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SARS-CoV-2: Immunoglobulins levels in Infected and Post-Vaccinated Subjects

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ABSTRACT

Background: There is currently a lack of clarity on the length and type of immune responses to SARS-CoV-2 infection. The predominant immune response to infection in humans is thought to be due to their presumed lack of immunological experience with SARS-CoV-2. **Objective:** This study aimed to use IgG and IgM titre of SARS-CoV-2 for vaccine efficacy assessment. **Materials and methods:** This study was conducted on a cohort of 82 individuals; 41 subjects were positive COVID-19 and 41 subjects taking COVID-19 vaccine in Zagazig, Sharkia. All patients had comprehensive medical histories taken, physical exams performed, and blood samples taken in order to evaluate the following: ELISA-based quantitative serological IgM and IgG levels were measured three weeks following the second dose of the Sinopharm and AstraZeneca vaccines, in addition to a full blood count.

Results: The results showed that IgM and IgG were significantly higher among Infected subjects compared to vaccinated subjects. We also noted that there was no significant association between IgM & IgG and age & gender or comorbidities. Regarding side effects, flu symptoms were the most prevalent side effects that were found in 26.8% of the patients, shoulder edema in 14.6% and local irritation in 2.4%.

Conclusions: IgG and IgM are good parameters for COVID-19 infection follow up and a significant indictor for vaccination effectiveness for prevention of transmission of SARS-Cov-2.

Keywords: SARS-CoV-2, COVID-19, COVID-19 vaccine, IgM, and IgG.

INTRODUCTION

Micron, a highly contagious new strain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has just emerged, dramatically increasing the frequency of infections around the world and causing significant alarm [1].

To strengthen the population's resistance, both natural SARS-CoV-2 infection and immunization against COVID-19 are used. Epidemiological studies use easily measurable components of immune responses, like antibodies, to answer new questions about the prevalence and features of antibody deficiency, as well as the duration of antibody responses induced by infection or vaccination [2].

IgM is the largest pentamer immunoglobulin, which is produced in the spleen via plasma blasts as an initial response to antigens. In COVID-19 and vaccinated subjects it starts appearing after just 72 hours. After that, long-term immunity is explained by the presence of IgG (four peptides and the most abundant (75%) in the human serum), which may stay in the circulation for over 2 months [3].

During COVID-19 infection of a human body, the IgM level starts to be significant on the 3rd day. It reaches its highest levels by the 2nd –3rd week, then drops to normal level. The IgG levels start to rise by the end of the 1st week and would be still detectable at a high level for 2 more months [4].

Regarding vaccinated subjects, the IgG levels are usually detectable 3 weeks after the 1st dose and to be boosted by the second dose, causing a rise after 4 weeks after the 2nd dose administration. The IgM levels were highest specifically after the 1st dose in seronegative individuals [5]. Serum levels of IgM and IgG measured using the ELISA binding antibody titer technique in vaccinated individuals may reflect the positive impact and vitality of total vaccination [6]. This study aimed to use IgG and IgM titre of SARS-CoV-2 for vaccine efficacy assessment.

MATERIALS AND METHODS

This retrograde study was conducted on vaccinated population in Zagazig, Sharkia, Egypt and the patients in the isolation sector of Zagazig University Hospitals from June 2022 to June 2023.

This study included 82 adult individuals; 41 subjects were positive COVID-19 and 41 individuals subjected to COVID-19 vaccine; 21 subjects used AstraZeneca vaccine, and 20 subjects used Sinopharm vaccine.

Exclusion criteria: Subjects with comorbidities affecting immunoglobulins, hepatic diseases, chemotherapy, radiotherapy, and autoimmune disease and subjects with contraindications to vaccine.

Patients' full medical histories were taken, including their names, ages, sexes, and any risk factors like obesity, smoking, and co-occurring disorders like diabetes and hypertension. The clinical examination included measuring arterial blood pressure, pulse, respiratory rate, temperature, and BMI. Additionally, blood samples were taken from every patient three weeks following the second dose of the Sinopharm and AstraZeneca vaccines to evaluate complete blood counts and quantitative serological IgM and IgG levels. We looked at adverse effects that occurred after the vaccine. Other standard tests included CBC, D-Dimer assay, CRP, ferritin, and evaluation of liver and kidney function.

The diagnosis of COVID-19 was achieved with the use of Primerdesign Ltd COVID-19 genesig® Real-Time PCR assay kits, which involved the real-time PCR of viral RNA using nasopharyngeal swabs. We used an

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Agilent Technologies "Stratagene Mx3005P" platform for real-time polymerase chain reaction. Total immunoglobulins levels (IgG and IgM), D-dimer and CRP were measured using serum samples by turbidimetric method, on Roche Cobas C501 chemistry module (Roche Diagnostics, Swtizerland) using dedicated reagents supplied by manufacturer. Kidney and Liver function tests were measured spectrophotometrically on Roche Cobas 8000-c702 chemistry module (Roche Diagnostics, Swtizerland) using dedicated reagents supplied by manufacturer. Ferritin was measured by electrochemiluminescence assay on Roche Cobas 8000-e602 module, using reagents supplied by manufacturer as well. CBC was performed on Sysmex XN cell counter (Sysmex corporation, Japan).

Ethical approval: The Research Ethics Board of The Medical School, Zagazig University gave its blessing to the study, and all participants gave written informed consents. The Helsinki Declaration was observed throughout the investigation conduct.

Statistical analysis

In order to gather, organize, and examine all of the data, we utilized SPSS V. 24.0 for Windows. We checked for normally distributed data using the Shapiro-Whitney U test. Frequencies and relative percentages were used to display the qualitative data. The X^2-test and Fisher exact were used to determine the difference between the qualitative variables, as indicated. Parametric quantitative data were expressed using the mean plus or minus the standard deviation, while non-parametric data were described using the median and range. For parametric variables, we used the Independent-T test, and for non-parametric variables, we used the Mann Whitney test to find out how the two sets of quantitative data separated. All of our statistical comparisons were conducted using two-tailed significance tests. A significant p-value was defined as ≤ 0.05.

RESULTS

Our results revealed that there is no significant difference between the groups regarding demographic and clinical data (Table 1).

Table (1): Baseline characteristics of the studied subjects

	Infected (n=41)	***Vaccinated (n=41)***	***P***
Age *(years)*	43.73 ± 5.91	41.88 ± 7.54	0.220
Males, *n (%)*	26 (63.4)	22 (53.7)	0.311
BMI *(kg/m^2)*	27.12 ± 2.84	26.53 ± 2.37	0.370
Comorbidities, *n (%)*			
DM	17 (41.5%)	10 (24.4%)	0.100
HTN	15 (36.6%)	18 (43.9%)	0.499
Temperature *(°C)*	37.59 ± 0.214	37.4 ± 0.258	**0.039**

BMI: body mass index, DM: Diabetes mellitus, HTN: hypertension

In our study, we noted that IgM and IgG were significantly higher among Infected subjects compared to vaccinated subjects. We also noted that there is no significant association between IgM & IgG and age & gender or comorbidities (Figures 1 & 2).

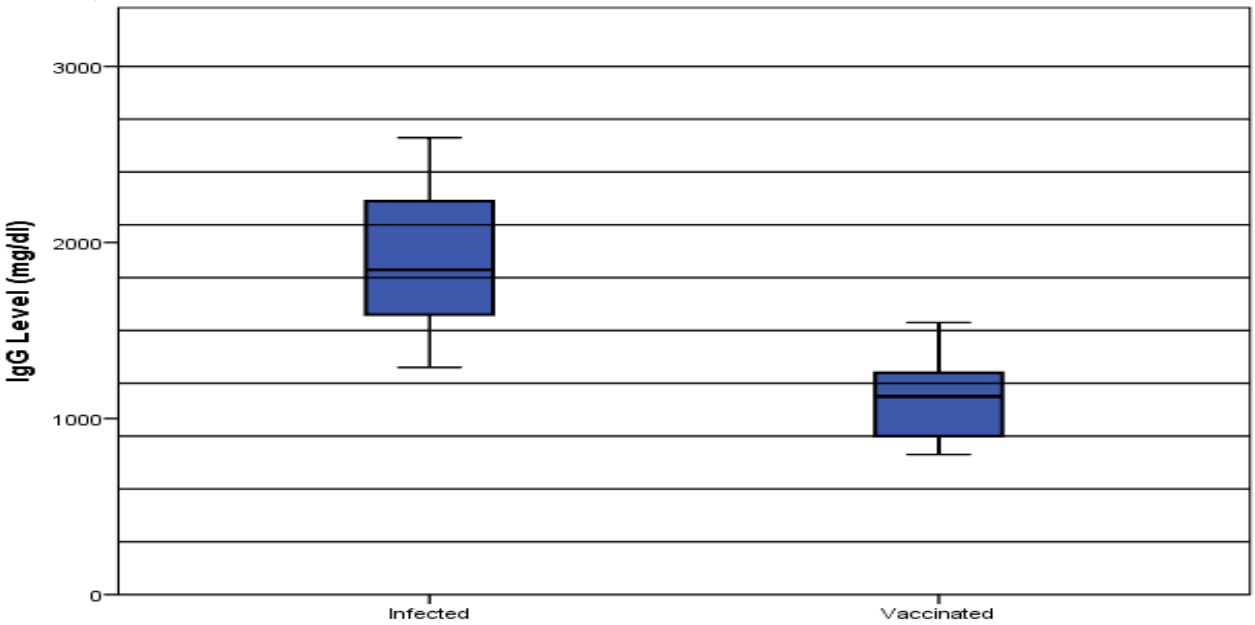

Figure (1): IgM level among cases.

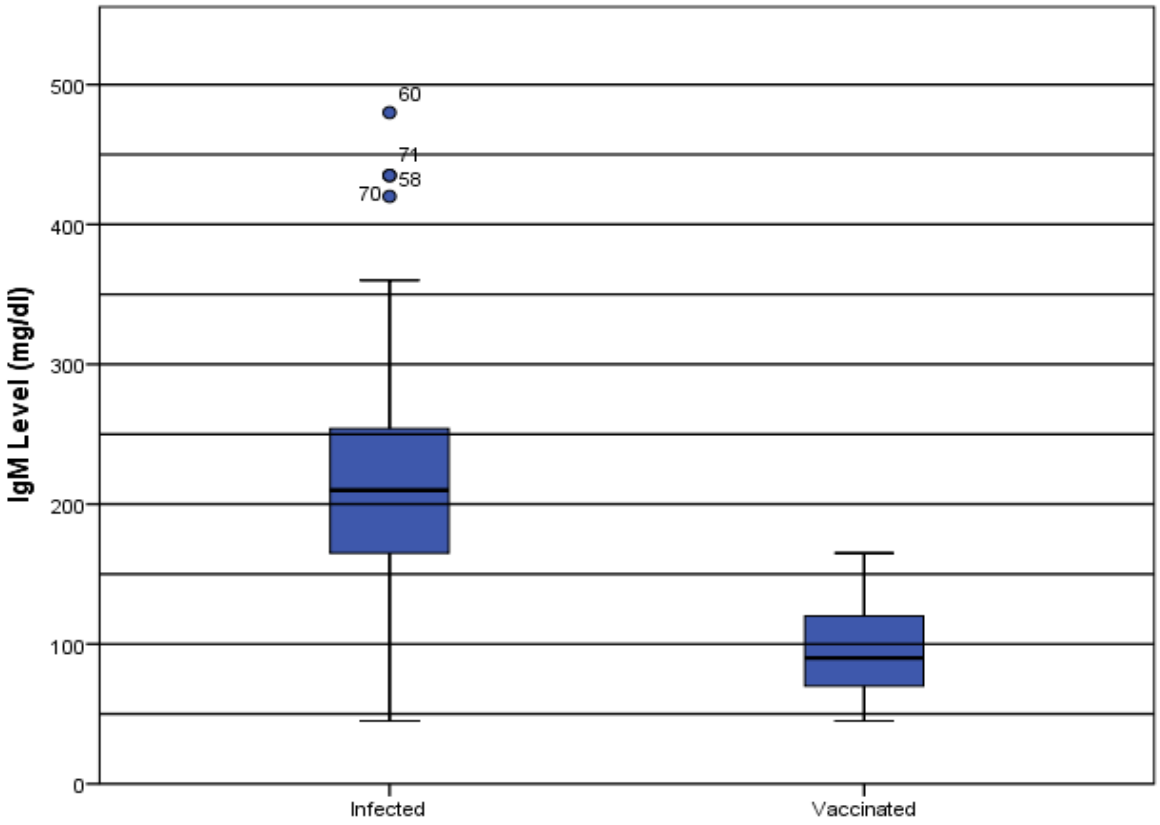

Figure (2): IgG level among cases

Regarding side effects, flu symptoms were the most prevalent that were found in 26.8% of the patients, shoulder edema in 14.6% and local irritation in 2.4%. While 56% reported no side effects (Figure 3).

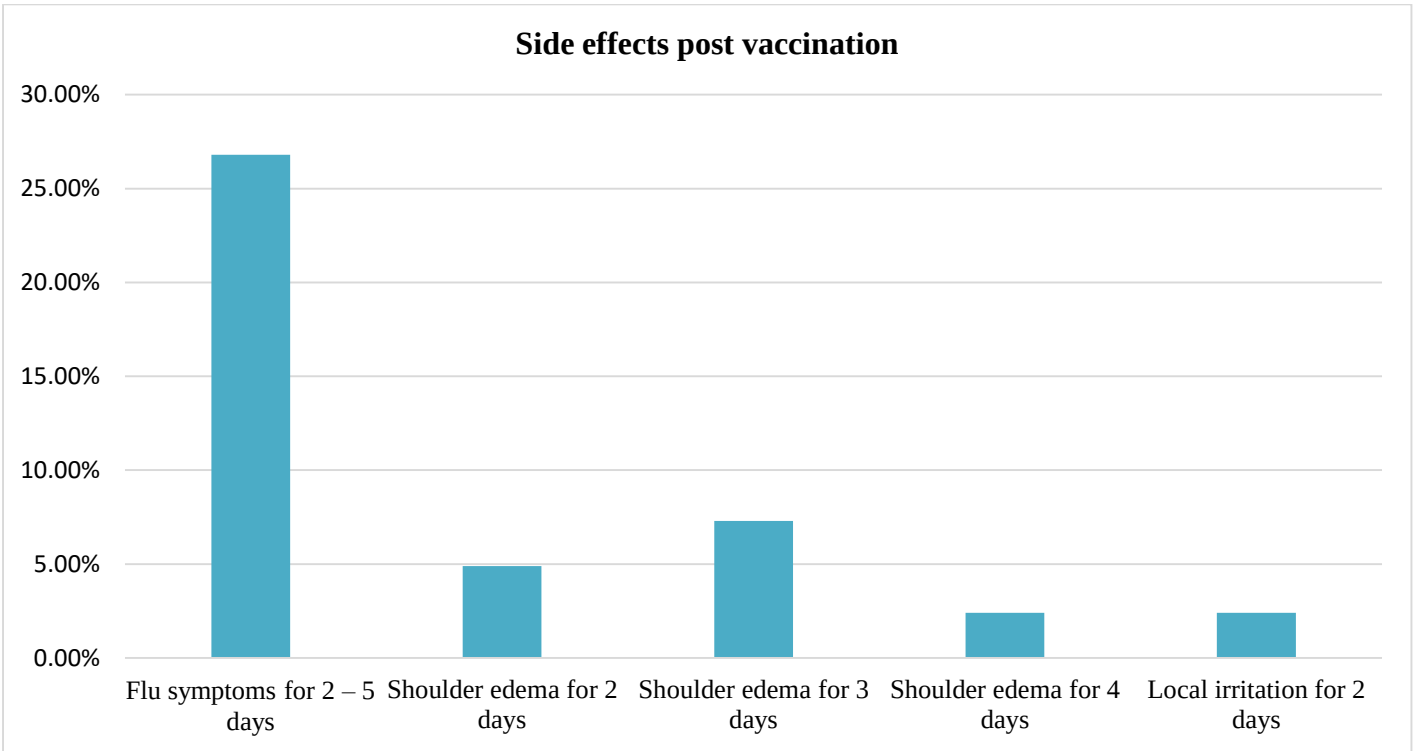

Figure (3): Side effects post vaccination.

DISCUSSION

Since the COVID-19 vaccine was introduced, the majority of sero-epidemiological research have narrowed their emphasis to certain populations, such as healthcare professionals. Very little is known about comparing the strength of antibody responses to various vaccinations and protocols in individuals with varying exposure histories (i.e., those who have never been exposed, those who have been sick but have different symptoms, etc.). So far, research indicates that mRNA vaccines outperformed other vaccination forms in terms of antibody affinity and IgG titers [2]. Vaccines can stimulate many immune responses, such as IgG anti-spike responses, variable amounts of IgM and IgA responses, and specific immunogens that could offer protection. From baseline to three weeks after the second dose, healthcare professionals vaccinated with Comirnaty showed different patterns of anti-spike IgG and IgM production, which was linked to virus-neutralizing activity, according to a recent longitudinal study [7].

Vaccination is a strong tool in the fight against infectious disease transmission; as a result, the 2019 coronavirus disease (COVID-19) vaccine has been advised that all citizens get the shot in order to boost their immune system's capacity to fight viruses and cut down on the number of cases [8].

Despite the administration of almost 10 billion doses of vaccine, additional research is necessary to determine why there remains a persistently high level of antibody in serum. According to the COVID-19 Diagnosis and Treatment Protocol published by the National Health Commission of China, clinical laboratories frequently employ SARS-CoV-2 specific antibody detection in conjunction with RNA detection and gene sequencing to establish the etiology of COVID-19. More than that, it's a way to track how many antibodies a person has after getting a shot. The most important intervention strategy for limiting the spread of COVID-19 is a safe and efficient mass vaccination, and the detection of antibodies serves as a reflection of the vaccine's efficacy [10].

Further research into the ways in which SARS-CoV-2 interacts with the immune system of the host and how this affects the development of disease and the functioning of organs is crucial. In the face of potentially fatal viral disorders like COVID-19, it is essential for all parties involved to maintain or enhance their immunity levels on a regular basis [11].

Our study found that compared to vaccinated patients, infected subjects had considerably greater levels of IgM and IgG. A sign of acute infection, IgM is released initially during the early stages of pathogen infection; however, it only exists for a short period. Indicators of mid- to late-infection or past infection can be seen in IgG, which is created later than IgM but can remain for a long period. Our results are consistent with those of previous research showing that SARS-CoV-2 infection was associated with an upward trend in IgM and IgG titres followed by a downward trend [12].

Those who are able to mount an immune response following vaccination (detectable IgM and IgG in vivo) may nevertheless have SARS-CoV-2 infection, according to earlier research. But compared to non-vaccinated individuals, they are able to quickly manufacture a substantial amount of IgG that acts as a protective barrier following infection [13].

As a therapy regimen utilizing COVID-19 survivors' plasma, the "COVID-19 survivors recovery plasma treatment " was published by the National Health Committee of China. This suggests that it has significant impact on COVID-19 patients with a high titre of plasma antibodies. The protective concentration of COVID-19 antibody is still unknown, while serum antibody level is generally considered a relevant indicator to assess the risk of epidemic disease. Someone who has been vaccinated against COVID-19 and yet got SARS-CoV-2 could have contracted the virus since some IgG antibodies aren't neutralizing antibodies and don't provide much protection. Secondly, due to the virus's mutation, an immunological escape reaction could occur. Third, over time, the antibody's protective titre decreases, but not to the point where it becomes vulnerable to the virus. Because of this, additional neutralizing antibody testing is necessary to ensure that the antibodies produced by vaccination are effective [14].

Chen *et al.* [14] found a similar pattern to ours: IgG is produced quickly in the first week following vaccination (on the first day), and 82.92% of the antibody-positive population had IgG (+) rather than IgM (-) antibodies. After reaching its maximum on day 13, the antibody titre progressively drops down to a very high level, maintaining it there while the proportion rises to 100%. On day seven following inoculation, IgM becomes positive; after a week or so, the antibody titre begins to decline and eventually turns negative. The trend of antibodies in patients infected with SARS-CoV-2 for the first time does not disagree with this change curve, but it does not match it. While IgM had become negative, it was possible that IgG was still present in their systems. However, IgG is positive on the first day after vaccination because it could be released in vivo soon after the second or third injection.

People who have recovered from COVID-19 typically have an IgG existence period of 3-8 weeks, according to studies by **Zhang *et al.*** [15] **and Long *et al.*** [16]**.** After being inoculated with the COVID-19 vaccine, the antibody levels can stable at a high titre for 1-2 weeks, according to **Chen *et al.*** [14]. After that, they start to fall slowly, and they continue to exist for 10-25 weeks. It is unclear if the antibody function is impacted, even though the drop amplitude is not large. So, even with universal vaccination coverage, SARS-COV-2

could still be able to spread, and effective public health initiatives are necessary to stop it.

The results showed that IgM could be quantified between the third and fifth day following SARS-CoV-2 infection, and IgG could be detected between the tenth and fifteenth day, as reported by **Zhou *et al.*** [9]. Although, it is slightly delayed following a natural infection, **Chen *et al.*** [14] found that IgM starts to exist on the seventh day after vaccination. By the time the second shot is administered, IgG has already been produced. Note that IgG would typically turn positive on day two, which is sooner than the time after a natural virus infection; however, more tests are required for confirmation. Because the immunological responses of the sexes are varied, the first two weeks following vaccination show a much greater IgM level in males than in females. Subsequently, IgG levels do not differ significantly between the sexes; they peak in the second week and then progressively decline, with no discernible variation in the rate of decline. The levels of IgM do not vary much between age groups; however, they do decline and become negative by the third week.

CONCLUSION

Infected individuals had substantially greater IgM and IgG titres than vaccinated ones. Compared to sick individuals, those who received the COVID-19 immunization had much reduced titre levels of IgG and IgM. Consequently, people infected with COVID-19 can be followed up with IgM and IgG. In addition, they work well as indicators of immunization, which is a powerful tool in the fight against the spread of SARS-CoV-2.

Conflict of interest: None.
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