Seroconversion in Newly Diagnosed Cases of Coronavirus Disease

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ABSTRACT

Objective: To determine the percentage of seroconverted real time reverse transcriptase polymerase chain reaction (RT-PCR) positive coronavirus disease (COVID-19) cases at different days post-symptom onset; and also find the agreement of chemiluminescence assay used for total antibody detection using RT-PCR as a reference method.

Study Design: Cross-sectional study.

Place and Duration of Study: Chughtai Institute of Pathology from April to May 2020.

Methodology: Fifty pre-pandemic samples (healthy population) and 75 COVID-19 patients were included in the study. RT-PCR confirmed COVID-19 patients were divided into 3 equal groups (25 each), according to the days of symptom onset. The samples were analysed using electro-chemiluminescence as assay principle. Positive and negative agreement of COVID-19 antibodies was calculated using EP evaluator to find out the sensitivity of chemiluminescence assay for total antibody detection. The results were analysed using SPSS version 23.0.

Results: All the pre-pandemic samples tested were negative for antibodies with a negative agreement of 100%. Total agreement at day 7 post-symptom onset was 84%; whereas, it was 94% at day 14 and increased rapidly to 100% at day 21 post-symptom onset. At day 7 post-symptom onset, 68% of patients were seroconverted; and this percentage was 88% and 100% at day 14 and 21 post-symptom onset, respectively.

Conclusion: Pre-pandemic samples were non-reactive for COVID-19 antibodies and seroconversion started within the first week post-virus exposure. There was 100% concordance between RT-PCR result and antibody positivity 21 days post-symptom onset.

Key Words: *COVID-19, SARS CoV-2, Seroconversion, Chemiluminescence.*

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INTRODUCTION

Twenty-seven mysterious cases of pneumonia were diagnosed in China on December 31, 2019. The causative organism was identified with the help of swab test (throat or nasopharyngeal) at Disease Control and Prevention Center in the month of January 2020.[1] WHO named this disease as coronavirus disease (COVID-19) and the organism was named as *SARS CoV-2*.[2] Mild symptoms like dry cough, fever and headache were present in many patients suffering from COVID-19; whereas, some of the cases remained asymptomatic and resolved spontaneously.

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On the other hand, some cases turned out with fatal complications such as multiorgan failure, sepsis, pneumonia, pulmonary edema and severe respiratory distress syndrome.[3] Uncertainty prevails regarding the mode of spread of this viral disease. According to the current knowledge, this virus is transmitted through respiratory fomites; and symptomatic patients are highly contagious.[4] On the other hand, asymptomatic patients can also spread virus in their incubation period.[5]

The presently reported figures can be an underestimation of the disease in Pakistan as the data depicts laboratory confirmed diagnosed cases only.[6] The only strategy to disrupt the spread of COVID-19 in Pakistan is early and correct diagnosis, isolation of infected cases, and timely treatment. Moreover, there should be a tracing system for close contacts of the infected cases.

Numerous healthcare companies have developed testing methodologies to diagnose COVID-19 infection. The gold standard diagnostic test available for COVID-19 is real time reverse transcriptase PCR (RT-PCR) having the highest sensitivity and specificity.[7] It detects viral genome and is widely used for diagnosis, monitoring disease spread, patient triage and current infection status. On the other hand, there are some serological tests also which comment on the immune status of the patient. These serological tests assess the immune response of the individual

against the virus by detecting the presence of antibodies in blood. The RT-PCR requires nasopharyngeal swab or throat swab sample; whereas, serological assays require blood sample.

There are numerous suggested uses of these serological tests including serosurveys and determining immune status of population. With the use of convalescent plasma to treat COVID-19 patients, it is of utmost importance to find out the sensitivity and specificity of available assays. Very recently, fully automated enzyme immunoassays using chemiluminescence as principle have been introduced in Pakistan to determine antibodies in COVID-19 patients claiming high sensitivity and specificity.

The aim of this study was to find out the positive and negative agreement of chemiluminescence assay used for total antibody detection using RT-PCR as a reference method in our laboratory; and to find out the percentage of population seroconverted after the onset of symptoms at different time periods.

METHODOLOGY

It was a cross-sectional study conducted at Chughati institute of Pathology, Lahore from April to May 2020. The study was approved by the Institutional Review Board. Blood samples were collected from 75 patients (18 to 60 years of age), both males and females, who were RT-PCR positive for *SARS CoV-2*, according to the days of post-symptom onset. These patients were admitted in corona isolation wards and high dependency units. The day when the first symptom appeared was considered to mark the onset of symptoms. Patients were having mild (fever, fatigue, myalgia, dry cough, sore throat), moderate (fever, fatigue, myalgia, productive or dry cough, sore throat, difficulty in breathing without respiratory distress, abdominal pain and diarrhea) to severe symptoms (development of dyspnea and hypoxemia along with diarrhea). Asymptomatic patients and cases with critical symptoms like acute respiratory distress syndrome, respiratory failure, encephalopathy, heart failure, coagulopathy and multiple organ failure were excluded from the study. None of the patients included in the study was on mechanical ventilation.

These patients were divided into three equal groups on the basis of days of post-symptom onset, but not severity. Group A consisted of 25 patients (PCR positive), whose sample were collected within 7 days of symptom onset. Group B consisted of 25 patients in which sample collection was done at 14 days of post-onset of symptoms. Group C consisted of 25 patients, whose sample was collected at 21 days of post-symptom onset. Three ml blood from each was collected and centrifuged at 3000 RPM for analyses. Control group included 50 serum specimens of healthy male and female adult population, collected and frozen at -80 degree Celsius, in November 2019, before the emergence of *SARS CoV-2* infection in Pakistan. The samples were analysed using fully automated analyser with electro-chemiluminescence as assay principle. This assay measured total antibody levels with a specificity of 99.8% and sensitivity of 100% at 14 days or more after RT-PCR confirmation of infection. After quality check, controls were within defined limit. A cutoff of 1.00 was established to see the presence or absence of COVID-19 total antibodies. Samples showing values greater than cutoff were labelled as reactive and those with values less than the established cutoff were labelled as non-reactive. Positive and negative agreement of COVID-19 antibodies was calculated using EP evaluator to find out the sensitivity of chemiluminescence assay at different days of post-symptom onset in COVID-19 patients and in pre--pandemic samples.

The results were analysed using SPSS version 23.0 to find out percentages and frequencies. Cohen's Kappa was calculated by EP evaluator, for agreement above what is expected by chance. A Cohen's Kappa >75% indicates high agreement.

RESULTS

All the pre pandemic samples were tested negative for antibodies with a negative agreement of 100%. In Group A, at day 7 post-symptom onset, 17 patients were reactive for antibody with a total agreement of 84% (positive agreement and Cohen's Kappa = 68%). The total agreement in Group B at day 14 post-symptom onset was 94% (positive agreement and Cohen's Kappa = 88%) which increased rapidly to 100%. In Group C at day 21 post-symptom onset (positive agreement and Cohen's Kappa = 100%) 95 percent confidence interval was calculated by score method.

The results of seroconversion in COVID-19 patients, according to the days after symptom onset, are given in Table I.

Table I: Percentage of seroconverted patients at different of days of post-symptom onset.

Seroconverted patients (n=75)	Number of patients	Percentage of patients
7 days after onset of symptoms	17	68%
14 days after onset of symptoms	22	88%
21 days after onset symptoms	25	100%

DISCUSSION

RT-PCR is the preferred molecular test of choice to confirm COVID-19, while serological tests act as supplemental tools. Serological response of the body takes time to develop. Therefore, serology has low sensitivity in the early course of the infection. In the recovered patients, viral RNA can no longer be detected *via* RT-PCR; and past exposure of the virus can only be detected with the help of serological testing. Moreover, the immune status of a population and its ability to resist or contract a viral attack is based on the antibody status of the population.[8] Due to the current emergency situation, US-FDA (United States Food and Drug Administration) has given emergency authorisation for the use of antibody testing for COVID-19. However, clinicians need to understand that most of the assays available today in the market still need Clinical Laboratory Improvement Amendments (CLIA) certification and these serological tests cannot be performed in a private office due to several complexities associated with these assays.[9]

All pre-pandemic samples were negative for the presence of

antibodies in the current study. This study revealed that the median time for seroconversion of patients was day 5 to 14. Within seven days of symptom onset, 60% of the infected patients developed antibodies. Low percentage of seroconversion in our study can also be related to the low sensitivity of assay at day 7 post-symptom onset (65.5%). A study conducted by Zhao *et al*. revealed that less than 40% of the patients who were tested within 7 days of symptom onset were positive for antibodies.[10] The authors stated that the most possible reason of this low percentage is sample collection at an early stage of disease. In this study, 88% of the patients became positive for antibodies against *SARS CoV 2* after day 14 of symptom onset and this percentage raised rapidly to 100% after 21 days of symptom onset.[10] This is in agreement with the above stated study in which 100% of the study subjects were tested positive for antibodies since day 14 after symptom onset (98% for IgM and 80% for IgG). Another study conducted in China stated that 100% of the patients were tested positive for IgG within 19 days of symptom onset and the antibody titers plateaued 6 days after seroconversion.[11] Another serological analysis conducted on COVID-19 patients in Paris, France revealed that seroconversion and virus neutralisation occurred on 5 to 14 days of post-symptom onset.[12] Rongqing Zhao along with his colleagues in China found that the average antibody levels of PCR confirmed COVID-19 cases, increased during hospital stay and two weeks after discharge. In this study, some of the asymptomatic medical staff involved in the study showed a raised antibody titer and one out of five PCR negative close contact of COVID-19 patients turned out to be reactive for antibody.[13]

In Pakistan, the pandemic is growing at an alarming rate and healthcare authorities along with government agencies may seek additional epidemiological solutions. Singapore and Taiwan's programmes can be followed by using surveillance programmes with enhanced testing facilities to trace and contain affected cases.[14] A recent report published by Yong and colleagues described the researchers to be able to trace the RT-PCR negative cases with the help of serological testing in Singapore. The researchers highlighted the success of serological assays in capturing the cases and slowing the spread of infection in Singapore.[15] This report emphasised that RT-PCR cannot be used as a sole method for diagnosis in surveillance studies because it is not meant to detect past infection. On the other hand, serological studies, if done within accurate timeframe after virus exposure, can detect recent and past exposure. This is evident from this study, where seroconversion was seen within first week of symptom onset, and antibodies were detected after 21 days of symptom onset when RT-PCR was negative for most of the patients.[16]

In Pakistan, serological analysis can be used to identify close contacts and can define affected population clusters. Contact tracing is very difficult in densely populated areas of Pakistan but by following the example of Singapore, it is possible to demarcate transmission chains and estimate the percentage of affected population. The serological assay data can help government officials to define control policies by assessing the number of individuals who have developed an immune response. Serological surveys can also help to decide the time to resume public activities and control infection spikes in the population.

CONCLUSION

Seroconversion starts within the first week of post-virus exposure and there is 100 % concordance between RT-PCR result and antibody positivity at 21 days post-symptom onset. However, samples were not collected subsequently so no information is available on duration of antibody positivity post-infection.

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ETHICAL APPROVAL:
Ethical approval was obtained by Institutional Review Board of Chughtai Institute of Pathology prior to initiation of the research work.

PATIENTS' CONSENT:
Informed consent was obtained from all patients to publish the data concerning this study.

CONFLICT OF INTEREST:
Authors declare no conflict of interest.

AUTHORS' CONTRIBUTION:
ORC: Orginal concept, study design, supervision.
HB: Paper write-up, data collection and analysis, literature review.
MDK: Proofreading, approval, discussion.
SA: Data collection, statistical analysis.

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