Comparative Study of Immunoglobulin G and Gender between COVID-19 Patients and Vaccinated Iraqi Individuals with Pfizer, AstraZeneca and Sinopharm Vaccine

1Zoubaida Kh. Ibraheem *, 1Raghad H. AL-Azzawy
1Department of Biology, Collage of Science, University of Baghdad
*Corresponding author: Zoubaida kh. Ibraheem E-mail: zoubaidakhalil963@gmail.com
Mobile: +9647708371837

ABSTRACT

Background: The coronavirus 2 that causes severe acute respiratory syndrome is the source of the contagious sickness known as coronavirus disease 2019 (COVID-19). The first case was detected in Wuhan, China, in December of this year. Since then, a pandemic has occurred due to the disease's global spread. The IgG antibody is a large, Y-shaped protein that aids in the immune system's ability to identify and eliminate foreign substances like bacteria and viruses. The antibody detects the antigen, a distinct molecular molecule of the virus.

Objective: This research aimed to know which were more infected males or females and the levels of antibodies produced when infected with the emerging virus as well as to compare the antibodies formed after taking two doses of its vaccines, which include Pfizer, AstraZeneca, and Sinopharm vaccines that used in Iraq by measuring the level of Serum IgG using enzyme-linked immuno-sorbent assay technology.

Materials and Methods: In the current study, 100 Iraqi patients with covid-19 disease and 30 Iraqi healthy control were included, and 20 previously uninfected individuals who received the vaccine, and 20 previously infected individuals who took the vaccine (for each type of the three vaccines). Samples were collected 21 days after the second dose and also from 3 to 6 months after the second dose by measuring the level of serum IgG using enzyme-linked immuno-sorbent assay technology. The study was conducted from September 2021 to February 2022. Blood samples were collected in Baghdad Teaching Hospital (Corona patients' isolation center) in Baghdad, Iraq.

Results: The study showed that infection with COVID-19 increases the proportion of antibodies IgG, and it was also found that the best type of vaccine is the AstraZeneca vaccine for raising IgG, and males are more susceptible for infection than females.

Conclusion: Elevation of immunoglobulin G in blood depends on the severity of the infection and the Effect of vaccine.

Keywords: Antibodies, ELISA, Second dose.

INTRODUCTION

Coronavirus 2 that causes severe acute respiratory syndrome is the source of the contagious sickness known as coronavirus disease 2019 (COVID-19) (SARS-CoV-2) (1).

The first case was detected in Wuhan, China, in December of this year. Since then, a pandemic has occurred due to the disease's global spread. Coronaviruses are a group of closely related RNA viruses that infect both mammals and birds (2). The IgG antibody is a large, Y-shaped protein that aids in the immune system's ability to identify and eliminate foreign substances like bacteria and viruses. The antibody detects the antigen, a distinct molecular molecule of the virus (3).

Antibodies, together with B and T cells, are the most essential component of the adaptive immune system. They come in two types: one that is linked i to a B cell and the other, which is soluble and found in extracellular fluids like blood plasma i. All antibodies start out as the first type, linked to the surface of a B cell, these are known as B-cell receptors (BCR). When an antigen binds to a BCR, the B cell activates and divides into plasma cells, which make soluble antibodies against the same paratope, or memory B cells, which persist in the body and provide long-lasting protection against the antigen (4).

Adaptive immunity to viral infection heavily depends on humoral responses. COVID-19 patients' gamma immunoglobulins (Ig) mediate viral neutralization and may have various roles in immunity at different stages of infection and at different anatomical sites (5). IgG levels are said to diminish significantly 8 weeks after the onset of symptoms, but treated individuals have high spike protein–specific IgG titers.

MATERIALS AND METHODS

Study Samples

A case study was conducted during September 2021–February 2022 to determine the levels of IgG in Iraqi patients with SARS-COV-2, vaccinated people with Pfizer, AstraZeneca and Sinopharm vaccines, and control subjects who were not infected and not vaccinated. The study samples included:

1. Patients

Consecutive 100 cases (62 males and 38 females) with positive PCR for SARS-COV-2 were randomly selected and recruited from Baghdad Teaching Hospital (Corona patients isolation center) that were diagnosed by PCR technique.
2. Normal Healthy Control Group

Thirty healthy individuals, males and females, without SARS-CoV-2 infection (not vaccinated) and other apparent diseases were randomly selected as the normal control groups during the period of this study.

3. Vaccinated Group included: two sub groups

1-Previous Infected Individuals included:
- 20 previous infected individuals (males and females) and vaccinated with Pfizer vaccine, serum was collected in period from 14 – 21 days after the second dose.
- 20 previous infected individuals (males and females) vaccinated with Pfizer vaccine, serum was collected in period from 3-6 months after the second dose.
- 20 previous infected individuals (males and females) and vaccinated with Sinopharm vaccine, serum was collected in period from 14 – 21 days after the second dose.
- 20 previous infected individuals (males and females) vaccinated with Sinopharm vaccine, serum was collected in period from 3-6 months after the second dose.
- 20 previous infected individuals (males and females) and vaccinated with AstraZeneca vaccine, serum was collected in period from 14 – 21 days after the second dose.
- 20 previous infected individuals (males and females) vaccinated with AstraZeneca vaccine, serum was collected in period from 3-6 months after the second dose.

2-Not-infected individuals included:
- 20 Not-infected individuals (males and females) and vaccinated with Pfizer vaccine, serum was collected in period from 14 – 21 days after the second dose.
- 20 Not-infected individuals (males and females) vaccinated with Pfizer vaccine, serum was collected in period from 3-6 months after the second dose.
- 20 Not-infected individuals (males and females) and vaccinated with Sinopharm vaccine, serum was collected in period from 14 – 21 days after the second dose.
- 20 Not-infected individuals (males and females) vaccinated with Sinopharm vaccine, serum was collected in period from 3-6 months after the second dose.
- 20 Not-infected individuals (males and females) and vaccinated with AstraZeneca vaccine, serum was collected in period from 3-6 months after the second dose.
- 20 Not-infected individuals (males and females) vaccinated with AstraZeneca vaccine, serum was collected in period from 3-6 month after the second dose.

Laboratory methods

My BioSource Company (USA) produced the Enzyme-linked Immunosorbent Assay (ELISA) kits, which were based on sandwich ELISA technology and were utilized for qualitative evaluations of IgG antibodies in patient and control samples.

Ethics approval:

The Ethics Committee at the Department of Biology (University of Baghdad) approved the study protocol (Reference: CSEC/0921/0096) In September 15, 2021. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

The Statistical Analysis

System-SAS (2012) application was used to determine how various study parameters were impacted by various circumstances. The T-test was utilized to significantly compare between means. The Chi-square test was used to compare percentages in a significant way (0.05 and 0.01 probability). The correlation coefficient between the study's variables was calculated.

RESULTS

Serum levels of IgG (ng/ml) were highly increased in the patient group (without vaccination), previously infected and vaccinated with the AstraZeneca vaccine. The results showed elevated IgG levels in patients after a short period of infection as shown in table (1), and figure (1).

The results of this study were based on a total of 100 cases with SARS-CoV-2 disease diagnosed by PCR technique (did not take a vaccine), with severe and acute symptoms, as well as a few with mild symptoms, and 30 healthy control (HC) subjects. The patients were divided into groups according to age distribution and gender. There were 62 (62.00%) males and 38 (38.00%) females with SARS-CoV-2 infection and also 30 as control, which were 8 (26.67%) males and 22 (73.33%) females, all of them were healthy individuals. This study involved a vaccinated group with Pfizer, Sinopharm, and AstraZeneca vaccines.

The vaccinated group was divided into those who had previously been infected with SARS CoV-2 and those who had not previously been infected but have been vaccinated as shown in table (3-1). The first group (G1) was previously infected and vaccinated with Pfizer, and the samples were taken in a period of 14 to 21 days after the second dose. There were 7 (35.00%) males and 13 (65.00%) females. The second group (G2) had not
previously been infected but had been vaccinated. Vaccinated with Pfizer, the samples were taken in a period of 14 to 21 days after the second dose. There were 11 (55.00%) males and 9 (45.00%) females. The third group (G3) was previously infected and took AstraZeneca, which included 10 (66.67%) males and 5 (33.33%) females. The fourth group (G4) had not previously been infected but had been vaccinated (AstraZeneca) and was distributed as 8 (53.33%) males and 7 (46.67%) females. The samples were taken from 14–21 days after second dose. And samples were taken from 14–21 days after the second dose in the fifth group (G5) that had not previously been infected but had been vaccinated (Sinopharm vaccine).

There were 10 (50.00%) males & 10 (50.00%) females. The sixth group (G6) was previously infected and took Sinopharm vaccine, 8 (40.00%) males and 12 (60.00%) females. This study also included groups that were vaccinated, and samples were taken three to six months after the second dose for individuals who were previously infected and had not been infected by three types of vaccine and divided to:

- Previously infected and vaccinated with Pfizer, 9 (45.00%) males and 11 (55.00%) females. AstraZeneca vaccine group 9 (45.00%) males and 11 (55.00%) females, Sinopharm vaccine 12 (60.00%) males and 8 (40.00%) females. Another group had not previously been infected but had been vaccinated with Pfizer 13 (65.00%) males and 7 (35.00%) females.
- For AstraZeneca vaccine, 11 (55.00%) males and 9 (45.00%) females. Sinopharm vaccine, 9 (45.00%) males and 11 (55.00%) females. The findings suggest that there were significant differences between patients’ groups and previous infected and vaccinated males and females in this study, as shown in table (2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE</th>
<th>IgG : normal value of cut off =29 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: control</td>
<td>23.93 ±0.63 g</td>
<td></td>
</tr>
<tr>
<td>G2: Patients (without vaccination)</td>
<td>87.90 ±3.49 a</td>
<td></td>
</tr>
<tr>
<td>G3: Non inf.+ Pfizer after 21 day</td>
<td>54.99 ±4.46 de</td>
<td></td>
</tr>
<tr>
<td>G4: Infected+ Pfizer after 21 day</td>
<td>70.11 ±4.14 bc</td>
<td></td>
</tr>
<tr>
<td>G5: Infected+ Astra. after 21 day</td>
<td>91.59 ±8.56 a</td>
<td></td>
</tr>
<tr>
<td>G6: Non inf.+Astra. after 21 day</td>
<td>47.42 ±4.78 ef</td>
<td></td>
</tr>
<tr>
<td>G7: Inf.+ Sinoph. after 21 day</td>
<td>66.17 ±3.95 cd</td>
<td></td>
</tr>
<tr>
<td>G8: Non inf.+ Sinoph. after 21day</td>
<td>38.14 ±2.58 fg</td>
<td></td>
</tr>
<tr>
<td>G9: Inf. + Pfizer after (3-6) months</td>
<td>46.50 ±2.78 ef</td>
<td></td>
</tr>
<tr>
<td>G10: Non inf. + Pfizer after (3-6) months</td>
<td>54.25 ±2.40 de</td>
<td></td>
</tr>
<tr>
<td>G11: Inf.+ Astra. after (3-6) months</td>
<td>82.38 ±4.97 ab</td>
<td></td>
</tr>
<tr>
<td>G12: Non inf.+ Astra. after (3-6) months</td>
<td>68.21 ±5.68 bcd</td>
<td></td>
</tr>
<tr>
<td>G13: Inf.+ Sinoph. after (3-6) months</td>
<td>66.54 ±3.98 cd</td>
<td></td>
</tr>
<tr>
<td>G14: Non inf. + Sinoph. after(3-6) months</td>
<td>44.52 ±4.78 ef</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
<td>14.94 **</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Means having with the different letters in same column differed significantly. ** (P ≤ 0.01).
G1: Control which was not infected and not vaccinated, G2: Patients with SARS CoV-2 (without vaccination), G3: Not infected + vaccinated with Pfizer after 14 – 21 day, G4: Infected + vaccinated with Pfizer after 14 – 21 day, G5: Infected + vaccinated with AstraZeneca after 14 – 21 day, G6: Not infected + vaccinated with AstraZeneca after 14 – 21 day, G7: Infected + vaccinated with Sinopharm after 14 – 21 day, G8: Not infected + vaccinated with Sinopharm after 14 – 21 day, G9: Infected + vaccinated with Pfizer after (3-6) months, G10: Not infected + vaccinated with Pfizer after (3-6) months, G11: Infected + vaccinated with AstraZeneca after (3-6) months, G12: Not infected + vaccinated with AstraZeneca after (3-6) months, G13: Infected + vaccinated with Sinopharm after (3-6) months, G14: Not infected + vaccinated with Sinopharm, after (3-6) months.

Table (2): Distribution of sample study according to Gender in difference groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: control</td>
<td>30</td>
<td>8 (26.67%)</td>
<td>22 (73.33%)</td>
<td></td>
</tr>
<tr>
<td>G2: Patients (without vaccination)</td>
<td>100</td>
<td>62 (62.00%)</td>
<td>38 (38.00%)</td>
<td>0.0084 **</td>
</tr>
<tr>
<td>G3: Non inf. + Pfizer after 21 day</td>
<td>20</td>
<td>11 (55.00%)</td>
<td>9 (45.00%)</td>
<td>0.082 NS</td>
</tr>
<tr>
<td>G4: Infected + Pfizer after 21 day</td>
<td>20</td>
<td>7 (35.00%)</td>
<td>13 (65.00%)</td>
<td>0.0061 **</td>
</tr>
<tr>
<td>G5: Infected + Astra. after 21 day</td>
<td>15</td>
<td>10 (66.67%)</td>
<td>5 (33.33%)</td>
<td>0.0054 **</td>
</tr>
<tr>
<td>G6: Non inf. + Astra. after 21 day</td>
<td>15</td>
<td>8 (53.33%)</td>
<td>7 (46.67%)</td>
<td>0.281 NS</td>
</tr>
<tr>
<td>G7: Inf. + Sinoph. after 21 day</td>
<td>20</td>
<td>8 (40.00%)</td>
<td>12 (60.00%)</td>
<td>0.021 *</td>
</tr>
<tr>
<td>G8: Non inf. + Sinoph. after 21 day</td>
<td>20</td>
<td>10 (50.00%)</td>
<td>10 (50.00%)</td>
<td>1.00 NS</td>
</tr>
<tr>
<td>G9: Inf. + Pfizer after (3-6) months</td>
<td>20</td>
<td>9 (45.00%)</td>
<td>11 (55.00%)</td>
<td>0.082 NS</td>
</tr>
<tr>
<td>G10: Non inf. + Pfizer after (3-6) months</td>
<td>20</td>
<td>13 (65.00%)</td>
<td>7 (35.00%)</td>
<td>0.0061 **</td>
</tr>
<tr>
<td>G11: Inf. + Astra. after (3-6) months</td>
<td>20</td>
<td>9 (45.00%)</td>
<td>11 (55.00%)</td>
<td>0.082 NS</td>
</tr>
<tr>
<td>G12: Non inf. + Astra. after (3-6) months</td>
<td>20</td>
<td>11 (55.00%)</td>
<td>9 (45.00%)</td>
<td>0.082 NS</td>
</tr>
<tr>
<td>G13: Inf. + Sinoph. after (3-6) months</td>
<td>20</td>
<td>12 (60.00%)</td>
<td>8 (40.00%)</td>
<td>0.021 *</td>
</tr>
<tr>
<td>G14: Non inf. + Sinoph. after (3-6) months</td>
<td>20</td>
<td>9 (45.00%)</td>
<td>11 (55.00%)</td>
<td>0.082 NS</td>
</tr>
<tr>
<td>P-value</td>
<td>--</td>
<td>0.0001 **</td>
<td>0.0001 **</td>
<td>---</td>
</tr>
</tbody>
</table>

* (P≤0.05), ** (P≤0.01).
DISCUSSION

The disease severity was associated with higher neutralizing antibody titers. Increased amounts of viral antigen can result in a more serious illness as well as a greater immune response. Alternatively, although there is currently no evidence of antibody-dependent enhancement in COVID-19, antibodies may play a causal role in the severity of the disease. Iwasaki et al. (6) in his study showed that almost all participants’ mount of detectable antibody response following two doses of the vaccine was stronger in people who have already had the infection (either known RT-PCR positive or seropositive at baseline). When people who had already been ill received the vaccine, studies have found that a significant synergy a “hybrid adaptive immunity”—occurs when natural immunity and immunity produced by vaccinations are combined Gazit et al. (7). A larger-than-expected immunological response develops when vaccine-generated protection and acquired natural immunity to SARS-CoV-2 are combined (8). It has been demonstrated that antibody levels decrease with time after vaccination but are still detectable more than six months after a second immunization. Neutralizing antibody types have been demonstrated to degrade more quickly in people who have never been infected before immunization than in people who have been infected previously (9). The results show that vaccination with the AstraZeneca COVID-19 vaccine in individuals who had previously contracted SARS-CoV-2 resulted in robust binding, cross-reactive, and cross-neutralizing antibody responses against a number of varieties of concern (VOCs), as shows in figure (1). In those who have already contracted SARS-CoV-2, the adenovirus vaccine increases levels of anti-Spike and anti-RBD antibodies that are cross-reactive against D614G, alpha, beta, gamma, and delta variants (10). Within two weeks of vaccination, spike protein-specific IgG formed at 14 days after the second dosage, its titers increased (11).

Potential participants who have recently experienced COVID-19-like symptoms or who have a history of positive PCR tests for SARS-CoV-2 are likely to have high levels of neutralizing antibodies. Real-world data from the United Kingdom is being used in conjunction with these publications to highlight how the second dosage of the vaccine boosted recipients’ protection against SARS-CoV-2 infection from 65% after the first dose to 70% after the second. A single dose of Pfizer or Oxford-AstraZeneca COVID-19 hospital admissions among vaccine recipients were decreased by 88 and 91%, respectively, by COVID-19 vaccines (12). After a year, antibody levels generated by a single dose of ChAdOx1 nCoV-19 gradually is reduced, although they were still higher than baseline levels. We have previously demonstrated that administering a second dose of the vaccine results in higher antibody responses 1 month after the second dose than before the second dose, with higher responses with a dosing interval of up to 3 months between the first two doses (13). Following vaccination with the AstraZeneca COVID-19 vaccine, the presence of cross-neutralizing antibodies in recovered patients may confer cross-protection against various variations. Following mild or moderate SARS-CoV-2 infection, levels of anti-pseudovirus antibodies significantly decreased within 6 months of diagnosis, which is consistent with previous studies (14). Within 28 days, the immune system began to work against the spike proteins and displayed cellular and humoral immunity. As a result, the goal was to fully express the SARS-CoV-2 spike in order to elicit an immune response. Within 28 days, significant humoral and cellular immune responses were seen, especially in the younger groups (15). The key benefits are that they are inexpensive and may be kept in an ordinary refrigerator at a temperature that will not affect their efficacy. The mRNA vaccine must be kept at −20°C (Moderna) or −80°C (Pfizer-BioNTech). Also enhancing the immune system’s cellular and humoral responses. Additionally vectors vaccine have the ability to act like adjuvants, activating the immune system through both TLR-dependent and TLR-independent routes (16).

According to the most recent statistics, COVID-19 transmission by droplet, direct contact, and aerosol transmissions are the main SARS-CoV-2 transmission routes. When hands come into contact with the eyes, nose, or mouth after touching a contaminated surface, infection may also result. Typically, droplets don’t move more than two feet, or about six feet (17). Also Males are more infected with covid19 than females, with one explanation including increased expression of angiotensin-converting enzyme-2 (ACE 2; coronavirus receptors) in males compared to females, as well as sex-based immunological differences mediated by sex hormone and the X chromosome. Furthermore, gender behavior (lifestyle), men’s irresponsible attitudes have a reversible effect on their compliance with preventive measures such as frequent handwashing, face mask use; and stay-at-home directives (18). After infection, biological sex differences can lead to differing immunological responses. Women, on average, are less susceptible to viral infections than men because their immune systems are more effective. During viral infection, men and women have different innate detection and adaptive immune responses. As a result, COVID-19 varied between men and women, which can be explained by variances in immunological responses between the sexes. Furthermore, estrogen has a key role in the female immune system (19). Because of their steroid hormone levels, the impact of X-linked genes, and sex-based immune responses, females are likely less susceptible to viral infections (20). The fact that women have two X chromosomes, one of which is inactive, emphasizes the system. And immunological checkpoints like the inhibitory CD200 receptor (CD200R) help to keep the immune system in check during microbial infection by
activating and regulating hyperimmune driven responses. In women, the levels of immune cell activation are higher than in men. It's linked to the toll-like receptor (TLR) 7 trigger, which causes interferon gamma (IFN) synthesis. TLR7 expression is higher in women than in males, and its biallelic expression leads to increased immunological responses and viral infection resistance Manry et al. (21). The ACE2 peptidase domain, which is responsible for converting angiotensin I to angiotensin 1-9, also provides a direct binding site for SARS-CoV-2 S-proteins. The lower ACE2 expression in females due to the mixture of the 2 X-linked genes compared to the expression arising from the X-linked and a Y homolog in males. Increased ACE2 activity in males, which is largely driven by sex hormones (22). The lack of increased ACE2 in men due to low estrogens would favor the ACE pathway in the RAS axis, which further promotes tissue injury and disease severity in men, compared with women with the same viral load (23).

**CONCLUSION**

We conclude from this study that:

1. The AstraZeneca vaccine has been shown to be the most effective vaccine in raising the immune system against COVID-19 in Iraqi individuals.
2. The IgG antibodies raise after infection with COVID-19.
3. Females are more protective than males.

**REFERENCES**