Abstract:
Background: Antibodies (Abs) are produced by B cells after infection with the SARS/COVID-19 virus. The presence of neutralizing antibody is an indicator of protective immunity for most viral infections. But, we still don’t know how long and how effectively this immune protection will cover.
Objectives: This study aimed to estimate the antibodies level in PCR-confirmed COVID-19 subjects in non vaccinated healthcare personnel.
Methods: SARS-CoV-2 specific total Abs (IgG and IgM), IgG of nucleocapsid (N) protein and spike (S) protein levels were estimated using two clinically validated and widely used serological assays, detecting antibodies against the Total Antibody, nucleocapsid(N) and spike(S) proteins.
Results: A total 130 subjects with PCR-confirmed SARS-CoV-2 infection were included in this study and all subjects were symptomatic and blood samples were collected between 3 to 24 weeks. Of all participants, about 52% were female and mean age was 43.2 years. The study found that the Total Abs, IgG of N protein and neutralizing Abs of S protein were developed 100%, 74.6% and 93.8% respectively. The study also found that the IgG titers of the N protein peaked at about 19 weeks after onset and decreased thereafter. The study also found that the neutralizing Abs of S protein were gradually increasing in the second phase of (9wks-19wks) weeks and in the third phase of (19wks -24wks) weeks after disease onset than compared to the first phase of weeks (3wks-9wks) and it was significant (p<0.001).
Conclusion: The study concluded that the antibodies, total Abs, IgG titer of N protein and neutralizing Abs of S protein were developed 100%, 74.6% and 93.8% respectively. The study also observed that IgG of N protein was decreasing within 19-24 weeks and neutralizing Abs of S protein peaked at 19-24 weeks after the onset of disease.
Key Words: Antibody; COVID-19; SARS-CoV-2; Health Care Personnel; Bangladesh

Introduction:
The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China at the end of 2019 and then rapidly spread throughout the world, resulting in a pandemic. In Bangladesh, the first case of COVID-19 was confirmed on March 8, 2020 and first death was confirmed on 18 March, 2020. From 8 March 2020 to 21 October 2021, there have been 1,566,664 confirmed cases of COVID-19 with 27,791 deaths in Bangladesh, reported by Institute of Epidemiology, Disease Control and Research. COVID-19 affects different people in different ways. Mostly infected people develop mild to moderate illness of pneumonia and most common symptoms of the disease include fever, dry cough, tiredness, sore throat, diarrhea, headache and loss of taste or smell and all ages have been affected by COVID-19. However, evidence suggested that two groups of people are at higher risk of getting severe COVID-19 disease, firstly people over 60 years old and those with co-morbidities such as cardiovascular disease, diabetes, chronic kidney disease, chronic respiratory disease, cancer and obesity. As of 21 October 2021, more than 242,809,062 cases of
COVID-19 including approximately 4,937,649 deaths have occurred globally.

Protection specific antibodies (Abs), including immunoglobulin G (IgG) antibody and neutralizing IgG antibody against the nucleocapsid (N) and spike (S) proteins of SARS-CoV-2 are extracted in most COVID-19 patients within 1-2 weeks after onset and contribute to viral clearance. An antibody is a large Y-shaped glycoprotein produced by B-cells and used by the immune system to identify and neutralize pathogens. SARS-CoV-2, the causative agent of COVID-19, gains entry to human cells by binding with angiotensin-converting enzyme 2 (ACE2) receptor with the receptor-binding domain (RBD) of its spike (S) protein.

Accordingly, the S protein, especially the RBD, is a major target to neutralizing antibodies and can neutralize SARS-CoV-2. Antibodies against the S and N proteins are detectable in individuals infected with the COVID-19 virus. It has been reported that the severity of disease affects the antibody titers and different studies showed that the presence of neutralizing antibodies are a good indicator of protective immunity for most viral infection and also true for SARS-CoV-2 infection.

Some studies have reported that the antibody levels are relatively stable for 2-5 months. Furthermore, other groups have reported that the antibody titer against SARS-CoV-2 declines during the early convalescent phase, especially in asymptomatic or mildly symptomatic individuals. Therefore, it has been suggested that humoral immunity in such individuals might not be long-lasting, and in few case scenarios, individuals with no symptoms or mild symptoms may be re-infected with SARS-CoV-2.

Materials and Methods:
Patient selection
The study was a cross-sectional and all participants were taken from May to November 2020, at BIRDEM General Hospital (Dhaka, Bangladesh) and all subjects were healthcare personnel. The study was reviewed and approved by Review Board from Diabetic Association of Bangladesh (BADAS) and patients were included after written informed consent (IRC No-00322).

Blood samples from PCR-confirmed COVID-19 subjects were collected and tested for Total serum antibody (IgM, IgG, IgA), IgG against nucleocapsid(N) protein and IgG against spike(S) protein (RBD) responses to SARS-CoV-2. All blood samples were collected between 3 to 24 weeks after confirmed diagnosis.

Detection of SARS-CoV-2 specific Total antibody assay
The Dimension EXL CV2T assay (Siemens, USA) is a chemiluminescent immunoassay used for the qualitative detection of total antibodies (including IgG, IgA and IgM) against SARS-CoV-2 in human serum from patients who have been diagnosed or infected with coronavirus disease (COVID-19). The SARS-CoV-2 Total Abs assay is designed to detect Nab antigen in human serum and plasma.

Detection of SARS-CoV-2 specific IgG antibody assay
Serum-IgG antibodies against SARS-CoV-2 were analyzed using commercially available serological CMIA kit (Architect i2000SR, Abbott Laboratories, USA). The SARS-CoV-2 IgG assay is designed to detect IgG antibodies to the nucleocapsid (N) protein of SARS-CoV-2 in serum from patients who were confirmed of COVID-19 by RT-PCR.

SARS-CoV-2 Neutralizing antibody assay
SARS-CoV-2 S1/S2 IgG is a chemiluminescence immunoassay (DiaSorin, Italy) technology for the quantitative determination of anti-S1 and anti-S2 (including RBD) specific IgG antibodies to SARS-CoV-2 in human serum samples. The specific recombinant S1 and S2 antigens are used for coating magnetic particles (solid phase) and mouse monoclonal antibodies to human IgG are linked to an isoluminol derivative.

Statistical analysis
Categorical variables were presented as frequency with percentage and continuous variables were expressed as median with interquartile range (IQR). Continuous and categorical variables were compared between different time points were expressed using ANOVA (analysis of variance) with 95% CI and chi-square test as appropriate. The level of statistical significance p<0.05 were used for all analysis. Statistical analysis was performed using SPSS version 20 (IBM).

Role of the funding
The funding source had no role in study design, data collection, data analysis, data interpretation, or writing.
of the paper. How assay kits were managed should be mentioned.

**Results:**
The study was a cross-sectional and conducted at BIRDEM General Hospital from May to November 2020. All study participants were physicians, nurses and health care workers working in this hospital. A total of 710 participants were screened for RT-PCR and 130 (18.87%) were confirmed to have SARS-CoV-2 infection. The blood samples were collected from all 130 individuals at various time points (between 3 to 24 weeks) after onset and all subjects were symptomatic. About 52% subjects were female and majority (91.5%) subjects had positive contract history. The mean $\pm$ SD of age was 43.2 $\pm$9.59. The median time from onset to antibody response was 10 weeks (IQR: 7-18) and the median IgG titer of N protein and neutralizing antibody of S protein were 3.08[1.33-4.75] and 80.45[40.30-124.50] respectively [Table 1]. The clinical characteristics and weeks after onset of the study participants were presented. Of the total participants, the IgG titer of N protein and neutralizing antibody of S protein to the RBD, 74.6% and 93.8% respectively were positive after 24 weeks after the onset of infection. [Table 2]

**Table-II**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG(N):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>33</td>
<td>25.4</td>
</tr>
<tr>
<td>Positive</td>
<td>97</td>
<td>74.6</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>100.0</td>
</tr>
<tr>
<td>IgG(S):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>6.2</td>
</tr>
<tr>
<td>Positive</td>
<td>122</td>
<td>93.8</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>100.0</td>
</tr>
</tbody>
</table>

To understand the overall trend of the antibody responses of these 130 subjects, we divided the total number of weeks into three parts, at 3-9 weeks, 9-19 weeks and 19-24 weeks respectively for IgG of N and S protein. The median value of IgG titer of N protein were 2.34(IQR: 1.13-4.22), 3.57(IQR: 1.89-5.09) and 3.46 (IQR: 1.58-4.77) respectively. These results indicated that the IgG titer of N proteins were peaked at 9 to 19 weeks than the median was slowly decreasing after 19 to 24 weeks post-onset. Similarly, the median and IQR of neutralizing antibodies of S proteins were 57.00(IQR: 33.30-107.00), 94.10(52.70-133.00) and 104.35(72.35-184.75) at three time points in the study. Of the three time points, the median neutralizing antibody of S protein was gradually increasing and these results was more remarkable than that of the IgG titer of N protein [Table 3].

**Table-III**

<table>
<thead>
<tr>
<th>No. of IgG antibody of N and S protein with COVID-19 subjects</th>
<th>Median</th>
<th>Q_1 - Q_3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG(N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-9</td>
<td>3.46</td>
<td>2.27-5.13</td>
</tr>
<tr>
<td>9-19</td>
<td>4.43</td>
<td>2.88-5.30</td>
</tr>
<tr>
<td>19-24</td>
<td>3.81</td>
<td>2.93-5.44</td>
</tr>
<tr>
<td>IgG(S)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-9</td>
<td>57.00</td>
<td>33.30-107.00</td>
</tr>
<tr>
<td>9-19</td>
<td>94.10</td>
<td>52.70-133.00</td>
</tr>
<tr>
<td>19-24</td>
<td>104.35</td>
<td>72.35-184.75</td>
</tr>
</tbody>
</table>
We compared immune responses of IgG antibody of N protein and neutralizing antibody of S protein at different time points and used chi-square test. The study revealed that the prevalence of IgG antibodies of N proteins were positive by 67.2%, 81.8% and 78.6% at 3-9wks, 9-19wks and 19-24 weeks respectively though it was not significant [Table 4]. They were peaked at 9 to 19 weeks after onset and decreased thereafter [Fig 1]. Similarly, the neutralizing antibodies of S proteins to RBD were positive by 87.9%, 97.7% and 100% at 3-9wks, 9-19 wks and 19-24 weeks respectively [Table 4] and they were gradually increased at the entire study period [Fig 2] and it was significant (p=0.039).

**Table-IV**

<table>
<thead>
<tr>
<th>Variables</th>
<th>IgG (N)</th>
<th>IgG (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive (%)</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>No of Weeks:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-9wks</td>
<td>39(67.2%)</td>
<td></td>
</tr>
<tr>
<td>9-19wks</td>
<td>36(81.8%)</td>
<td>3.10</td>
</tr>
<tr>
<td>19-24wks</td>
<td>22(78.6%)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig-1:** The changes in IgG antibody of N protein, the mean level at three time points between 3 to 24 weeks after post-onset (weeks:3-9, 9-19 and 19-24; mean:2.84, 3.63 and 3.57; p>0.05). After 9 to 19 weeks, the IgG of N protein was decreased.

**Fig-2:** The changes in neutralizing antibody titers against the RBD, the mean level of IgG antibody of S protein at three time points between 3 to 24 weeks after post onset (weeks:3-9, 9-19 and 19-24; mean:66.38, 102.61 and 133.87; p<0.001).

**Discussion:**

All 130 subjects were RT-PCR confirmed against SARS-CoV-2 and they showed mild to moderate symptoms. Majority (91.5%) of subjects had positive contact history and blood samples were collected at various time points from 3 to 24 weeks. This study evaluated the changes and persistence of IgG antibody of N and S protein in the recovered subjects against SARS-CoV-2. It has been observed that, the IgG titer of N proteins were developed in 74.6% and peaked at 9 to 19 weeks after the disease onset and decreased thereafter. The levels were maintained for several months after onset and the results similar with other studies 3, 4, 5, 6. The study also revealed that the neutralizing antibodies of S protein were developed in 93.8% and peaked at about...
19 to 24 weeks and gradually increasing during the entire study period (24 wks). Others also reported the similar results for neutralize antibodies. Another cohort study of SARS-CoV-2 infected individuals indicated that antibodies to the RBD of spike antigens were detectable up to 8 months in the majority of recovered patients regardless of age or co-morbidities. This study also found that the neutralizing antibodies titers to RBD of S protein were positive in 87.9%, 97.7% and 100% at 2-9 wks, 9-19 wks and 19-24 weeks respectively. The neutralizing antibodies of S protein were significantly increased during the entire study period (3-24 wks). Similar results in antibody titers have been reported in COVID-19 patients and these studies also observed that antibodies against SARS-CoV-2 do not drop significantly during this time. However, follow-up assessments of recovered individuals for more than a year may be considered to better understand the kinetics. These finding also suggest that the virus growth in the respiratory organs could be suppressed by the immune response. Our results also suggested that compromised immune response to the SARS-CoV-2 S protein to be a major trait of COVID-19 patients and thereby inform on the planning of COVID-19 patients care.

Our study has some limitations. Firstly, the study was cross-sectional and didn’t classify the disease severity and it is difficult to determine the association between antibody response and clinical course. Secondly, in this study we didn’t include any type of co-morbidities and therefore it was not possible to find any relation between COVID-19 disease and co-morbidity.

**Conclusion:**

The study found that the IgG titer of N protein and S protein were developed 74.6% and 93.8% respectively. The study also observed that the IgG antibody of N protein was decreasing within 19-24 weeks and neutralizing antibody of S protein was peaked at 19-24 weeks after the onset of disease. The study concluded that the presence of neutralizing antibody gives humoral immune protection in affected individuals approximately 6 months. We also need to check uninfected, vaccinated people to evaluate the humoral immune response. This may allow us to assess the efficacy of the vaccine, duration of the presence of protective antibody.

**Acknowledgement:**

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**References:**


