The relationship between epidemiological factors and seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients from Al-Diwaniyah and Al-Najaf governorates

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Abstract

The current study is an attempt to determine the levels of IgM immunoglobulins, IgG, and CRP levels after SARS-CoV-2 infection. And the effect of viral infection on the level of indicators IgM, IgG, CRP, as well as its effect on the demographic distribution of patients. Our study started from September 2020 to April 2021, and blood samples (2 mL of venous blood) were collected by disposable syringe in Materia Medica Containers. Of the patients between the ages of 19 and 72 years, represented by 116 samples, the samples were subjected to an immunofluorescence assay by enzyme-linked fluorescence assay (ELFA), to detect the levels of IgM and IgG immunoglobulins in the patient’s blood serum. Samples were submitted for immunoassay using the ELFA technique used on the VIDAS machine. The levels of IgM and IgG antibodies were detected. As well as the use of the Finecare™ CRP Rapid Quantitative test that uses Fluorescence Immunoassay (FIA) technology, which is used to determine the level of CRP in the blood of patients after they have contracted COVID-19.

The study concluded that there were statistically significant differences according to demographic characteristics, age, gender, and disease severity. Also, strong correlations exist between CRP, IgM, and IgG levels in COVID-19 patients after they have been infected with SARS-CoV-2.
Introduction

SARS-CoV-2, the virus that causes COVID-19, is a respiratory disease. SARS-CoV-2 belongs to a large family of viruses called the betacoronavirus family. And that this virus can infect humans and some animals. The first infection with SARS-CoV-2 was recorded in 2019 (1). It was isolated for the first time in the city of Wuhan, China, from the respiratory system, and it caused an acute respiratory infection, and later spread throughout the world, as it spreads through coughing, sneezing, and spraying during a speech, as well as through direct contact and social relations (2). Like other viruses such as SARS-CoV and MERS, it has receptors that it attaches to in target cells. It is similar to SARS-CoV in the type of receptor, angiotensin-converting enzyme (ACE2), found on the surface of the cells that the virus attacks, the epithelial cells (3). After infection with the virus, the body begins to form the primary antibodies represented by IgM immunoglobulin. The presence of IgM antibodies indicates a recent infection. IgG antibodies are then produced, indicating that a person infected with SARS-CoV-2 has developed antibodies against the virus, but it is not known how long these antibodies persist (4). Also, the level of CRP increases, which indicates the presence of inflammation in the body.

COVID-19, an infectious disease caused by the SARS-CoV-2 virus, infects respiratory cells because they contain ACE2, which is present on the surface of target cells. Like the lungs, heart, kidneys, and brain (5).

Because SARS-CoV-2 is considered a common virus among humans and animals, the virus is believed to have evolved from bats and then transmitted through an intermediate host. Humans are a frequent host of the virus (6).

The SARS-CoV-2 virus is the third virus to attack humans after SARS-CoV and MERS. The SARS-CoV virus was transmitted from bats to humans in Guangdong, China in 2002, while MERS was transmitted by Arabian camels to humans in Saudi Arabia in 2012 (7).

Research into the effect of the SARS-CoV-2 virus focused on the immunological and hematological parameters of patients after contracting Covid-19 disease (8).

Materials and methods

Sample collection

The study was conducted on 116 patients suffering from Covid-19 disease. The samples were collected from Al-Diwaniyah and Najaf governorates in Iraq, from September 2020 to April 2021, and blood samples were collected by
venipuncture according to procedures from 116 patients (2 ml of venous blood), which was withdrawn by a disposable syringe into medical waste containers. Each blood sample was collected directly into a sterile tube, one containing a gel tube and the other containing sodium citrate, where they are used to measure the antibodies formed in the blood as well as the level of C-Reactive protein that indicates the presence of inflammation in the body.

Detection of IgM and IgG immunoglobulins

The VIDAS® SARS-COV-2 IgM and VIDAS® SARS-COV-2 IgG assays from BIOMÉRIEUX in France were used. It is designed to use enzyme-linked fluorescence assay (ELFA) technology to detect IgM and IgG antibodies to SARS-COV-2 in human serum. In less than 30 minutes, these tests provide trustworthy results, which were performed according to the company's instructions.

Principle of VIDAS® SARS-COV-2 IgM and IgG Immunoassay Method

The assay combines a two-stage sandwich enzyme immunoassay with an end-fluorescence detection (ELFA) step. The machine performs all scans automatically. Several times, the reaction medium is cycled in and out of the SPR device. SARSCoV2 IgM and IgG are collected by recombinant SARS-CoV-2 antigen precipitated on the interior of the wall of the SPR device after the sample dilution phase. During the washing process, unbound components are removed.

In the second phase, anti-human IgM and anti-human IgG tagged with alkaline phosphatase are used to specifically detect IgM and IgG. During the washing process, unbound components are removed. The substrate (4-methylumbilivryl phosphate) is cycled in and out of the SPR device during the final detection process. The conjugate enzyme catalyzes the hydrolysis of this substrate to produce a luminous product (4-methyl-umbelliferone) with a flash of 450 nm. The results are automatically calculated by the instrument based on the S1 parameter stored in memory at the end of the test, and the test value is obtained.

Procedure VIDAS® SARS-COV-2 IgM and IgG Immunoassay Method

1. Leave the reagents and serum at room temperature for 30 minutes.

2. To perform the examination, a VIDAS® SARS-COV-2 IgM and IgG test strip and solid-phase supplies (SPR) were used, one for each sample or control being tested, and then placed in the device space.

3. The type of test was determined by selecting the COM 9 or 9 COG test from the device. The sample, control solution, and titrant solution were mixed using a shaker.

4. 100 μl of blood serum was taken and then placed in the first slot on the SPR
strip.

5. Starting the examination procedure, where all the examination steps are carried out automatically by the device.

6. The scan will take about 30 minutes to complete. Remove the SPRs and strips from the instrument once the scan is finished.

7. The result was automatically calculated by the machine based on the standard curve stored in the machine's memory, and then the result was printed by the machine.

8. Place the used SPRs and reagent strips into a suitable container.

The Result of the VIDAS® SARS-COV-2 IgM and IgG Immunoassay Method

When the scan is finished, the computer analyzes the results automatically. For each sample examined,

Table 1: Interpretation of the results of the VIDAS® SARS-COV-2 IgM and IgG Immunoassay Method

<table>
<thead>
<tr>
<th>Index</th>
<th>Test Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>i &lt; 1.00</td>
<td>Negative</td>
<td>IgM and IgG antibodies to SARS-CoV-2 not detected</td>
</tr>
<tr>
<td>i ≥ 1.00</td>
<td>Positive</td>
<td>IgM antibodies to SARS-CoV-2 detected</td>
</tr>
<tr>
<td>i ≥ 1.00</td>
<td>Positive</td>
<td>IgG antibodies to SARS-CoV-2 detected</td>
</tr>
</tbody>
</table>

Detection of a quantitative CRP rapid test

The Finecare™ CRP Rapid Quantitative Test, developed by Guangzhou Wondfo Biotech Co., Ltd in China, is a fluorescence immunoassay for the quantitative measurement of C-reactive protein (CRP) in human serum using the Finecare FIA System. Infections and infections are also detected with this test.

CRP principle

Fluorescent immunoassay technology is used in the rapid quantitative CRP assay. The sandwich immunoassay is used in the test. CRP antibodies to the fluorescence-labeled reagent on the sample pad bind to the CRP antigens in the blood samples and form immune complexes when the sample is placed in the test cartridge sample well. The anti-CRP reagent and anti-CRP antibody complexes
immobilized on the test strip are captured by capillary action as the complexes migrate onto the nitrocellulose matrix of the test strip. As a result, the higher the concentration of CRP antigens in the blood sample, the more complex aggregated on the test strip. The amount of CRP collected is reflected in the signal intensity of the reagent antibody.

**Sample collection and preparation**

1. A sample of venous blood is drawn and placed in tubes containing anti-sodium citrate.

2. Separate the serum from the blood as quickly as possible. The test should be performed as soon as the sample is collected. Avoid storing samples at room temperature for an extended time. Samples should be stored at between 2 and 8 °C for up to 7 days. Samples should be kept below -20°C for long-term storage.

3. Before testing, bring samples to room temperature. Frozen samples should be completely thawed and mixed well. Repeat freezing and thawing of samples are not recommended. Only clear, non-hemolytic samples are allowed.

**Perform the Finecare™ CRP Rapid Quantitative Test**

1. The test must be carried out at room temperature, and before the test begins, the equipment must be turned on.

2. 5 μL of serum is withdrawn into the tube using a transfer pipette.

3. Close the cap and shake the tube 10 times to properly mix the sample mixture.

4. Pipette 75 μl of the sample mixture into the test well of the sample cartridge.

5. After adding the sample mixture to the sample well, we put the test cartridge into the Finecare FIA System Test Cartridge Holder. And click on the "Test" button to start the testing process. The results can be displayed on the main screen or printed by pressing "Print". After release from the Finecare FIA System, dispose of the used test cartridges under local regulations and procedures.

**Interpretation of the score for CRP**

The Finecare™ FIA system automatically calculates CRP test results and displays exact CRP concentrations on the screen in mg/L units.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Clinical Reference</th>
</tr>
</thead>
</table>

**Table 2: Interpretation of the results for CRP**
There may be other infections (bacterial infections or viral infections).

10-20 mg/L: Generally indicates viral infections or mild bacterial infection.

Results

Demographic characteristics of the studied groups

Immunological parameters of patients according to gender

<table>
<thead>
<tr>
<th>Groups</th>
<th>Male</th>
<th>Female</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>9.54±1.93</td>
<td>9.14±2.05</td>
<td>0.335(NS)</td>
</tr>
<tr>
<td>IgM</td>
<td>3.44±1.54</td>
<td>3.04±1.28</td>
<td>0.212(NS)</td>
</tr>
<tr>
<td>CRP</td>
<td>38.48±8.19</td>
<td>40.33±6.55</td>
<td>0.267(NS)</td>
</tr>
</tbody>
</table>

Results of immunological indicators in patients according to age groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>19-28</th>
<th>29-38</th>
<th>39-48</th>
<th>49-58</th>
<th>59-68</th>
<th>69 or more</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>10.53±2.19</td>
<td>10.17±2.01</td>
<td>9.55±2.07</td>
<td>8.88±1.59</td>
<td>9.15±2.09</td>
<td>8.85±0.85</td>
<td>0.112(NS)</td>
</tr>
<tr>
<td>IgM</td>
<td>2.46±1.51</td>
<td>2.30±1.2</td>
<td>3.02±0.63</td>
<td>3.6±1.14</td>
<td>3.79±1.66</td>
<td>5.55±1.41</td>
<td>&lt;0.0001(HS)</td>
</tr>
<tr>
<td>CRP</td>
<td>26.2±3.53</td>
<td>30.9±3.74</td>
<td>35.4±2.04</td>
<td>40.9±2.83</td>
<td>45.6±3.88</td>
<td>53.3±2.43</td>
<td>&lt;0.0001(HS)</td>
</tr>
</tbody>
</table>

The severity of the disease according to the immunological indicators in the patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>69 or more</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>8.85±0.85</td>
<td>0.112(NS)</td>
</tr>
<tr>
<td>IgM</td>
<td>5.55±1.41</td>
<td>0.0001(HS)</td>
</tr>
<tr>
<td>CRP</td>
<td>53.3±2.43</td>
<td>&lt;0.0001(HS)</td>
</tr>
</tbody>
</table>
Study of the correlation between immunological parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CRP</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>-0.120</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>0.268**</td>
<td>-0.435**</td>
<td>1</td>
</tr>
</tbody>
</table>

* significant correlation (P<0.05)       ** highly significant correlation (P<0.01)

Discussion

Distribution of COVID-19 patients and control group healthy people by gender

The results of the current study showed the relationship between gender and changes in some levels of the studied immune indicators (IgG, IgM, CRP) after infection with the SARS-CoV-2 virus. The statistical analysis of the results showed that there was no significant difference between the average levels of immune indicators for males and females. The SARS-CoV-2 virus infects both males and females equally.

And the rate of antibody formation did not show a statistically significant difference between male and female patients. As there are no statistically significant differences between IgG and IgM levels and gender when analyzing the results statistically for antibodies according to gender.

The results of the current study showed that there were no statistically
significant differences between the average level of CRP and gender.

Results of immunological indicators in patients according to age groups

The current study revealed that the SARS-CoV-2 virus has affected all ages, and after statistical analysis of the results, it was found that the levels of IgG between the age groups did not register any change, as the age group (more than 69 years) recorded a decrease in the average level of IgG.

While the age group (19-28 years) recorded the highest mean level for IgG antibodies. Age is one of the main factors affecting levels of immune markers in COVID-19 patients. In the current study, the results indicated that there were no differences between IgG levels and groups.

On the other hand, there were significant differences between IgM levels and age groups, in the age group (greater than 69 years).

As for CRP, the results showed that there were significant differences between the average level of CRP and between the age groups.

CRP is an inflammatory marker protein identified during tissue damage or inflammation. It increases with age and linearly in older age groups when compared to younger age groups. It can be considered an independent inflammatory marker associated with disease severity and is age-dependent and indicates tissue and organ damage (9).

That is, the concentration of CRP in the blood serum increased in older age groups as a result of the SARS-CoV-2 infection. The results suggest that the systemic inflammatory response to COVID-19 is also age-dependent as SARS-CoV-2 infection leads to increased serum CRP levels for age groups of 65 years and onwards.

The severity of the disease according to the immunological indicators in the patients

The results of the current study showed some aspects of the immunological criteria, which included all Immunoglobulin M IgM, Immunoglobulin G IgG, and C-Reactive Protein CRP according to the severity of the disease condition of COVID-19 patients. IgM and IgG immunoglobulins are used to detect the presence of SARS-CoV-2 antibodies in sera. The level of CRP, which is a non-specific sign of infection, is also being investigated, as its level increases significantly in the presence of infections.

In this study, IgM and IgG levels of SARS-CoV-2 were measured in patients with varying severity of COVID-19, and the relationship between specific
antibody levels and disease severity was categorized and the importance of antibody detection was clarified.

We aimed to determine the levels of IgM and IgG antibodies that are specific to SARS-CoV-2, changes in their concentration based on the severity of COVID-19, and to determine the significance of their detection. COVID-19 patients were included and divided into groups according to the severity of the disease into critical, severe, and mild groups.

As for the level of CRP, the results showed that patients with severe symptoms had higher levels of CRP compared to patients with mild symptoms. CRP is a non-specific marker of inflammation, with levels of this inflammation significantly elevated in severe COVID-19 cases, and it is one of the first markers to be considered in response to SARS-CoV-2 infection. Elevated levels of CRP in serum are a major marker of disease progression and a risk factor for severe COVID-19 patients and an indication of the development of a cellular storm in COVID-19 patients. Following virus infection, a cytokine storm destroys bone marrow progenitor cells and leads to a decrease in platelet production (10,11).

Study of the correlation between immunological parameters

The results of the statistical analysis of the correlation coefficient showed that there was no significant correlation between the levels of CRP and immunoglobulin M IgM, and we did not find any relationship between the presence of IgM antibodies and the future course of the disease.

While we found a significant positive correlation between CRP and Immunoglobulin G levels, where IgG antibodies were positively associated with CRP levels in patients. As for the correlation between the levels of Immunoglobulin M and Immunoglobulin G, a highly significant negative correlation was observed.

References


