The correlation between humoral immune responses and severity of clinical symptoms in COVID-19 patients

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Abstract

The SARS-CoV-2 pandemic commenced in 2019 and with high global mortality still ongoing. Early symptoms of COVID-19 include pneumonia, fever, myalgia, and fatigue. The human immune system produces IgM and IgG antibodies as a response to SARS-CoV-2 exposure. Despite many previous studies, there are limited information on the relation between clinical features and the humoral immune response of patients. The aim of this study was to investigate the correlation between level of serum IgM/IgG and severity of clinical symptoms in patients with COVID-19. From 188 patients with COVID-19 recruited to Ganjavian hospital in Dezful, finally 134 patients were fully included in the study. The patients were divided into three (including mild, moderate and severe) groups based on their symptoms. The levels of IgM and IgG in serum were measured on three occasions, one month apart using the ELISA method. The results showed that serum IgG level was significantly higher in patients with moderate symptoms in comparison with patients with mild symptoms (p<0.001). IgG production was also significantly higher in patients with severe symptoms in comparison with mild (p<0.0001) and moderate (p<0.05) groups. IgM and IgG titers were highest in the first samples and decreases over time. Although it is expected that the anti-SARS-CoV-2 antibodies have protective role against the virus, the direct association between IgG levels and the severity of symptoms could be due to the reason that the immune system has acted late and tried to produce antibodies in a burst against the virus in patients with severe compared to the mild symptoms. As evidences indicate that the late functioning of the immune system is associated with a dysregulated innate immune response, so, to complete the data it is suggested to measure not only the serum IgG and IgM, but also the serum type I interferons earliest at the onset of the symptoms.

Keywords: COVID-19; Clinical Symptoms; IgG; IgM; Immune System
1. Introduction
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel beta-coronavirus responsible for the coronavirus disease 2019 (COVID-19) pandemic which first occurred in patients with pneumonia symptoms in Wuhan, China in December 2019 (1). Coronaviruses belong to the family of Coronaviridae, the order Nidovirales, and the genus Coronavirus. The family contains two subfamilies, the Coronavirinae and the Torovirinae. Coronavirinae are categorized into four important genera that include Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. With the discovery of the genomic sequence of SARS-CoV-2, this virus has also been placed in the betacoronavirus genus (2). SARS-CoV-2 is a positive-sense single-stranded enveloped RNA coronavirus with one of the largest known RNA viral genomes (~29.8 kb). The genome of this virus is strikingly similar to those of other coronaviruses, especially the SARS-CoV and bat coronavirus. There are various proteins, as antigens, in the structure of this virus, including spike (S), membrane (M), envelope (E), and nucleocapsid (N) antigens (3-4).

An efficient immune response is essential to control and eradicate this coronavirus. In COVID-19 infection, any immune system dysfunction can lead to morbidity and mortality. A better understanding of how the immune system works against COVID-19 can be very effective in controlling the disease. Similar to other viruses, the immune system can detect coronavirus via innate immune receptors such as Toll-like receptor (TLR), RIG-I-like receptor (RLR), NOD-like receptor (NLR), and C-type lectin-like receptors. Type I interferons (INFs) are at the forefront of defense against viruses (5). Studies have demonstrated that even though SARS-CoV, SARS-CoV-2 and other coronaviruses are sensitive to IFN-α and IFN-β, they remain pathogenic. These viruses escape the host immune system using various methods such as inhibiting the JAK-STAT pathway downstream of type I interferons (6). On the other hand, the virus interferes with the differentiation and function of dendritic cells, thereby affecting specific immune responses (5). CD4+ T cells play an important role in immunity against SARS-CoV-2 by stimulating the production of virus-specific antibodies by B cells. CD8+ T cells are cytotoxic lymphocytes and manage to kill virus-infected cells. The antibody responses in the body also includes a dynamic and complex set of antibodies that target different antigens on the surface of SARS-CoV-2. The virus uses its surface proteins as an adhesion factor to enter host cells through a special receptor called angiotensin-converting enzyme 2 (ACE2) (7). Studies have shown that in the primary immune response, IgM antibodies are produced in low quantities. In contrast, IgG production is delayed but due to the creation of immune memory, the production of this class of antibodies is higher. These antibodies remain in the serum for a
longer period and even after the infection is resolved. Therefore, the detection of IgM in a patient's serum could be immunological evidence for a recent infection, whereas, the detection of IgG in the serum of a person who has no clinical symptoms often indicates a previous infection.

COVID-19 causes a variety of symptoms, especially in the respiratory system. Clinical symptoms vary from asymptomatic to acute respiratory syndrome and dysfunction of several organs, but common clinical symptoms include fever, cough, sore throat, headache, fatigue, shortness of breath, and conjunctivitis. Some patients may not have obvious symptoms, so a computerized tomography (CT) scan may be a suitable approach to diagnose the disease in the early stages when the clinical symptoms are nonspecific or rare. In 2020, Zhou et al. reported bilateral changes in the lung of most COVID-19 patients. These bilateral changes were evident on chest X-ray or CT scans (8-10).

Considering that the humoral immune response, especially specific antibodies, plays a prominent role in neutralizing viruses, so the purpose of this study was to investigate the relationship between the severity of clinical symptoms and the level of specific antibodies in serum of COVID-19 patients.

2. Materials and Methods

2.1. Study design and patient enrollment

This cross-sectional study was conducted between May 25, 2020, and October 19, 2020. A total of 188 patients with COVID-19 were recruited to Ganjavian hospital in Dezful, out of 188 called people, finally 134 patients were fully included in the study (Fig1).
After obtaining informed consent from the participants, they entered the study. The amount of 3 ml of blood sample was taken from the patients on three occasions, one month apart. The first blood sample was taken at least 25 days after the onset of symptoms. The second and third sampling were one month and two months after the first sampling, respectively.

Study participants were adults, male and female, unvaccinated against COVID-19 with a positive quantitative reverse transcription polymerase chain reaction (RT-qPCR) test. The patients were subdivided based on type of and the severity and duration of the clinical symptoms, duration of hospitalization and oxygen requirement. The severity evaluation was occurred at the time of the first blood sample based on the patient's registered file at hospital.
2.2. Detection of IgG and IgM against SARS-CoV-2

Blood samples were used to evaluate IgM and IgG using enzyme-linked immunosorbent assay (ELISA) kits (Euroimmun, Lubeck, Germany). The commercial Anti-SARS-CoV-2 S1 ELISA IgG kit and nucleocapsid protein-specific IgM kit were used. Results were analyzed and interpreted according to the manufacturer’s instructions.

2.3. Statistical analysis.

Results were expressed as mean ± SD. Differences in mean values between groups were analyzed with Wilcoxon signed rank test, Mann–Whitney U test and Kruskal Wallis test to reveal significant differences between IgG and IgM level in patients’ serum. Differences were considered to be significant at P < 0.05. Statistical calculations were performed with SPSS 19 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Grouping samples

The patients were divided into 3 groups based on their symptoms (Table 1). Common symptoms included headache and anosmia, observed in almost all patients. As we go from the first to the third group, the condition of the patient with SARS-CoV-2 becomes worse and the clinical symptoms become more severe. More than 50% of group C patients were hospitalized, while only 16% of group A patients were hospitalized. The group C patients were overweight and had underlying health-related conditions such as diabetes or heart disease. According to the results 5.9%, 11.9% and 29.7% of the patients from group A, B and C, respectively, required respiratory support and oxygen therapy. The difference between age of the group A, B and C was not significant.

Table 1. Grouping of COVID-19 patients based on symptoms.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age (Average ± SD)</th>
<th>Gender</th>
<th>Respiratory support %</th>
<th>Hospitalization %</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>Total</td>
<td>Temperature</td>
<td>Symptoms Count</td>
<td>Percentage</td>
<td>Severity</td>
<td>Symptoms</td>
</tr>
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<td>------------</td>
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<td>---------------------------------</td>
</tr>
<tr>
<td>A (mild symptoms): flu-like without fever</td>
<td>51</td>
<td>36.6±9.7</td>
<td>11 (22%)</td>
<td>40 (78%)</td>
<td>5.9</td>
<td>Headache, anosmia, muscle aches, cough, sore throat, hoarseness, loss of appetite</td>
</tr>
<tr>
<td>B (moderate symptoms): flu-like with fever, gastrointestinal symptoms, fatigue</td>
<td>38</td>
<td>37.7±9.8</td>
<td>8 (19%)</td>
<td>30 (79%)</td>
<td>13.2%</td>
<td>Headache, anosmia, loss of appetite, diarrhea, sore throat, cough, fever, hoarseness, chest pain, fatigue</td>
</tr>
<tr>
<td>C (severe symptoms): flu-like with</td>
<td>45</td>
<td>37.4±9.5</td>
<td>9 (20%)</td>
<td>36 (80%)</td>
<td>31.1%</td>
<td>Headache, anosmia, loss of appetite, cough, fever, hoarseness</td>
</tr>
</tbody>
</table>

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fever, dizziness, severe abdominal and respiratory symptoms

, sore throat, chest pain, fatigue, dizziness, muscle aches, confusion, dyspnea, diarrhea, abdominal pain

3.2. IgG and IgM levels in COVID-19 patients with mild (A), moderate (B) and severe (C) symptoms

As indicated in Figure 2, COVID-19 patients with severe symptoms (group C) had the highest serum IgG level and the mild group (group A) had the lowest IgG level. Statistical comparison of group A, B, and C revealed that the serum IgG level was significantly higher in group B in comparison with group A (p-value=0.0009). IgG production was also significantly higher in group C in comparison with group A and B (p-value=0.00009, p-value=0.03 respectively).

As Figure 3 indicates the difference between serum level of IgM in group A, B and C is not significant.
Figure 2: The average of IgG level in the mixed three (first, second and third) samplings of group A, B and C. 
*p < 0.05, **p value < 0.001, ***p value < 0.0001
Figure 3: The average of IgM level in the mixed three (first, second and third) samplings of group A, B and C.

ns= Non significant

3.3. Comparing the IgG levels in COVID-19 patients with mild (A), moderate (B) and severe (C) symptoms based on the sampling time interval from the onset of the disease

The time interval of the first sampling was at least 25 (34.2±7.8) days after the onset of the disease, and the second and third sampling were one month and two months from the first sampling, respectively.

As indicated in Fig4, at the first sampling, the difference of IgG level between groups A (Mild), B (Moderate) and C (Sever) was significant while at the second sampling, the
difference of IgG level between group B and C was not significant. Furthermore, at the third sampling, the difference of IgG level between none of the groups was significant. In other words, the earlier we take the samples, the difference between the IgG level of group A, group B and group C becomes more significant.

In the severe group (group C) and the moderate group (group B), the IgG level at the first sampling was significantly higher than the second sampling, and the IgG level in the second sampling was significantly higher than the third sampling, while in the mild group (group A), there were no significant differences in the first, second and third samplings. In other words, the decrease of IgG level from the first to second, second to third and from first to third samples in group A was not significant (Fig4).

![Graph showing IgG levels in groups A, B, and C at three samplings](https://example.com/graph.png)

**Figure 4:** The IgG level in first, second and third serum samples of group A, B and C.

ns= Non significant, * p-value < 0.05
As indicated in Fig5, the average of IgG level of all the patients (A, B and C groups) at the third samples was significantly lower than the first and second samples. Also, the average of IgG level of all the patients at the second samples was significantly lower than the first samples.

Figure 5: The average of IgG secretion of all the patients (A, B and C groups) at the first, second and third samples. *p-value<0.05

3.4 Comparing the IgM level in COVID-19 patients with mild (A), moderate (B) and severe (C) symptoms based on the sampling time interval from the onset of the disease

As indicated in Figure 6, the difference between secreted IgM level in serum of the group A (Mild), B (Moderate) and C (Severe) in the first and second sampling was not significant. Just
the difference between secreted IgM level in serum of the group A and B at third sampling is statistically significant.

Figure 6: The IgM level in first, second and third serum samples of group A (Mild), B (Moderate) and C (Severe).

ns= Non significant, * $p$-value < 0.05

As Figure 7 indicates, the average of IgM level in all the COVID-19 patients non-significantly decreased in the third samples in comparison with the second samples ($p$-Value =0.6556). The IgM level also decreased in the second samples in comparison with the first samples ($p$-Value =0.9347) which was not statistically significant.
Figure 7: The average of IgM level of all the patients (A, B and C groups) at the first, second and third samples. ns = Non-significant
4. Discussion
This study aimed to evaluate the level of antibodies and describe clinical manifestations and the relationship between antibody levels and severity of disease symptoms in COVID-19 patients.

COVID-19 is a life-threatening infectious disease whose clinical symptoms include fever, cough, shortness of breath, loss of sense of smell and taste, and fatigue. The disease is mostly transmitted through breathing and close contact and is a major threat to global health. Incidence and severity of the disease depends on the interaction between the virus and the human immune system. Factors such as the state of the human immune system, age, gender, physical condition, nutrition, hygiene, homeostasis (between the immune system, the nervous and endocrine systems), virus mutations, and the number of virus particles entering the human body are all important factors involved in the emergence, severity, and relapse of the disease (11).

It is widely known that once a virus enters the body, the innate immune system detects it and immediately activates the specific immune system. Innate immune system cells, such as macrophages, take up viral particles and, after processing, present them to specific lymphocytes. Then, the IgM antibodies are produced first, which are in low quantity and quality and their production time is short. Then, as the immune response progresses and receives the appropriate signals, B lymphocytes do class switch and produce mainly IgG-antibodies, which will remain in the serum for a longer period, even after the infection is resolved (12).

Various studies have demonstrated that the seroconversion of COVID-19 is very similar to other acute viral infections, meaning that as IgM level approaches its maximum concentration, IgG level begins to increase. However, it has been reported that the increase in IgM and IgG titers against SARS-CoV-2 is slower than other respiratory system viruses (13).

Some studies indicate that anti-SARS-CoV-2 IgM antibody appears about 5 days after the onset of symptoms and its titer increases rapidly, and the maximum anti-virus IgM titer is observed on days 18-22 after which IgM level decreases. Anti-SARS-CoV-2 IgG also appears about 9 days after the onset of disease symptoms and its titer increases rapidly and the maximum anti-SARS-CoV-2 IgG titer is observed in 24 days and then remained high for a long time. However, for both types of immunoglobulins, patients with severe forms of the disease showed higher titers of antibodies compared to patients with mild forms of the disease at all times of antibody level assessment (14).
In this study, blood samples were taken from 134 patients with COVID-19 on three occasions, one month apart. IgG and IgM levels were measured and patients were divided into 3 groups based on their severity of clinical COVID-19 symptoms. The more we move from the group A to the group C, the worse the condition of the patient with SARS-CoV-2 becomes. As indicated in the results section, more than 50% of group C patients were hospitalized while only 16% of group A patients were hospitalized. Most commonly, patients in groups B, and C were less able-bodied, overweight, and had underlying health-related conditions such as diabetes or heart disease.

In this study, the titers of IgG and IgM were assessed on three occasions, one month apart. As the results indicated, the IgG level significantly decreased over time (p-Value <0.05) but the reduction of IgM over time was not statistically significant (p-Value> 0.05) which can be due to the small number of samples.

In another comparison, the levels of IgM and IgG were evaluated based on the severity of the disease symptoms (severe, moderate, and mild) at different time points from the onset of the disease.

IgM is the first antibody that is produced and has a lower affinity than IgG. The increased level of IgM is an indicator of disease onset and its level may vary from person to person. IgM levels were expected to decrease over time in patients with different symptoms levels (severe, moderate, and mild), but no significant changes in IgM levels were observed in this study. This may be due to the small number of samples. Moreover, when measuring IgM using ELISA-based methods, if the amount of IgG in the serum is higher than IgM (in this study we measured IgM and IgG at least 25 days after the onset of the disease, then, the amount of IgG is so higher compared to IgM), therefore, IgG (because of higher affinity) occupies the epitopes and no longer allows IgM to bind to the epitopes. Since the level of IgG at the second and third samples is decreased, so the amount of the measured IgM in the third samples is close to the real titer. Thus, the amount of IgM at the first samples compared to the second, and the second compared to the third samples is calculated lower than the actual titer. As a result, it could be another reason that the IgM level in the first, second and third samples are not significantly different (15-16).

As we move from the first samples to the third, the IgG levels in patients with different symptoms severity levels (severe, moderate, and mild) significantly decreased. According to the measured IgG levels in patients with mild symptoms, it was observed that the level of IgG
decreased over time but it was not statistically significant. It could be due to the fact that in these patients, initially, the immune system functioned well and anti-virus antibodies were produced in a timely and sufficient manner. Therefore, it did not require the explosive production of antibodies. It means that from the very beginning, the immune system of these people stopped the virus and did not allow the virus to multiply and cause severe damage to the body tissues thus the disease did not shift towards severe symptoms. In fact, the level of antibodies was not very high at first. Therefore, the amount of IgG decreased over time in a gradual fashion. On the other hand, in patients with severe symptoms, the level of IgG was higher than the other patient groups and decreased overtime significantly. As a possible explanation, the authors hypothesize that at the onset of the disease, the immune system of these patients does not stop the virus, probably due to IFN-I response impairment, so it multiplies in the body tissues widely and leads to the severe clinical symptoms (17). Thereafter, the immune system tries to produce antibodies in a burst, the higher secreted IgG may cause more inflammation, most likely through complement activation and opsonization, than protection. In agreement with this, Long et. al. showed that IgG level in the serum of symptomatic group was significantly higher than those in the asymptomatic group in the acute phase. Also, Long et. al. showed the IgG level in the symptomatic group was still significantly higher than those in the asymptomatic group till 8 weeks after they were discharged from the hospital (18).

Some evidences indicate that the disease severity of SARS-CoV-2 infection is associated with a dysregulated innate immune response. In innate immunity against SARS-CoV-2 infection, IFN-I plays a critical role, since it inhibits viral replication in infected cells and has a defensive role in uninfected cells (17, 19). Impairment of the IFN-I response due to the suppression of the immune system of the infected person by the SARS-CoV-2 or due to the inherent weakness in the host's innate immune system, or both, relative to the onset of symptoms probably results in high viral replication and produces an exaggerated inflammatory response including a burst, but late, high IgG secretion. So, in this study, it would be good if we measured not only the serum IgG and IgM, but also the serum type I interferons (IFN-α and IFN-β) earliest at the onset of the symptoms.

5. Conclusion
Although we usually expect that the anti-SARS-CoV-2 antibodies have protective role against the virus, the direct association between IgG levels and the severity of symptoms could be due to the reason that the immune system has acted late against the virus in patients with severe symptoms. Therefore, although, at a later, the immune system tries to produce antibodies in a burst, the higher secreted IgG may cause more inflammation than protection. On the contrary, in patients with mild symptoms, when the immune system reacts against the virus on time, the antibodies are produced in sufficient quantities and protect the host cells from the virus infection.

Author Contributions: AS conceived the study; AS and PK supervised the project; SA, MS, NB and FKS performed experiments; HA and SA provided patient samples; AS, SA, HAK and MS analyzed the data and prepared the figures; AS, SA, MS, HAK, MRD and NB wrote the paper. All authors edited the manuscript and approved the final version. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: There is no any conflict of interest.

The data of the current paper are available at:
https://www.cambridge.org/core/journals/epidemiology-and-infection/information/transparency-and-openness-policy
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