SARS-COV-2 Neutralizing IgG Antibodies in Patients Recovered from COVID-19 in a Hospital-Based Study: From Predictions to Reality

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¹,²,³ Conception of study
¹,²,³ Experimentation/Study conduction
¹,³,⁴ Analysis/Interpretation/Discussion
¹,²,³ Manuscript Writing
³,⁴ Critical Review

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Abstract

Introduction: The duration and immunity nature in form of antibodies to COVID-19 is still not clear. But so far we know that an immune response protects the individuals from future illness for some specific time. The presence or absence of immunity tells us about the future transmission of illness.

Objectives:
1. To determine the frequency of antibodies to COVID-19 after three weeks of negative PCR report.
2. To predict the association of the antibodies values with the severity of the disease the patient had in the recent past.

Material and Methods: We did a cross-sectional study in the department of Pathology on 36 patients recovered from COVID-19 from 3rd July 2020 to 31st July 2020. Antibodies level was measured by electro-chemiluminescence immunoassay using Roche Cobas E411 Chemistry Analyzer for which commercial kits of Roche diagnostics were used as per the instructions of the manufacturer, in respondents after 3 weeks of a negative PCR report.

Results: The mean with standard deviation for numerical variables i.e. (age and antibodies) was 34±11 years and 34.52±12.71 respectively. 29(80.55%) were males and 7(19.45%) females. The mean antibodies level was statically significantly higher in patients with age less than 50 years (p-value: 0.01). Patients who had severe symptoms at the time of being reported positive by PCR had significantly higher antibody cut-off values as compared to asymptomatic positive patients (p-value:0.04). Clinically severe Covid-19 patients positively moderates with an increase in the values of the post-infection anti-SARS-COV-2 antibodies supported by an interaction term with ($\beta=0.388**; \Delta R^2=0.126, p-value=0.02$). Male gender supported by the results of an interaction term with ($\beta=0.388**; \Delta R^2=0.126, p-value=0.02$) had the severe disease as compared to females.

Conclusion: The higher anti-SARS-COV-2 antibodies were reported in patients with age <50 years and in patients who had severe symptoms or a history of hospitalization during the infectious period. There was a statistically significant relationship supported by the interactions in terms of higher post-infection antibodies level with the male gender, an increase in age and with history of severe disease or hospitalization.

Keywords: COVID-19, Pandemic, SARS-CoV-2 Antibodies.
Introduction

COVID-19 (Coronavirus disease) reportedly originated from the wild meat market of a metropolitan city, Wuhan, China. It was reported to the World Health Organization in Dec 2019. It causes severe respiratory disease/pneumonia. It clinically manifests with high-grade fever, cough, generalized body aches, loss of taste and smell and dyspnea. By date April 15, 2020, globally 19 million people are infected with Coronavirus disease (COVID-19) with more than 120000 deaths from all over the world. In Pakistan the so far (24th July 2020) reported data from government sources to declare 270,400 confirmed cases with 5763 deaths. 219783 cases are recovered so far and 1316 cases are under treatment in high dependency units countrywide. Sindh is the province with the highest number of corona cases crossing 115883.

The response to the pandemic was tremendous from all over the world focusing mainly on the treatment options, vaccination and acquiring the ability for serological tests to identify those with immunity to this deadly virus. That was also the main goal of the plasma convalescent therapy approach worldwide used to some extent for patient management. The dynamics and nature of the immune response to the COVID-19 are still limited. Antibodies’ testing is done to detect immunoglobulins that are produced in human blood in response to infection with COVID-19. However, the issue is the time period that for how long these antibodies are effective to protect an individual against the COVID-19. The antibodies production is related to the severity of the infection and reportedly last for 3 months after the onset of the first symptoms. Again currently researchers know very little about the longevity of these neutralizing antibodies to respond, a study suggesting that re-infection with corona is possible after 80 days in patients who developed antibodies against COVID-19. That is worrisome for many scientists expecting a second wave of COVID-19 due to re-infection of those who were positive by PCR with mild symptoms and low levels of neutralizing antibodies. The possible explanation to how much extent the males are more susceptible to acquire infection with more consequences is that the number of death reported is caused by the gender behaviour (lifestyle) i.e. smoking, drinking, and working abroad is more among men as compared to women. The IgG antibodies production in mild, moderate and severe COVID differs in gender groups. Antibodies levels in the female gender tend to be rise as compared to males which may account for a different outcome of COVID.

The present study was therefore designed to determine the frequency of antibodies to COVID-19 after three weeks of negative PCR report and to predict the association of the antibodies values with the severity of the disease the patient had in the recent past.

Materials and Methods

This cross-sectional study was conducted in the Department of Pathology on 36 patients recovered from COVID-19 from 3rd July 2020 to 31st July 2020 as we have been able to purchase one kit (200 tests) on administration, that had an expiry of one month only and we have been able to perform more than 150 tests out of which 36 were the patients who were reported negative on PCR, 3 weeks before. Those patients were only picked up from the data and further studied. Ethical Approval was taken from the ethical review board of Nowshera Medical College Nowshera. Verbal consent was taken from all the respondents that their confidentiality shall be maintained.

The criteria for selection of the candidates was that only those patients who were reportedly negative on PCR for 2019-nCoV done 3 weeks ago, were included for their antibodies status. All those candidates who came for antibodies test without being reported with infection with confirmed PCR test for virus detection were excluded. Similarly, patients with a duration less than three weeks of being reported negative for coronavirus were also excluded from the study.

The procedure was to collect 3ml of the blood in Gel tube under aseptic conditions. Antibodies level was measured by electro-chemiluminescence immunoassay using Roche Cobas E411 Chemistry Analyzer for which commercial kits of Roche diagnostics were used as per the instructions of the manufacturer, in respondents after 3 weeks of a negative PCR report.

Data was uploaded in the SPSS version 25. Numerical variables like age and anti-SARS-COV-2 antibodies levels were presented as mean with standard deviation and range. We applied the Shapiro-wilk test to see the distribution of data and with an insignificant p-value, we observed a normal distribution of data to develop a baseline for further analysis.

Categorical variables like age categories, gender and clinical presentation were presented with percentages. An independent t-test was used to determine the
difference in mean antibodies level in different groups/categories like gender, age categories and categories of clinical presentation.

Linear regression test was used using SPSS version 25 with the task to predict a positive link of the dependent variable, input (antibodies levels, age and gender) with the independent variable; output (clinical presentation at the time of being reported positive on PCR). Interaction terms/values like beta value, variation and significance were used to predict the relationship of different variables with the severity of the disease the patient had at the time of active infection.

Results

The mean age of patients with standard deviation was 34±11 years while mean antibodies cut off values of patients with standard deviation was 34.52±12.71. (Table 1)

We included 36 respondents who attended the Pathology laboratory of QHAMC for testing their anti-SARS-COV-2 antibodies after 21 days of being negatively reported for 2019 nCoV on PCR. 29 (80.55%) were females and 7 (19.45%) females. On the Shapiro-wilk test to see the distribution of data and with an insignificant p-value we observed a normal distribution of data to develop a baseline for further analysis. (Figure 1)

A statically significant difference in mean antibody cut-off values was noted in age groups of patients. We observed that patients with age<50 years had developed higher antibodies as compared to patients with age>50 years of age (p-value: 0.01). No significant difference of antibodies in gender groups was observed with insignificant (p-value=0.61). The difference in mean post-infection antibodies level was statistically significant with higher cut off values in a patient who had severe symptoms at the time of being reported positive by PCR as compared to an asymptomatic patient (p-value=0.04). (Table 2)

Clinically severe COVID-19 positively moderates with an increase in the values of the post-infection anti-SARS-COV-2 antibodies supported by the results of an interaction term with (β=0.388**, ΔR²=0.126, p-value=0.02). The value of R², i.e. (R²=0.126), shows that exposure (in terms of production of antibodies) is accounted for the variability of 12.6% in the cases of severe COVID. The p-value which is (p=0.02), supported the statistically significant relationship. The stated results showed that post-infection antibodies cut off values are positively associated with the severity of the disease the patient had. One unit increment in the antibodies cut off values predicts an increment of 3.8% in the rate of the severity of the disease the patient had.

We observed that an increase in age has been shown to have a predictive value for severe COVID-19 as supported by the linear regression analysis. Beta value, i.e., (β=0.064**), showed that there was a positive link between an increase in age and severe Covid-19. One unit increment in the age in years will cause a 6.4% increment in the rate of the severity of the disease. The value of R², i.e. (R²=0.004), shows that exposure is accounted for the variability of 0.4% in the cases of severe COVID. The p-value which is (p=0.001), supported the statistically significant relationship. (Table 3)

Clinically severe Covid-19 positively moderates with gender. Male gender supported by the results of an interaction term with (β=0.388**, ΔR²=0.126, p-value=0.02) had the severe disease as compared to females. (Table 3)

Table 1: Descriptive statistics of age and antibodies cut off values

<table>
<thead>
<tr>
<th>Descriptive statistics</th>
<th>Mean with SD</th>
<th>Range (Max-Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34±11 (years)</td>
<td>45 (65-20)</td>
</tr>
<tr>
<td>Antibodies</td>
<td>34.52±12.71</td>
<td>126.92 (127-0.08)</td>
</tr>
</tbody>
</table>

Table 2: Mean difference in Anti-SARS-COV-2 antibodies in age and gender groups and based on a clinical presentation at the time of positive PCR reporting. (Independent T-Test)

### 2.1. Age Categories

<table>
<thead>
<tr>
<th>Age</th>
<th>Number</th>
<th>Mean ±SD of anti-SARS-COV-2</th>
<th>p-value (Independent t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age&lt;50y</td>
<td>32(88.88%)</td>
<td>35±13</td>
<td>0.01</td>
</tr>
<tr>
<td>Age&gt;50y</td>
<td>4(11.12%)</td>
<td>28±2.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2. Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
<th>Mean ±SD of anti-SARS-COV-2 Antibodies</th>
<th>p-value (Independent t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>29(80.55%)</td>
<td>34±20</td>
<td>0.61</td>
</tr>
<tr>
<td>Female</td>
<td>7(19.45%)</td>
<td>35±13</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.3. Clinical presentation

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Number</th>
<th>Mean ±SD of anti-SARS-COV-2 Antibodies</th>
<th>p-value (Independent t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinically asymptomatic</td>
<td>18(50%)</td>
<td>22±14</td>
<td>0.04</td>
</tr>
<tr>
<td>With severe symptoms/hospitalized</td>
<td>18(50%)</td>
<td>47±23</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Linear regression analysis to predict severity of Covid-19 against different variables like age, gender, post-infection antibodies cut-off values

<table>
<thead>
<tr>
<th>Name of predictor</th>
<th>B (β-value)</th>
<th>ΔR² (Variability)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibodies value</td>
<td>0.038**</td>
<td>0.126</td>
<td>0.02</td>
</tr>
<tr>
<td>Age</td>
<td>0.064**</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.089**</td>
<td>0.024</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Significance at p=0.01**, Significance at p=0.05*

Figure 1: Distribution of cut off antibodies values in gender groups

Discussion

As the pandemic is almost declining in terms of severity and mortality in Pakistan, we tried to estimate the neutralizing antibodies levels in patients recovered from COVID-19 in our teaching hospital. The majority of the recovered patients were the health care workers of the teaching hospital with a mean and standard deviation of the age was 34±11 years while mean antibodies cut off values of patients with standard deviation was 34.52±12.71. Age factor matters in the COVID-19 infection, its severity and mortality. In one of our interventions published in the journal of World Family Medicine, we had concluded that a statistically significant difference was noted among the age groups (p=0.024). Studies from China have reported a high rate of the severity of infection with high mortality risk...
of 0.48 in an increase in age. The antibodies are less useful for diagnosis and screening for early infection, however, these are helpful to protect the individual from re-infection. It is a known fact from the so for research that a higher antibody title was directly associated with a worse clinical classification (p=0.006) that was consistent with an understanding that patients with severe disease have a higher antibody titre in their plasma to neutralize the antigenic virus along with high levels of other inflammatory markers like d-dimers, ferritin and c reactive proteins.

We observed that patients with age<50 years had developed higher antibodies as compared to patients with age>50 years of age (p-value: 0.01) that indicates a high immunity level of the younger population. In another study that we published in Microbes and infectious disease (MID), we observed that the rate of infectivity and exposure to come across in contacts with positive patients with a probability of 2 times more than in people with age<55 years (p=0.005, OR=2.01) while the case fatality was three times more than in patients with age >55 years (p=0.001, OR=3.16). The mortality rate was 5.41%,

No significant difference of antibodies in gender groups was observed. However clinically severe Covid-19 positively moderates with male gender supported by the results of an interaction term with (β=0.388**; ΔR²=0.126, p-value=0.02) as compared to the female gender. Studies have reported that women exhibit greater antibody response as compared to their counterparts. Antibodies production is higher in the female gender in an early phase of the disease which is one of the possible mechanisms that mediates wishful recovery in females from COVID-19.

Clinically severe disease moderates with an increase in the values of the post-infection anti-SARS-COV-2 antibodies supported by the interaction terms with (β=0.388**; ΔR²=0.126, p-value=0.02). The IgM appears after 5-7 days of the initial symptoms and disappears in the second week, where the IgG antibodies start to appear and reach a peak at 35 days after the onset of symptoms and peaking at 4 months after the resolution of the infection and then gradually decreases but can remain for up to 2 years. Studies reveal that quantitative detection of antibodies against SARS-COV-2 has a strong potential for evaluating the severity and prognosis of the disease which shows a concordance with our findings.

Using an independent t-test we observed that patients who reportedly had severe symptoms or hospitalization history had a higher level of antibodies as compared to the patients without symptoms at the time of being reported positive for COVID-19 (p=0.04).

We are aware of the findings of the other researchers who reported high levels of IgG in mild and severe cases but lowers when the disease enters the critical phase (Critical vs mild (p=0.03), critical vs severe (p=0.02)). The possible explanation would be because of high disease activity and low immune response in critically ill patients.

We concluded that the higher anti-SARS-COV-2 antibodies were reported in patients with age <50 years and in patients who had severe symptoms or a history of hospitalization during the infection period. There was a statistically significant relationship supported by the interactions in terms of higher post-infection antibodies level with the male gender, an increase in age and history of severe disease or hospitalization.

The study is the first to report the clinical relevance of the use of IgG neutralizing antibodies against the SARS-COV-2 in our province and probably in the country. We covered a small sample that can not be sufficient to represent a population, however, it directs the researcher to conduct larger population studies to see the clinical impact, protective role, correlation of these antibodies with the severity of the disease, duration of protection etc.

## Conclusion

The higher anti-SARS-COV-2 antibodies were reported in patients with age <50 years and in patients who had severe symptoms or a history of hospitalization during the infectious period. There was a statistically significant relationship supported by the interactions in terms of higher post-infection antibodies level with the male gender, an increase in age and with history of severe disease or hospitalization.

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