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Article

Comparative Study of IgG Between Iraqi Covid-19 Patients and Vaccinated Individuals

Sally Taleb Mutlaq[1,], and Raghad Harbi Mahdi[2]*
[1] College of Science, Baghdad University, Iraq.
[2] College of Science, Baghdad University, Iraq.
* *Correspondence: Sally.bio88@gmail.com*
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ABSTRACT

COVID-19 is a coronavirus disease caused by the severe acute respiratory syndrome. According to the World Health Organization (WHO), coronavirus-2 (SARS-CoV-2) was responsible for 87,747,940 recorded infections and 1,891,352 confirmed deaths as of January 9, 2021. Antibodies that target the S-protein are efficient in neutralizing the virus. **Methodology:** 180 samples were collected from clinical sources (Blood and Nasopharyngeal swabs) and from different ages and genders at diverse hospitals in Baghdad / IRAQ between November 5, 2021, to January 20, 2022. All samples were confirmed infected with COVID-19 disease by RT-PCR technique. Haematology analysis and blood group were done for all samples, and Enzyme-Linked Immunosorbent Assay used an IgG test. **Results:** Complete blood count showed white blood cells, haemoglobin and platelets were higher in group 1 (without vaccine) than in control. In contrast, lymphocytes showed a lower ratio, ABO group showed type A, and O has more susceptible than other types. IgG level showed a high ratio in group one. **Conclusions:** Vaccination plays an important role in COVID-19 patients and maintains normal levels of the parameters under study.

Keywords: COVID-19; Vaccinated Individuals; IgG; CBC; Blood group

INTRODUCTION

Since its discovery in Wuhan, China, on December 12, 2019, the severe acute respiratory syndrome coronavirus 2 (SARSCoV2) has spread rapidly worldwide and become a pandemic [1]. The disease has been renamed coronavirus disease (COVID19) by the World Health Organization (WHO). More than 700,000 COVID19 cases had been confirmed in over 100 countries and regions by March 31, 2020 [2]. SARSCoV2's fast spread has caused significant harm to public health and the economy too far [3]. COVID19 has a 3 to 7-day incubation period, with typical clinical signs including fever, cough, shortness of breath, and exhaustion [4]. More than 84 000 confirmed cases and 4600 deaths had been reported in China by April 22, 2020 [5]. Approximately 80% of individuals developed mild or nonspecific symptoms, with 20% of patients progressing to severe or critical disease [6]

SARS-CoV-2, the COVID-19 causal agent, enters human cells by attaching the angiotensin-converting enzyme 2 (ACE2) receptor to the receptor-binding domain (RBD) of its spike (S) protein [7]. Other beta-coronaviruses elicit different serological responses. Patients infected with SARS-CoV developed IgG antibodies in all cases [8]. Antibodies that target the S-protein are efficient in neutralizing the virus. SARS-CoV-2 S- and nucleocapsid (N-) protein-specific IgG antibodies are typically measured in seroprevalence investigations; however, whether these antibodies correlate with protective immunity is uncertain [9]. A link between the ABO blood group and vulnerability to Covid-19 has been found in studies from China and other world regions (USA, Turkey, Europe, and Middle Eastern nations) [10]. Despite some differences, they demonstrated a general tendency that those with type A blood had a higher probability of contracting SARS-CoV-2 infection than those with type O blood [11].

Vaccines are designed to trigger a primary immune response by introducing altered or weakened antigens (or components thereof) that cause disease, allowing the host to establish immunological memory without becoming sick naturally [12]. Ideally, vaccines should stimulate B- and T-cell responses. The primary mechanism of vaccine effectiveness is the induction of antigen-specific antibodies. The efficacy of antibodies is determined by their quality (affinity, specificity, and/or neutralizing capacity). Long-term protection requires the persistence of vaccination antibodies above protective thresholds and/or the preservation of immunological memory cells capable of quick and effective reactivation following subsequent exposure [13].

MATERIALS AND METHODS

Samples Collecting

One hundred and eighty samples (patients and healthy) were collected from clinical sources (Blood and Nasopharyngeal swabs) and different ages and genders at Ibn Zohr Hospital, Ibn Al-Khatib Hospital, Dar Al Attaa Hospital and Economic Council Hospital between November 5 and January 20, 2022. All samples were confirmed infected with COVID-19 disease by Reverse Transcriptase -Polymerase Chain Reaction technique and distributed according to:

Patients

1. 30 Patients who lie in the hospital after 15-21 days of being diagnosed with COVID-19 through a PCR smear (G1).

2. 30 Pfizer vaccinated and previously infected after 21 days, the date of taking the second dose(G2).

3. 30 Previously infected patients 3-6 months after their last infection (whether vaccinated or not) (G3).

4. 30 Pfizer vaccines 3-6 months after taking the second dose(G4).

Control

1. 30 Previously uninfected persons vaccinated with Pfizer, a second dose after 21 days from the date of the second dose (positive control) (G5).

2. 30 Unvaccinated uninfected persons (negative control) (G6).

Laboratory Analysis

All samples (patients and control) were tested for CBC and ABO group as follow:

Complete Blood Count (CBC): Use of an auto analyzer Manufactured by a haematology company (Sysmex Kx2In Japanin) for blood cell count for all samples. The tests under study were done, including Hemoglobin (HB), White blood cells (WBCs), Lymphocyte (LMY) and Platelet (PLT).

ABO Group: This test was used according to the Manufactured company to determine the type of blood for all samples.

Human anti-SARS-CoV2(N) IgG Enzyme-Linked Immunosorbent Assay

This test was used according to the human anti-SARS-CoV2(N) IgG ELISA Kit (Cat. No.: MBS7608188, MyBioSource, CAL, UAS) For quantitative detection of Anti-SARS-CoV2(N) IgG in serum as follow :

Step1: Wash plate 2 times before adding Standard, Sample (diluted at least 1/50 with Sample Dilution Buffer) and Control (blank) wells.

Step2: Add 50ul standard or sample to each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 3 times.

Step3: Add 50ul HRP-labeled antibody working solution to each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step4: Add 50ul TMB Substrate Solution. Incubate for 10-15 minutes at 37°C.

Step5: Add 50ul Stop Solution. Read at 450nm immediately and calculate.

Statistical Analysis:

The Statistical Analysis System- SAS (2012) application was utilized to determine the effect of different components in research parameters. To make a significant comparison between means, the Least Significant Difference –LSD test (ANOVA) was performed. This study used the Chi-square test to compare percentages (0.05 and 0.01 likelihood) [14].

RESULTS

The relation of Complete Blood Count (CBC) with COVID-19

The results shown in Table 1 showed CBC for all study samples as follows: the WBC ratio at G1, G2, G3, and G4 were (12.73 ±0.32,6.61 ±0.24,7.15 ±0.26,6.62 ±0.19) µl respectively while in case of control, G5 and G6 showed (7.01 ±0.29,7.18 ±0.22) µl respectively with significant (P-value = 0.0001).

The levels of lymphocytes of all study participants (infected and control) as follow: G1, G2, G3 and G4 showed (2.75 ±0.21, 2.17 ±0.12, 2.75 ±0.12, 2.33 ±0.11, 2.50 ±0.12) µl respectively while in the case of control, G5 and G6 shown (2.50 ±0.12, 2.37 ±0.09) µl respectively with significant (P-value = 0.0996).

Hemoglobin in study group (patients) G1, G2, G3, and G4 showed (12.83 ±0.19 ,13.27 ±0.21 , 13.60 ±0.20 , 13.58 ±0.18) g/dl respectively while in the control, G5 and G6 showed (13.73 ±0.19 ,13.52 ±0.18) g/dl respectively with significant (P-value = 0.0051).

Platelet at G1, G2, G3 and G4 showed (285.60 ±8.01, 266.28 ±9.45, 269.79 ±11.06, 253.07 ±10.04) µl respectively, while in the case of control, G5 and G6 showed (280.36 ±9.41, 264.29 ±9.51) µl respectively with significant (P-value = 0.048).

Group	Mean ± SE			
	WBC	Lymphocyte	Hb	PLT
G1: Patients	12.73 ±0.32 a	2.75 ±0.21	12.83 ±0.19 b	285.60 ±8.01 a
G2: V21	6.61 ±0.24 b	2.17 ±0.12	13.27 ±0.21 ab	266.28 ±9.45 ab
G3: P3-6M	7.15 ±0.26 b	2.75 ±0.12	13.60 ±0.20 a	269.79 ±11.06 ab
G4: V3-6M	6.62 ±0.19 b	2.33 ±0.11	13.58 ±0.18 a	253.07 ±10.04 b
G5: CO+	7.01 ±0.29 b	2.50 ±0.12	13.73 ±0.19 a	280.36 ±9.41 ab
G6: CO-	7.18 ±0.22 b	2.37 ±0.09	13.52 ±0.18 a	264.29 ±9.51 ab
LSD value	0.847 **	0.612 NS	0.585 **	27.546 *
P-value	0.0001	0.0996	0.0051	0.048
This means having the different letters in the same column differed significantly. * (P≤0.05), ** (P≤0.01).				

Table 1. Comparison between different groups in WBC, Lym, Hb and PLT

The relation of Blood group with COVID-19

The results study shown in table 2 showed the ABO group associated with sample study (patients and control) at Significant (P≤0.05) as follow: G1 (24 (24.00%) A, 25 (25.00%)B, 22 (22.00%)AB, 29 (29.00%) O), G2 (15 (30.00%)A, 12 (24.00%)B, 14 (28.00%)AB,9(18.00%) O), G3 (12 (24.00%)A, 12 (24.00%)B, 11 (22.00%)AB, 15 (30.00%) O), G4 (15 (30.00%) A,12 (24.00%)B, 10 (20.00%)AB, 13 (26.00%) O).

In the case of control, G5 and G6 showed 14 (28.00%)A, 12 (24.00%)B, 9 (18.00%)AB, 15 (30.00%) O) and (13 (26.00%)A,13(26.00%) B, 8 (16.00%) AB, 16 (32.00%) O) respectively.

Group	No	A No. (%)	B No. (%)	AB No. (%)	O No. (%)
G1: Patients	100	30 (30.00%)	21 (21.00%)	20 (20.00%)	29 (29.00%)
G2: V21	50	15 (30.00%)	12 (24.00%)	14 (28.00%)	9 (18.00%)
G3: P3-6M	50	12 (24.00%)	12 (24.00%)	11 (22.00%)	15 (30.00%)
G4: V3-6M	50	15 (30.00%)	12 (24.00%)	10 (20.00%)	13 (26.00%)
G5: CO+	50	14 (28.00%)	12 (24.00%)	9 (18.00%)	15 (30.00%)
G6: CO-	50	13 (26.00%)	13 (26.00%)	8 (16.00%)	16 (32.00%)
*** (P≤0.05).**					

Table 2. Distribution of sample study according to ABO in different groups

The relation of IgG with COVID-19

The results study shown in Figure 1 showed a high ratio of G1, G2, G3 and G4 (12.83, 66.15, 45.8, 50.27) ng/ml, respectively. In the control case, G5 showed a high level (55.12) ng/ml, and G6 showed a normal ratio (23.69) ng/ml.

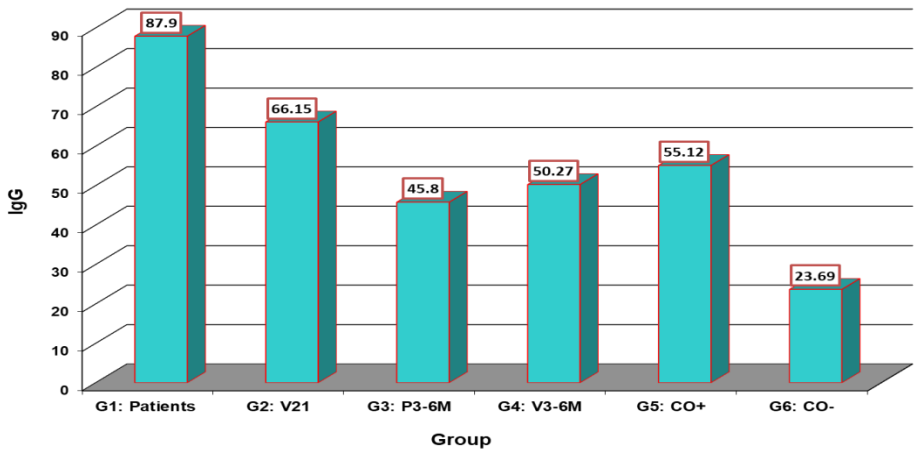

Figure 1. Comparison between the different groups in IgG

DISCUSSION

In many nations, the global COVID-19 epidemic is still spreading. To suppress the COVID-19 pandemic, a protective vaccination will be needed to achieve substantial herd immunity to SARS-CoV-2 infection [15]. More than 200 COVID-19 vaccines are now in development, according to the World Health Organization (WHO), and there are great hopes for effective preventive COVID-19 vaccinations [16].

From Table 1, this study disclosed the ratio of increased white blood cell count at G1 was the highest at 12.73 among the other groups, and the results were highly significant P-value = 0.0001. The current results agree with many studies. WBC count increased relatively slightly in individuals with severe illness (0.41 109 /L), while it increased clinically significantly in patients who died (4.15 109 /L). As a result, a considerable rise in WBCs in patients with severe illness may indicate clinical deterioration and an increased likelihood of a poor outcome [17]. A large increase in WBC counts in patients with severe COVID-19 may indicate clinical deterioration and poor prognosis [18].

Leukocytosis, whether it be neutrophilia, lymphocytosis, or both, is seen in a small percentage of COVID-19-infected patients and appears to be a sign of bacterial infection or superinfection. Leukocytosis was found in 11.4 percent of patients with severe disease, compared to 4.8 percent of patients with mild-to-moderate disease [19]. Another meta-analysis found that patients with severe sickness were more likely to have leukocytosis (11.4 percent) than those with mild to moderate illness (4.8 percent) [odds ratio, 2.54; 95 percent confidence interval, 1.43–4 [20]. In COVID-19-infected patients, worsening leukocytosis could indicate bacterial infection or superinfection. If the patient is currently on antibiotics, any increase in leukocytosis is a warning flag that should be investigated for bacterial superinfection or resistance [18]. The clinical significance of growing white blood cells (WBC) found that a WBC >10.0 109/L (adjusted HR 2.0; 95 percent CI 1.3–3.3) was linked with an increased risk of death in severe instances [21].

Another research [22] found lower LYM levels and higher PLT levels in swab-positive cases and a significantly decreased platelet count (P = 0.001). Previous investigations comparing RT-PCR positive and RT-PCR negative patients found that the positive patient had a considerably lower lymphocyte count [23].

The following common haematological abnormalities were described in the early reports: leukopenia (33.9%), thrombocytopenia (36.5%), and lymphopenia (82.3%) [24]. A low platelet count is normally linked to a viral infection that isn't COVID-19-specific [18]. Similarly, the impact of low platelet counts in predicting prognosis has been extensively documented in some investigations involving critically ill patients. Several studies have found that certain patterns, such as a low nadir platelet count or a rapid drop in platelet count, are significant [24]. The low platelet count has been confirmed as a significant predictive factor of severity and mortality in patients with COVID-19 [20]. Disturbed myelopoiesis in patients with severe, systemic COVID-19 infection is suggested by alterations in circulating neutrophils and platelets [25].

The lymphopenia is a typical symptom of COVID-19 infection and is thought to indicate a faulty immunological response to the virus. An absolute lymphocyte count of 1.0 10^9/L) in 26 (63%) of 41 people with RT-PCR–confirmed COVID-19 infection in an early study of 41 adults with RT-PCR–confirmed COVID-19 infection [26]. According to a recent meta-analysis [27], 35 to 75 percent of individuals had lymphopenia, which was more common in dying patients. A lymphocyte count of 0.6 10^9/L was predictive of admission to the intensive care unit in 67 COVID-19 patients from Singapore (ICU) [28]. Our results agree with a study done by [29], who found that in COVID-19 patients, platelet, leukocyte, and haemoglobin were found to be higher in patients with positive test results as follows: PLT (288.0 ± 72.3), Hemoglobin (14.9 ± 1.8), Leukocytes (11.2 ± 3.7), and lower with Lymphocytes (2.6 ± 2.5) [30] . COVID-19-positive patients' haemoglobin levels were considerably higher; other factors, such as comorbidities or anemia and habits like cigarette smoking, may impact these results [30].

In terms of blood groups, patients with positive COVID-19 (G1) were shown to be more likely to have blood group types A and O (30 instances and 29 cases, respectively), which was statistically significant (Table 2). These findings align with other studies conducted in various countries [31], which found that SARS-COV-2 patients with blood group type A had a greater hospitalization rate following infection. In China, SARS-COV-2 patients with blood group A had a greater fatality rate than non-A patients [32].

The current findings were comparable to those of [33], who examined the clinical aspects of 134 COVID-19 cases in China and found that males were more prevalent than females and that the percentage of older patients with the underlying condition was relatively high. Importantly, the researchers revealed that A: 43.82 percent, B: 26.91 percent, O: 19.21 percent, and AB: 10.1 percent of COVID-19 patients belonged to the ABO group. People with blood group type A are more susceptible to infection than others but not to hospitalization, according to studies conducted in the United States [34]] and Denmark [35].

A recent analysis shows SARS-COV-2 infection and blood group exhibit significant variances. Unlike some studies [36] that say there is no substantial difference between patients regarding disease severity and mortality regarding the ABO system, Turkey found no correlation between clinical outcomes like intubation, intensive care unit stay, and mortality.

ELISA measured the serum levels of immunoglobulin (IgG) against SARS-CoV2. Our results showed a high level of IgG at G1(before vaccination) from other groups vaccinated with Pfizer Figure 1, several studies indicated the important role of IgG in VOVID-19 infection. SARS-CoV-2 spike-specific IgG levels in the blood grew dramatically after the first mRNA vaccine dose and peaked 18–21 days later. SARS-CoV-2 spike-specific blood IgG increased even more after the second vaccine dose, peaked 7 days later, and remained elevated

(58 percent of peak values) for the remainder of the >100-day follow-up period [37].

Anti-SARS-CoV-2 S-specific IgG antibodies were detected starting on day 7 and peaked around day 25. After 4 weeks of infection, serum IgG antibodies were still at a high level [38]. Following vaccination, the preservation of spike antigen-specific serum IgG is hoped to be a favorable indicator of successful long-term immunity and a clinical indicator of vaccine response [39]. Humoral responses are important components of viral infection adaptive immunity [40]. COVID-19 patients' alpha and gamma immunoglobulins (Ig) mediate viral neutralization and may play diverse roles in immunity at different stages of infection and different anatomical sites [41]. IgG2 antibodies are mostly elicited in response to bacterial infection, whereas IgG1 and IgG3 antibodies are elicited in reaction to viral infections [42].

Vaccines provide altered or weakened antigens (or parts of antigens) that ordinarily cause disease, allowing the host to acquire immunological memory without becoming infected naturally [43]. It's vital to develop an effective vaccination against SARS-CoV-2, the virus causing the coronavirus disease-2019 (COVID-19) pandemic, but more research is needed to determine the safety of various vaccines [44].

CONCLUSIONS

Longitudinal serology of COVID-19 mRNA vaccination recipients raises crucial questions about immunity and vaccine response monitoring. Following vaccination, the preservation of spike antigen-specific serum IgG is hoped to be a favorable indicator of successful, long-lasting immunity and a clinical indicator of vaccine response. Haematology analysis showed WBC, HB and PLT were higher in group 1 when infected with COVID-19, while Lymphocyte was lower. ABO group showed O and A-type has more susceptible to COVID-19 disease. Immunoglobulin IgG was higher in group 1 and decreased after being vaccinated.

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