tertiary Care Hospital

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### ABSTRACT

**Objective:** To compare the frequency of SARS-CoV-2 infection between Sero-positive and Sero-negative healthcare workers after 6 months in a tertiary care hospital.

**Study Design:** Prospective Cohort study.

**Place and Duration of Study:** Combined Military Hospital, Kohat Pakistan, from Aug 2020 to Jan 2021.

**Methodology:** Two Hundred and eighty-eight healthcare workers were included by non-probability consecutive sampling. They were divided into two groups according to SARS-CoV-2 IgG-Ab status at baseline as sero-positive and sero-negative. They were followed for six months period and symptomatic healthcare workers were tested for SARS-CoV-2 infection by RT-PCR. Individuals from all departments were included and used standard personal protective equipment.

**Results:** Out of 288 Healthcare workers 240(83.33%) were male and 48(16.67%) were female. At baseline, 42(14.58%) individuals were sero-positive and 246(85.42%) were sero-negative for SARS-CoV-2 Ab. During 6 months of follow up 28(11.38%) symptomatic sero-negative Health Care Workers were tested positive for COVID-19 infection by RT-PCR. None of the sero-positive Health Care Workers was tested positive for SARS-CoV-2 infection. Risk of infection was higher in sero-negative group (Odds ratio 1.19 with 95% CI; 1.13- 1.26).

**Conclusion:** SARS-CoV-2Ab may offer immunity against COVID-19 infection as no infection was observed in sero-positive individuals as compared to 11.2% in sero-negative individuals.

**Keywords:** COVID-19, Healthcare workers, SARS-CoV-2 Ab, Sero-negative, Sero-positive.

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### INTRODUCTION

Over 0.5 million cases of current COVID-19 pandemic have been reported across Pakistan with over twelve thousand deaths and it continues to increase day by day. It is a great challenge worldwide for health perspective and economic threat as well.<sup>1</sup> Detectable immune response against infection is produced in most cases, but the extent to which this immune response offer protection to re-infection in previously infected person is yet not clear. Whether postinfection immunity exists or not, for how long it protects from re-infection and the extent to which it can prevent symptoms or reduce its severity are the major questions that are yet to be answered during SARS-CoV-2 pandemic.<sup>2</sup>

Humoral and cell-mediated immunity are the major post infection protective responses developed after an infection. Important considerations while

investigating post infection immunity are identifying functional correlates of protection, identifying measurable surrogate markers, and defining end points, such as prevention of disease, hospitalization, death or further transmission. B cells produce antibodies against organism that are specific to organism. Initially IgM antibodies are produced which disappear in few weeks time and 2-3 days later IgG antibodies are developed. These antibodies are more mature, specific, and produced in high titers and offer long term immunity for months or years.<sup>3-4</sup> Antibodies are produced against different proteins of organism. Same is true for SARS-CoV-2. Assays have been developed for SARS-CoV-2 anti-spike and anti-nucleocapsid antibodies with different dynamics.<sup>5</sup> Association between antibody titers and neutralizing ability against virus is assay and time dependent. Different assays have been developed for SARS-CoV-2Ab detection. These assays may detect IgM-Ab, IgG-Ab or both against one of its proteins. The association between antibody titers and protection offered is dependent on assay type and time of infection.<sup>6-8</sup> The

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assays that detect more mature IgG-Ab are more accurate, precise and can quantify Ab titer. Roche diagnostics also developed a highly specific quantitative SARS-CoV-2 Ab assay for detection of anti-nucleocapsid Ab on a sophisticated automated immunoassay analyzer. This assay is primarily designed to detect more mature high affinity Ab for return work fitness, donor fitness for convalescent plasma and to estimate sero-prevalence studies. Evidence for postinfection immunity against current SARS-CoV-2 infection is emerging. More than 100million people have been infected worldwide and transmission is still going on, but reported cases of re-infections are rare. These cases of re-infection are reported mostly after asymptomatic or mild primary infection.<sup>9</sup> The data from most studies suggests that SARS-CoV-2 infection offers some immunity against re-infection.<sup>10</sup>

We performed a prospective cohort study in health care workers to assess the relative risk of incidence SARS-CoV-2 in sero-positive and sero-negative individuals. Anti nucleocapsid IgG-Ab were measured at baseline and SARS-CoV-2 polymerase-chain-reaction (PCR) test was carried out in symptomatic HCW during follow up for six months. Frequency and odds ratios for SARS-CoV-2 infection were calculated for sero-positive and sero-negative HCW.

**METHODOLOGY**

The prospective cohort study was performed at the Department of Pathology, Combined Military Hospital Kohat, Pakistan from August 2020 to January 2021 after approval by IERB (Certificate no E-2005/A dated 25 Feb 2021). Sample size was calculated using WHO sample size calculator for hypothesis test for relative risk estimation with 95% confidence interval incidence in sero-positive individuals 0.13% as compared to 1.09% in sero-negative individuals.<sup>2</sup>

**Inclusion criteria:** All adult HCWs regardless of age and their prior COVID-19 status (either sero-positive or sero-negative at baseline) were included.

**Exclusion criteria:** Individuals who could not provide baseline serum samples for SARS-CoV-2 specific quantitative antibody assay, did not complete the informed consent process or had incomplete baseline or follow-up data, were excluded.

A total of 288 Health Care Workers (HCW) from the hospital were included by non-probability consecutive sampling after informed consent of

patients regardless of prior COVID-19 status. Selected subjects were divided into two groups as Sero-positive (HCW positive for SARS-CoV-2 Ab at base line) and Sero-negative (HCW negative for SARS-CoV-2 Ab at base line). Base line Anti SARS-CoV-2 IgG Ab was measured. Selected individuals were followed by for 6 months and symptomatic subjects were tested for COVID-19 by Reverse Transcriptase-Polymerase Chain reaction (RT-PCR) for SARS-CoV-2.

Baseline SARS- CoV-2 specific quantitative antibody assay was done from serum samples and analyzed on Roche E411 automated immunoassay analyzer by electrochemiluminescent method. RT-PCR for SARS-CoV-2 RNA was performed on Cepheid smart cycler after auto extraction from Nasopharyngeal swabs samples.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Odds ratios with 95 % confidence interval for the risk of COVID-19 infection were calculated between sero-positive and sero-negative HCW.

**RESULTS**

Out of 288 HCW 240(83.3%) were male and 48(16.7%) were female with mean age of 34.48±7.15. Of these 39 were doctors and 249 were paramedical staff including nurses as shown in table. At baseline 42(14.6%) subjects were positive (Sero-positive) and 246(85.4%) negative (Sero-negative) for SARS- CoV-2 specific IgG Ab. Baseline data of different qualitative variables is given in Table-I.

**Table-I Baseline SARS-CoV-2Abstatus in Different Qualitative Variables (n=288)**

Variables	SARS-CoV-2Ab status	
	Sero-positive	Sero-negative
All cases	42(14.6%)	246(85.4%)
Male	31(12.91%)	209(87.09%)
Female	11(22.91%)	37(77.09%)
Doctors	5(12.82%)	34(87.18)
Paramedics	37(15.86%)	212(84.14%)

After 6 months of follow up no case of COVID-19 was reported in sero-positive subjects as compared to 28(11.38%) cases in sero-negative subjects (Odds ratio with 95% confidence interval 1.19)as shown in Table-II.

We also observed that frequency of COVID-19 was higher in doctors as compared to paramedical staff

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and female were more likely infected as compared to male as shown in Table-III.

**Table-II: Odds ratio estimation for Risk of COVID-19 in Sero-positive and Sero-negative HCW with 95% Confidence Interval (CI) (n=288)**

Risk of infection	SARS-CoV-2Ab status	
	Sero-positive	Sero-negative
PCR Positive	00	28(11.38%)
PCR Negative	42(100%)	218(88.62%)
Odds ratio with 95% CI	00	1.19(1.13-1.26)

Medicine reported low incidence of re-infection in sero-positive healthcare workers over 31 weeks of follow up. They used anti nucleocapsid IgG and Anti spike IgG antibodies as a marker of sero-conversion. Incidence of infection was 1.09% in sero-negative HCW as compared to 0.13% in sero-positive individuals. This also revealed the possibility of protection against SARS CoV2 among the persons who were sero-positive in the beginning.<sup>2</sup> Another study showed That incidence of SARS-CoV-2 infection amongst the health care

**Table-III Odds ratio for Risk of COVID 19 among Gender and Professional Groups with 95% CI (n=288)**

Risk of infection	Groups			
	Male VS. female		Doctors VS. Paramedics	
PCR Positive	23 (9.58%)	5 (10.41%)	7(17.94%)	21(8.43%)
PCR Negative	217 (90.42%)	43 (89.59%)	32(82.06%)	228(91.57%)
Odds ratio with 95% CI	0.984(0.821-1.179)	1.080(0.466-2.501)	2.031(0.990-4.169)	0.855(0.687-1.064)

### DISCUSSION

SARS CoV- 2 (COVID-19) has emerged as global pandemic and a global challenge for this world. Health care workers, researchers, scientists all are very keen to explore the dynamics of the disease and bring the facts on the surface.<sup>11</sup> Evidence for post infection immunity against current SARS-CoV-2 infection is emerging. Cases of re-infection are reported but are rare. Different anti body assays are being used for diagnosis and for possible immunity in exposed persons.<sup>12-14</sup> In our study, we compared two groups of health care workers which were actively involved in patient management in their respective departments in a prospective cohort study during 6 months of follow up. The group which was Sero-positive in the beginning for anti-SARS-CoV-2 nucleocapsid IgG antibodies were associated with a high protection against SARS-CoV-2 re-infection. No case of PCR Positive re-infection was reported in 42 Sero-positive HCW as none of them developed any symptom related to SARS-CoV-2 infection. On the other hand 28 out of 246(11.38%) HCW who were sero-negative at beginning developed RT-PCR positive SARS-CoV-2 infection (Odds ratio 1.19; 95% confidence interval 1.13-1.26). The health care workers of both the groups in our study followed the similar standard protocols like using PPE as per their nature of duty, social distancing and frequent hand washing and almost had the similar type of exposure but the outcome was different. Our results also showed higher incidence of infection in females (10.41%) as compared to the males (9.58%) and higher among doctors (17.94%) as compared to the other HCW (8.43%). Lumley *et al.* in a similar study published in New England Journal of

workers is inversely related to baseline anti-nucleocapsid antibody titers.<sup>15</sup>

Another study conducted by Lumley *et al.* reported that Anti-spike IgG Ab were detectable for a median of 180 days while Anti-nucleocapsid IgG Ab were detectable for a median of 121 days. Higher maximum observed anti-nucleocapsid titers were associated with longer estimated antibody half lives. Old age, Asian ethnicity and prior reported symptoms were associated with higher levels anti-nucleocapsid Ab levels.<sup>16</sup> In our study, we found a lower risk of re-infection with SARS-CoV-2 up to six months among health care workers who were positive for antinucleocapsid IgG antibodies as compared to those who were sero-negative at baseline. Although we did not retest but we assumed them to be protected as they remain symptom free. A study conducted by Dong *et al* demonstrated that SARS-CoV-2 patients who were discharged with elevated levels of neutralizing antibody levels had protective humoral immunity to re infection.<sup>17</sup>

Zhang *et al.* reported re-infection in two patients who showed reductions in IgG anti-SARS-CoV-2 Ab. Both of them were sero-positive at the time of first infection but at re-infection one of them was sero-negative and other was only weak positive before throat swab viral RNA was detected again.<sup>18</sup> A study conducted by Seow *et al.* showed that high Neutralization Ab (nAb) response was seen post infection in all individuals with peak at 23.1 days (range 1-66 days). Only two individuals (3.1%) did not show any nAb response. After 65 days potent nAb response was observed in only 16.7% individuals, which shows decrease in protection beyond this

period.<sup>19</sup> These results were in contrary to our results which showed protection up to 6 months, however we did not measure AB response in asymptomatic individuals due to financial constraints. Differences may also be attributed to different strains of viruses infecting different regions and different assays and protocols used for testing.

Long *et al*, reported COVID-19 IgG levels in the asymptomatic patients (median 3.4; IQR, 1.6-10.7) were significantly lower ( $p=0.005$ ) compared to the symptomatic group (median 20.5; IQR, 5.8-38.2). There result suggested that asymptomatic individuals had a weak immune response to SARS-CoV-2 infection<sup>20</sup>. This may be one of the reason of differences in results of different studies because severity of infection may affect Ab response.

#### LIMITATIONS OF STUDY

The limitations of our study were that we used only one marker i.e. antinucleocapsid IgGAb for the sero-conversion. It is however suggested that other markers like anti spike IgG antibodies and markers of cellular immunity may further be explored in future studies to establish their roles in the prevention of re-infection against SARS CoV2. Furthermore, our cohort was relatively a younger age group of less than 60 years of age, this opens the window for further research on persons who are over 65 years of age and became seropositive. SARS CoV2 being the novel disease is a challenge and needs a lot of research on its distinct aspects in future.

#### CONCLUSION

SARS-CoV-2Ab may offer immunity against COVID-19 infection as no infection was observed in sero-positive individuals as compared to 11.2% in sero-negative individuals.

**Conflict of Interest:** None.

#### Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

MA & MYR: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

TA & ML: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

SN & MSJ: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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