ESTIMATING SENSITIVITY AND SPECIFICITY OF COVID-19 ANTIBODIES ASSAY POST RT-PCR TESTING

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ABSTRACT

Objective: To determine sensitivity and specificity of Roche Sars-cov-2 antibodies assay using real time polymarase chain reaction (RT-PCR) Covid-19 as standard in Pakistani population.

Methodology: It was a cross-sectional study conducted in Rehman Medical Institute Peshawar from 1st January 2021 till 15th February 2021. This study include 192 suspected Covid-19 patients. Serum samples set consisted of 122 symptomatic RT-PCR positive patients and 70 negative RT-PCR were used for qualitative detection of Antibodies (CoVID-19 IgG, IgM). Overall and period wise (Post RT-PCR) diagnostic accuracy was determined by comparing results of antibodies assay to Rt-PCR. Chi square test was applied to assess the correlation between post-PCR duration and Anti-SARS-Cov-2. A p-value of ≤0.05 was considered significant.

Results: On post-PCR duration and Anti-SARS-Cov-2 analysis, it was evident that the sensitivity of detection increased steadily with increase in duration after viral detection reaching a value of 94.5% after 20 days. Overall sensitivity was 86.9% with PPV of 97.3%. There was a significant level (X2= 6.846, p =.033) of correlation between detection probability of Antibodies and post-PCR duration at 95% CI. Anti-SARS-Cov-2 showed a specificity of 95.7% and a NPV of 81.7%.

Conclusion: Our study demonstrated that in middle and later stages of disease antibodies to SARS-CoV-2 can be detected. This testing strategy can be utilized complementary to molecular based testing of CoVID-19 diagnosis. Also, this can help in determining the seroprevalence of CoVID-19 in community.

Key Words: COVID 19, Anti-SARS-CoV-2, RT PCR

INTRODUCTION

Severe acute respiratory syndrome due to SARS-CoV-2 was identified in China in December 2019.[1] The disease COVID-19 began in Wuhan city of Central China and rapidly spread to involve the whole world. On 11th March, 2020 the WHO declared this outbreak a pandemic.[1] Worldwide, WHO reports172,630,637 cases of COVID-19, including 3,718,683 deaths.[2] Coronavirus has led to 1229347 confirmed cases of COVID-19 with 2238 deaths in Pakistan till date.[3] Till 18 November 2021 according to statistics of government of Pakistan more than 96% patients have recovered from corona virus and death rate is around 2.2%.[3]

SARS-CoV-2 is a single-stranded, enveloped, RNA virus in the Coronaviridae family. Coronaviruses are structurally similar to each other, consisting of 16 non-structural proteins and 4 structural proteins: spike, envelope, membrane and nucleocapsid proteins. The presentation of Covid 19 infection has got a wide variety of symptoms starting from mild to severe to critical cases; most of the infections are not of severe intensity.[4-10] Majority of patients are asymptomatic which creates confusion regarding the diagnosis of the disease. Prompt and accurate diagnosis of COVID-19 infection is necessary especially in asymptomatic subjects to limit further spread of the virus. Serological assays can quickly identify people who have been infected to the virus and quantify the level of exposure in a community, thus helping to decide whether to impose, implement or relax containment measures.[11] Diagnostic accuracy of real time polymarase chain reaction (RT-PCR) relies on variables like sample type, its collection, transportation, storage and RT-PCR assay quality.

Tremendous progress has been made in the domain of in vitro diagnostic assays for COVID-19 infection. Various serological immunoassays are being developed in order to complement the molecular diagnostic assays and to provide a quick and cheaper way to identify the patients. These include chemiluminescent immunoassay (CLIA), rapid lateral flow immunoassay (LFIA) and ELISA which are based on detection of immunoglobulins (IgM and IgG).12 In the first 10 days of

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COVID-19 infection antibodies to anti-SARS-CoV-2 are detectable in most patients. Antibody testing demand is increasing, and a significant number of studies are being conducted. Regarding quality assurance of anti SARS- antibody tests, there is an imminent requirement for multiple validation studies and global quality assurance programs. Sensitivity, specificity and cross-reactivity are the variables that can affect the interpretation of test results. Comparing total antibodies test-to a solitary IgM or IgG test, the combined IgM-IgG assay has better utility and sensitivity.13 The main aim of this study was to determine the diagnostic accuracy (Sensitivity, specificity, positive and negative predictive values) of this qualitative detection assay(Anti SARS-CoV-2).

METHODOLOGY

This cross-sectional descriptive study was performed in Rehman Medical Institute Peshawar, from 1stJanuary till 15thFebruary 2021. The research and ethical committee of the Rehman Medical Institute Peshawar approved the study. Participants were included by nonprobability convenient sampling technique. Patients of both genders were included in study. Informed consent was taken from all the patients. Serum samples of 192 patients who were suspected for Covid-19 were included in this study. Demographic data (Age, sex) and clinical information of the study group was obtained from Record section RMI. Cough was the most prevalent symptom (60%) followed by fever (54%) and shortness of breath (22%), loss of smell (45%), and body aches 25%). All the suspected patients were already tested by RT-PCR were also analyzed for antibodies (CoVID-19) to assess the diagnostic accuracy of the test. It is a qualitative test to detect antibodies (CoVID-19) in the patient's serum. Instrument used was Cobas e 411 auto analyzer that uses Electro-chemiluminescent immunoassay (ECLIA) technique to detect the target. ECLIA is a double-antigen sandwich assay. The assay utilizes a recombinant protein which represents nucleocapsid protein of the virus. Antibodies present in patient's specimen attaches tobiotynylated/ recombinant nucleocapsid antigen and a ruthenium labelled recombinant antigen to form a sandwich complex. This complex is made to unite with streptavidin coated microparticles and these microparticles are then captured on the surface of an electrode. When voltage is given to electrode, chemiluminescence is generated which is measured by a photomultiplier tube.[14] Results are generated in the form of a cutoff index (COI), reactive (COI >1.0; positive) and non-reactive (COI < 1.0; negative).[14] Serum samples were segregated into 3 groups on the basis the number of days between the RT-PCR and the antibody test. Group 1: 0 to 10 days, Group 2: 11 to 20 days and Group 3: More than 20 days. All the data collected was analyzed using SPSS version 23. The demographics like age and gender were calculated as mean and standard deviations. The correlation of Antibodies test with the PCR and post PCR duration (in positive cases only) was analyzed for the derivation of Antibodies test sensitivity, specificity, positive predictive and negative predictive values. Chi square test was used to assess the correlation between Antibodies test and post PCR duration, a p value of $\leq$0.05 was considered significant at 95% CI.

RESULTS

The number of males was greater (139) as compared to females (53). Gender-wise distribution of patients is shown in Table No 1. On post-PCR duration and Anti-SARS-CoV-2 analysis, it was evident that the sensitivity of Anti-SARS detection increased steadily with the increase in duration after PCR starting from 74.1 % and reaching a maximum value of 94.5 %, with an overall sensitivity of 86.9% (Table 2). There was a significant level (X2= 6.846 and p =.033) of correlation between the detection probability of Antibodies detection and post PCR duration at 95% confidence interval on applying chi-square test. Overall sensitivity, specificity, NPV and PPV is shown in table.

For specificity we analyzed RT-PCR negative cases by antibodies detection assay (Roche) which showed an overall specificity of 95.7%. Positive predictive value (PPV) and Negative predictive value (NPV), of antibodies detection assay (Roche) were 97.3% and 81.7% respectively

DISCUSSION

Our study revealed that the sensitivity of antibodies detection assay (Roche)in the Group 1 (post PCR duration 0-10 days) was 74.1% which was higher than sensitivity of Roche Anti-SARS-CoV-2 (65.5 %) in the 0 – 6 post PCR days category as claimed by the manufacturer[15] and even much higher than that deduced by Lau et al (48.2 %).[16] Similarly, in Group 2 (post PCR duration 11-20 days) sensitivity was 85% which was comparable to 81.1% by Roche diagnostics in the 7-13 days category and higher than sensitivity derived by Lau et al (75.6%). In Group 3 (post PCR duration > 20 days) the sensitivity was 94.5% which was lesser than that of Roche diagnostics (100%) in the >14 days post PCR duration category and also lesser than that derived by Lau et al with a post PCR duration of >21 days. Overall sensitivity of our study was 86.9% which was comparable to that derived by diagnostic support group public health England (83.7%).[14] Our study concluded a specificity of 95.7% for the assay which was lower than 100 % specificity of manufacturer and the 100% specificity derived by diagnostic support group public health England.[14,15] Andrew et al reported a 100% sensitivity and specificity of Anti SARS IgG assay of ABBOT diagnostics [17] which was higher than the values of our study. According to a study conducted in Germany, the overall specificity for the Roche Anti-SARS-Cov-2 was 99.82 %

Table 1: Mean Age and Gender Distribution

Gender	Number	% age	Mean Age ± Std. dev.
Male	139	72.4	39.86 ± 14.697
Female	53	27.6	40.62 ± 17.866

Table 2: Correlation of Post Pcr Duration with Anti-Sars-Covid-2

Post PCR duration of Anti SARS Covid-2 in days	Number	Reactive	Non-Reactive	Sensitivity at 95 %CI
(Group 1) 0-10	27	20	7	74.1%
(Group 2) 11-20	40	34	6	85%
(Group 3) >20	55	52	3	94.5%

Table 3: Comparison of Results of Anti Sars-Cov-2 with Sars Rt Pcr

	SARS-COV-2 PCR Positive	SARS COV-2 PCR Negative
Anti SARS-Cov-2 Positive	106	3
Anti SARS-Cov-2 Negative	16	67

Table 4: Diagnostic Performance of Anti Sars-Cov-2 Assay

Anti SARS-Cov-2 Sensitivity	86.9%
Anti SARS-Cov-2 Specificity	95.7%
Anti SARS-Cov-2 PPV	97.3%
Anti SARS-Cov-2 NPV	81.7%

which was comparable to 95.7% specificity of present study.[18] This study demonstrated a positive predictive value(PPV) of 97.3% which was lower than a PPV of 100% given by diagnostic support group England and a negative predictive value of 81.7% which was again lower than a value of 98.6% given by the diagnostic support group.[14]

The current study and the studies in comparison concluded that sensitivity of the ECLIA Antibodies detection assay increased considerably after 14 days of PCR confirmation, which denotes the mounting of considerable antibody response after this period of time. This fact is further strengthened as a result of study conducted by Zhao et al revealing that after 15 days of symptoms onset antibody response was 100.0 %.[19] Herroelen et al reported that Wantai SARS-COV-2 Ab ELISA and Antibodies detection assay (ROCHE) have 95 % sensitivity and 100% specificity10 days after onset of symptom.[20] This again supports the diagnostic ability of Roche Total antibodies detection assay. However further studies on this assay can help us understand the presence of these antibodies and detection ability of this assay beyond the period which is not covered in this study. Further research is required to completely understand in terms of the severity of sickness once exposed, following recovery, and future reinfection, how the presence or lack of SARS-CoV-2 antibodies influences continuous vulnerability to the virus and possible immunity. Limitation of the study was that we used a qualitative assay for the determination of immune status of the patients and the second limitation was that assay was a total antibody assay and it could not differentiate the disease stage (acute, chronic or convalescent) of the patient. Such assays that identify other parameters linked with the immune response, such as quantified antibody levels and neutralizing antibodies, are resource expensive and not yet commonly convenient in the present pandemic.[21]

CONCLUSION

Our study demonstrated that antibodies detection assay (Total antibodies) performs excellent in middle and later stages of disease (CoVID-19). Consequently, this study advocates the routine application of serological testing for diagnosis along with molecular based testing. After the advent of vaccination against CoVID-19 this test can help in assessment of the immune status of the population.

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Author's Contribution

BI wrote the article and collected the data, KB designed study and wrote the article, MH analyzed the data and searched the literature, MMD proof read the article and collected the data, MUF analyzed the data and searched the literature. 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.

Conflict of Interest

Authors declared no conflict of interest

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None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.