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Frequency of SARS-CoV-2 Antibodies in PCR-Positive COVID-19 Patients and High-Risk Exposed Subjects at Multan

Sana Iqbal, Muhammad Younas, Raja Kamran Afzal*, Asma Khan*, Farhat Yasmeen, Muskan Younas*****

Department of Pathology, Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Department of Pathology, Combined Military Hospital, Multan/National University of Medical Sciences (NUMS) Pakistan, **Combined Military Hospital/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, ***Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To investigate the degree of association between Reverse Transcription Polymerase Chain Reaction positivity and seroconversion after natural COVID-19 infection in Multan, City of Pakistan.

Study Design: Cross-sectional study.

Place and Duration of Study: Combined Military Hospital, Multan Pakistan from Apr 2021 to Sep 2021.

Methodology: In this study, 219 Healthcare Workers with suspected SARS-CoV-2 infection were screened via Reverse Transcription Polymerase Chain Reaction for viral genome, followed by detection of corresponding antibody response in serum samples within ten weeks of their first exposure against spike protein via Chemiluminescence immunoassay.

Results: There was a significant association between positive RT-PCR and detectable corresponding antibodies (p=0.001). However, we found no evidence of an association between age and RT-PCR positivity and between age and detectable antibodies (p=0.874 and 0.842, respectively). Furthermore, results indicated no association between gender and RT-PCR positivity and between gender and detectable antibodies (p=0.536 and 0.285, respectively).

Conclusions: It is concluded that antibody detection against SARS-CoV-2 virus spike protein is a useful laboratory tool for screening for COVID-19 infection.

Keywords: Antibody test, Asymptomatic cases, COVID-19, Healthcare workers, PCR, SARS-CoV-2.

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INTRODUCTION

Corona Virus Disease-19 (COVID-19), a highly contagious infectious disease, was first identified as an outbreak of respiratory illness in Wuhan City of China in late 2019. First, on 30 January 2020, World Health Organization (WHO) declared it a global health emergency, but later, on 11 March 2020, it was declared a global pandemic.[1] The pandemic attributed to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), a novel strain of coronaviruses which continues to reshape the word.[2] This unexpected and unprecedented illness has intensely harmed humans, the economy and healthcare systems worldwide. Isolation strategy, personal protection measures, and social distancing have proven effective in confining the spread of disease.[3] However, some decision-makers prefer to relax the strict social distancing due to economic constraints and political pressure. In such a scenario, asymptomatic and pre-symptomatic infected cases are the hidden contaminants and weaken the viral spread control. Studies have shown that about thirty to forty-five per cent of confirmed COVID-19 patients have either mild or no symptoms at all but are potential sources of infection.[4] Situation becomes more challenging in hospital settings, where COVID-19 is highly infectious during patients' asymptomatic and pre-symptomatic periods. The nosocomial transmission of COVID-19 to healthcare workers (HCWs) and other patients can severely impact hospital performance. HCWs are the most exposed and vulnerable group to acquiring COVID-19 infection.[5] Asymptomatic and pre-symptomatic infected HCWs themselves are the potential source of transmission. The majority of hospitals have developed preadmission screening strategies for COVID-19. As this disease's clinical presentation is highly variable, the role of laboratory findings is pivotal for diagnosis and screening.[6] Most agencies, including Centres for Disease Control and Prevention (CDC) in the United States, have recommended COVID-19 Polymerase Chain Reaction (PCR). In COVID-19 PCR, SARS-CoV-2 RNA is detected by reverse transcription real-time polymerase chain reaction (RT-PCR) in nasopharyngeal and oropharyngeal swab specimens. SARS-CoV-2 migrates downward from the nasopharyngeal mucosa to the throat

Correspondence: Dr Sana Iqbal, Department of Pathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan

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and pulmonary alveoli. Therefore, the number of viral genomes copies tend to be higher in the nasopharyngeal compartment at the onset of infection and then declines progressively.[7]

Type of specimen, site of specimen and the time since onset of symptoms are critical parameters for COVID-19 diagnosis. In addition, the sampling technique, extraction method and type of analysis also greatly influence the PCR results. With limited test availability, the requirement of specialized instruments, experienced technical staff, increased turnaround time and a high proportion of mild and asymptomatic infections, more than the COVID-19 PCR test is needed as a screening tool. On the other hand, an immunoassay-based COVID-19 antibody test for the qualitative detection of human immunoglobulin G (IgG) in serum and plasma specimens is rapid, cost-effective and relatively easy to perform even in peripheral laboratories.[8,9] However, limited data is available on the development of antibody response against SARS-CoV-2 antigens, particularly the formation of IgG. The efficacy of antibody tests as a laboratory tool for diagnosing SARS-CoV-2 is also a concern, as little is known about long-lasting immunity. Antibody test has low sensitivity, particularly in the early stage of infection, to diagnose COVID-19. However, they complement other testing in individuals presenting later when RT-PCR tests are negative or not done. Furthermore, the antibodies test can be a useful tool to determine the prevalence of COVID-19 infection, which can be unobserved by inadequate/unreachable RT-PCR testing, particularly among asymptomatic patients. Our study aims to evaluate the relationship between COVID-19 RT-PCR positive results and corresponding antibodies in patient serum, mainly in asymptomatic or pre-symptomatic patients.

METHODOLOGY

The cross-sectional study was conducted at Combined Military Hospital, Multan Pakistan after the approval of the Institutional Ethical Review Board Committee (ERC No. 10/2020 dated 20th September 2020) from April 2021 to September 2021. The study comprised 219 HCWs enrolled through a non-probability consecutive sampling technique. The sample size was estimated by using WHO calculator with a 7% prevalence of COVID-19.[10] All individuals were enrolled after taking informed written consent.

Inclusion Criteria: Health care workers suspected of SARS-CoV-2 infection who were recently exposed to COVID-19 RT-PCR-positive patients, including doctors, nurses, paramedics and janitor staff, were enrolled by non-probability sampling. Only asymptomatic and individuals with mild or vague symptoms with a strong history of recent exposure or positive contact history were included in this study.

Exclusion Criteria: Participants who developed positive findings on HRCT for COVID-19 infection or well-established symptoms during the study were excluded.

Individuals were tested for SARS-CoV-2 RNA in respiratory samples, then detected corresponding antibody responses in serum. Samples for SARS-CoV-2 RNA detection were taken aseptically after taking all necessary protection precautions from the posterior pharyngeal wall and the nasopharynx by trained laboratory staff. SARS-CoV-2 RNA extracted with fully automated extractor Super Extract 32 by SYSTAAQ Diagnostic Products, USA. After extraction, the detection was done by using CFX96 Real-Time PCR Detection System, BIORAD. Immunoglobulin G (IgG) samples against SARS-CoV-2 spike protein were taken within ten weeks of exposure in a plain gel tube and spun at 3400 rpm for four minutes to get the serum. Tests were run on the principle of electrochemiluminescence immunoassay by using a CLIA kit on a fully automated analyser Cobas E411 by Roche Diagnostics.

Results were analysed using Statistical Package Social Science (SPSS) version 23:00. For categorical variables, and Chi-Square tests were applied to evaluate the association of gender, RT-PCR, and antibody response for the SARS-CoV-2 virus. For a continuous variable, an independent sample t-test was used. The p-value <0.05 is considered significant.

RESULTS

Two hundred-nineteen HCWs suspected of COVID-19 infection were included in our study, with a mean age of 32.73±9.7 and 128(58.45%) males and 91(41.55%) females. Out of 219 participants, 79(36.07%) were found to be positive for SARS-CoV-2 on RT-PCR, and 140(63.93%) were negative for SARS-CoV-2 on RT-PCR. Among RT-PCR positive cases for SARS-CoV-2, 45(56.96%) out of 79 had reactive and 35(44.30%) had non-reactive anti-Spike antibodies in their serum. Among RT-PCR negative cases for SARS-CoV-2, 105(75.00%) out of 140 had non-reactive anti-Spike antibodies in their serum. Detailed data describing the association of RT-PCR with anti-spike antibodies is presented in Table-I. There was a significant association between the positive RT-PCR for SARS-CoV-2

and reactive antibody response against spike protein (p-value<0.001). Interestingly out of 140 patients who were negative for SARS-CoV-2 RNA on RT-PCR, 35(25.00%) had reactive antibodies against SARS-CoV-2 spike protein in their serum. Seroprevalence among asymptomatic and pre-symptomatic exposed HCWs (219) was 80(36.53%). There was an association between positive RT-PCR and detectable antibodies. However, we have no evi-dence of an association between gender and RT-PCR positivity (p=0.536). Our study comprised 128(58.45%) male HCWs and 91 (41.6%) females. Among the males positive percentage for RT-PCR is 35.94%, and for females was 36.26%.

Similarly, no significant association was found between gender and detectable antibodies against SARS-CoV-2 spike protein (p=0.177). Of 128 male HCWs, 43(33.59%) have reactive anti-spike antibodies in their serum, and 85(66.41%) have non-reactive anti-spike antibodies. Among females, 37(40.66%) have reactive, and 54(58.45%) have non-reactive anti-spike antibodies in their serum. Detailed data is presented in Table-II. Furthermore, we have found no evidence of an association between age and RT-PCR positivity and between age and detectable antibodies. The only significant association was between positive RT-PCR and reactive anti-Spike antibodies for SARS-CoV-2 (Table-III).

Table I: Comparison between RT-PCR positive and negative results for COVID-19 (n=219)

Parameters		Total (n=219)	PCR Positive (n=79)	PCR Negate (n=140)	p-value
Age (year)	Mean±SD	32.73±9.7	32.69±9.5	32.91±9.76	0.874
Gender	Male	128(58.45%)	46(35.94%)	82(64.06%)	0.536
	Female	91(41.55%)	33(36.26%)	58(63.74)	

Table-II: Association of Gender with reactive Anti-Spike Antibodies (n=219)

			Anti-spike Antibodies		p-value
			Reactive	Nonreactive	
Age (years)		Mean±SD	32.66±10.29	32.94+9.4	0.842
Gender	Males	n(%)	43 (33.59%)	85(66.41%)	0.285
	Females	n(%)	37(40.66%)	54(59.3%)	
Total		n(%)	80 (36.53%)	139 (63.47%)	

Table-III: Comparison of COVID-19 RT-PCR with corresponding Antibodies (n=219)

		RT-PCR for Covid-19		p-value
		Positive (n=79)	Negative (n=140)	
Anti-spike antibodies	Reactive	45(56.96%)	35(25%)	<0.001
	Non-reactive	34(43.04%)	105(75%)	

DISCUSSION

COVID-19 is a highly contagious disease; therefore, an early and accurate diagnosis is critical not only for treatment but also to identify the potential sources of spread, particularly in asymptomatic or pre-symptomatic patients.[11,12] HCWs are the most exposed group to COVID-19 infection and can be the potential source of transmission. Symptoms of the illness usually develop 8-16 days after the infection. Significant spread of infection can occur before the development of the symptoms (pre-symptomatic). Furthermore, some fractions of infected individuals do not develop symptoms (asymptomatic). Such conditions demand aggressive testing to identify the potential source of infection early. The standard diagnostic tests used to confirm SARS-CoV-2 infection are based on detecting viral genome in upper airway samples by real-time reverse-transcriptase polymerase chain reaction.[13] The RT-PCR is a time-consuming test creating diagnostic delays and requires expensive specialized equipment, reagents, and skilled laboratory staff. These factors limit the use of RT-PCR in peripheral laboratories of underdeveloped countries and in situations when rapid diagnosis is required. Antibody assay, on the other hand, is a simple, inexpensive, and rapid alternative. Our study suggested that there is a high probability of developing antibodies after COVID-19 infection, and it can be a useful laboratory tool as it is a simple, inexpensive, rapid, and easily available test. A study by Chughtai *et al*. conducted in Lahore city of Pakistan, included 154 participants to find out the frequency of IgG antibodies against COVID-19 in exposed asymptomatic individuals and showed that 24 individuals had reactive antibodies despite being asymptomatic, which supports our findings.[14] A large cohort study conducted in New York City, USA, on the prevalence of SARS-CoV-2 antibodies in healthcare personnel (n =70, 812) showed a seroprevalence of 13.7%. In this study, 2186(36.0%) healthcare personnel were PCR positive; out of these, 2044(93.5%) had positive corresponding antibodies.[15] This finding strongly correlates with our study.

The specimens used for RT-PCR are swabs taken from the upper respiratory tract, which is another limitation to this test as false negative results can be yielded due to the poor quality of the sample or attaining the sample at an incorrect timeframe.[16,17] False-negative results could hamper the efforts to prevent and confine the infection, particularly when this test plays a key reference role in deciding hospital

admission, discharge, quarantine, or emergency interventions. Li *et al*. conducted at the Fever Clinic of the Beijing Haidian Hospital in January 2020 indicated that two out of ten cases which were negative on the RT-PCR test were finally confirmed to be positive for SARS-CoV-2 infection, yielding a false-negative rate of around 20% for RT-PCR.[18] Serological assays based on different antibody types used to diagnose SARS-CoV-2 can compensate for the limitations of RT-PCR. These tests are less expensive, relatively easy to perform and may not require specialized instruments as they can be done on routine chemistry analysers. Antibodies are rapidly produced after the infection, and their detection could be a more practical alternative to RT-PCR, particularly in small peripheral laboratories. It is also a good tool to evaluate the overall rate of COVID-19 infections and particularly the prevalence of infection among asymptomatic. However, our data showed that the sensitivity of antibody tests is comparatively less in asymptomatic or pre-symptomatic patients. Its yield may be increased in symptomatic patients. Sensitivity of antibodies test may also vary with different manufacturer kits. Certain studies state that some antibody tests (that use specific antigens or epitopes) are more suitable to diagnose SARS-CoV-2 infection in asymptomatic cases than others. However, more serological data is needed to compare antibody test yield in symptomatic and asymptomatic patients.

LIMITATIONS OF STUDY

Our study has a limitation that only asymptomatic or pre-symptomatic patients were examined. Some studies have shown that symptomatic COVID-19 patients have a high yield. For infection control, which necessitates screening asymptomatic subjects, an antibody test alone might not be sufficient and may be used in combination with RT-PCR in doubtful cases. The time interval between the exposure and conduction of the antibody test is also a limiting factor for the yield of this test. Nonetheless, antibody tests could be helpful for physicians in diagnosing COVID-19 infection where an RT-PCR facility is unavailable.

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CONCLUSION

SARS-CoV-2 detection by RT-PCR is the cornerstone for the infection diagnosis but has limitations due to false negative results. Antibodies test is a useful alternative tool for cost-effectiveness, availability at peripheral laboratories, throughput, low sampling errors, and high sensitivity with techniques like ECLIA and large-scale utility. Nevertheless, its sensitivity is comparatively low in asymptomatic and pre-symptomatic patients. We recommend the collection of more serological data to establish the clinical and epidemiological usefulness of antibody tests to detect infection in asymptomatic and pre-symptomatic patients.

Conflict of Interest: None.

Authors Contribution

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

SI: & MY: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

RKA: & AK: Study design, drafting the manuscript, data interpretation, approval of the final version to be published.

FY: & MY: Concept, 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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