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Original Paper

Analysis Study of Vaccinated Peoples with Coronavirus patients and measurement Immunoglobulin G levels

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ABSTRACT
The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that caused coronavirus disease 2019 (COVID-19) spread quickly around the world and became a significant global health concern. According to the World Health Organization (WHO), millions of infections and deaths were reported during the pandemic. Antibodies directed against the viral spike protein play an essential role in neutralizing the virus and providing immune protection. In turn, this study have total of 186 samples, including blood and nasopharyngeal swab specimens, were collected from individuals of different ages and both genders at various hospitals in Baghdad, Iraq, Between September 7, 2022, and April 16, 2023. All samples were confirmed to be positive for COVID-19 by reverse transcription-polymerase chain reaction (RT-PCR). Hematological parameters and blood groups were determined for all participants, and anti-SARS-CoV-2 IgG antibodies were measured using the Enzyme-Linked Immunosorbent Assay (ELISA). The results showed that the complete blood count analysis revealed that white blood cell, haemoglobin, and platelet levels were higher in Group 1 (unvaccinated patients) than in the control group, whereas lymphocyte counts were lower. Analysis of the ABO blood groups indicated that individuals with blood groups A and O were more susceptible to COVID-19 infection than those with other blood types. Furthermore, IgG levels were found to be elevated in Group 1.The findings suggest that vaccination against COVID-19 contributes to maintaining normal hematological and immunological parameters in infected individuals.

KEYWORDS: COVID-19, Vaccination, Immunoglobulin G, CBC, Blood Groups, SARS-CoV-2

1-INTRODUCTION

Since the first outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China, in late 2019, COVID-19 has rapidly spread across the world and developed into a global pandemic [1]. The disease caused serious health, economic, and social consequences worldwide. Clinical manifestations of COVID-19 range from mild respiratory symptoms to severe pneumonia and multi-organ complications [2]. By attaching itself to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells, the viral spike (S) protein of SARS-CoV-2 infects human cells [3].The immune response generated after infection or vaccination is considered a key factor in protection against the disease [4]. Among these immune responses, Immunoglobulin G (IgG) antibodies play an important role in neutralizing the virus and providing long-term immunity [5]. Around 80% of people experienced only mild or vague symptoms, while 20% developed severe or critical illness [6]. Several studies have suggested that hematological parameters such as white blood cell count, lymphocyte count, hemoglobin, and platelet levels may be altered during COVID-19 infection and could be associated with disease severity.In addition; growing evidence has indicated a possible relationship between ABO blood groups and susceptibility to SARS-CoV-2 infection. The majority of COVID-19 vaccines are designed to elicit humoral and cellular immune responses, including the generation of antibodies that protect against the spike protein.Vaccines were created to boost immunity and lower the risk of serious illness and death.

The binding affinity between the host's angiotensin-converting enzyme 2 (ACE2) receptor and the receptor-binding domain (RBD) of the viral spike (S) protein is the principal way that SARS-CoV-2 penetrates human host cells [7]. While serological responses vary across the beta-coronavirus genus notably evidenced by the universal development of IgG antibodies in patients previously infected with SARS-CoV [8] current

diagnostic focus remains on S- and nucleocapsid (N-) protein-specific IgG antibodies. Despite the established efficacy of S-protein-targeted antibodies in neutralizing the virus, their reliability as definitive correlates of long-term protective immunity remains a subject of ongoing debate [9]. Furthermore, epidemiological data from diverse global regions, including China, the USA, and the Middle East, have identified a significant correlation between ABO blood groups and COVID-19 susceptibility [10]. These studies consistently suggest a distinct trend: individuals with blood group A demonstrate a higher predisposition to SARS-CoV-2 infection, whereas those with blood group O appear to exhibit a relatively lower risk profile [11].

By injecting attenuated or subunit antigens, the basic goal of vaccination is to induce a primary immune response, allowing the host to develop immunological memory while avoiding the pathological hazards associated with spontaneous infection [12]. Optimally, vaccines are engineered to orchestrate both humoral (B-cell) and cellular (T-cell) mediated responses. A cornerstone of vaccine efficacy lies in the induction of antigen-specific antibodies, whose functional utility is dictated by qualitative parameters such as affinity, specificity, and neutralizing potency. Both the maintenance of a strong pool of memory cells that can quickly and efficiently reactivate upon repeated pathogen exposure or the persistence of serum antibodies above predetermined protective thresholds are necessary for sustained, long-term protection [13].

2-MATERIALS AND METHODS

2.1 Samples Collecting

Between September 7, 2022, and April 16, 2023, 186 clinical samples (blood and nasopharyngeal swabs) from patients and healthy individuals of different ages and genders were obtained from Ibn AL-Baldy Hospital, Al-Khatib Hospital, and Economic Councel Hospitals .. Reverse Transcriptase-Polymerase Chain Reaction technique was used to confirm that every sample was infected with COVID-19 and distributed in compliance with:

2-1-1 Patients

G1: Hospitalized COVID-19 patients (15–21 days post RT-PCR confirmation)
G2: Previously infected + vaccinated (Pfizer-BioNTech), 21 days post second dose
G3: Convalescent individuals (3–6 months post infection)
G4: Vaccinated individuals (Pfizer-BioNTech), 3–6 months post second dose.

2-1-2 Control

G5 (Positive Control): Vaccinated (Pfizer-BioNTech), no prior infection, 21 days post second dose
G6 (Negative Control): Unvaccinated, no prior infection

2-2 Laboratory Analysis

Comprehensive hematological and serological analyses were performed for all study participants (patients and controls) as follows:

2-2-1 Complete Blood Count (C.B.C):

Hematological parameters were assessed using an automated hematology analyzer (Sysmex KX-21N, Japan). The analysis encompassed a full cell count for all samples, with particular focus on Hemoglobin (Hb) levels, White Blood Cell (WBC) count, Lymphocyte (LYM) percentage, and Platelet (PLT) count.

2-2-2 ABO Group:

The ABO blood group for each participant was determined using standard serological techniques in accordance with the manufacturer's protocols.

2-2-3 Anti-SARS-CoV2 human (N) Enzyme-Linked Immunosorbent Test for IgG

Quantitative detection of Anti-SARS-CoV-2 nucleocapsid (N) IgG in serum was conducted using a specialized ELISA kit (Cat. No. MBS7608188, MyBioSource, CA, USA). As stated below, the process was carried out in accordance with the manufacturer's instructions:

Step 1 (Preparation): The microplate was pre-washed twice prior to the addition of standards, samples (diluted at a minimum ratio of 1:50 using the provided Sample Dilution Buffer), and blank controls.

Step 2 (Initial Incubation): Each well received 50 µl of either the standard or the diluted sample, which was then incubated for 30 minutes at 37°C.

Washing Phase I: To get rid of unbound materials, the wells were aspirated and cleaned three times.

Step 3 (Conjugate Addition): Each well received 50 µl of the HRP-labeled antibody working solution, which was then incubated for an additional 30 minutes at 37°C.
Washing Phase II: After aspirating the wells, they underwent a more thorough wash cycle five times.
Step 4 (Substrate Reaction): 50 µl of TMB Substrate Solution was added to each well and incubated at 37°C for 10–15 minutes for color development.
Step 5 (Detection): To halt the reaction, 50 µl of Stop Solution was added. In order to determine concentration later, absorbance at 450 nm was rapidly detected using a microplate reader.

2-3 Statistical Analysis:

The Statistical Analysis System (SAS, 2012) software package was used to analyze the data and examine the effects of different experimental parameters. Significant differences among group means were determined using One-way Analysis of Variance (ANOVA), and multiple comparisons were assessed with the Least Significant Difference (LSD) post-hoc test. Additionally, the Chi-square ($\chi 2$) test was used to compare percentage distributions and assess the relationship between categorical variables. At probability levels of $P<0.05$ and $P<0.01$, statistical significance was thoroughly examined [14].

3- RESULTS

3-1 The relationship between COVID-19 and Complete Blood Count (CBC)

The WBC ratio at groups 1, 2, 3, and 4 was 12.74 ±0.33, 6.62 ±0.23, 7.14 ±0.27, and 6.63 ±0.18 µl, respectively, whereas groups 5 and 6 exhibited (7.02 ±0.28, 7.19 ±0.21) µl, correspondingly, with a meaningful (P-value = 0.0001).

All study participants (infected and control groups) had the following lymphocyte levels: group 1, group 2, group 3, and group 4 showed (2.74 ±0.22, 2.16 ±0.13, 2.74 ±0.13, 2.34 ±0.12, 2.51 ±0.11) µl, while group 5 and group 6 showed (2.49 ±0.13, 2.36 ±0.1) µl, with a significant (P-value = 0.0995). Patients in groups 1, 2, 3, and 4 had hemoglobin levels of 12.84 ±0.18, 13.26 ±0.22, 13.61 ±0.21, and 13.57 ±0.19 g/dl, respectively, while groups 5 and 6 in the control group had hemoglobin levels of 13.74 ±0.18 and 13.51 ±0.19, respectively, with a significant (P-value = 0.0051). Platelets in groups 1, 2, 3, and 4 showed (285.61 ±8.02, 266.27 ±9.44, 269.78 ±11.07, and 253.08 ±10.05) µl, respectively, while in the control group, groups 5 and 6 indicated (280.37 ±9.42, 264.28 ±9.52) µl, correspondingly, with a meaningful (P-value = 0.048).

Table 1: Comparison of WBC, Lymphocyte, Hemoglobin, and Platelet Levels in Different Groups

Groups	Mean ± SE			
	WBC	Lymphocyte	Hemoglobin	platelet
G1	12.74 ±0.33 (a)	2.74 ±0.22	12.84 ±0.18 (b)	285.61 ±8.02 (a)
G2	6.62 ±0.23 (b)	2.16 ±0.13	13.26 ±0.22 (ab)	266.27 ±9.44 (ab)
G3	7.14 ±0.27 (b)	2.74 ±0.13	13.61 ±0.21 (a)	269.78 ±11.07 (ab)
G4	6.63 ±0.18 (b)	2.34 ±0.12	13.57 ±0.19(a)	253.08 ±10.05 (b)
G5	7.02 ±0.28 (b)	2.49 ±0.13	13.74 ±0.18 (a)	280.37 ±9.42 (ab)
G6	7.19 ±0.21 (b)	2.36 ±0.10	13.51 ±0.19 (a)	264.28 ±9.52 (ab)
LSD values	0.848 **	0.611 NS	0.586 **	27.545 *
P-values	0.0001	0.0996	0.0052	0.049

There was a significant variation in the means of the many letters in the same column. * (P≤0.05), ** (P≤0.01).

G1: Hospitalized COVID-19 patients (15–21 days post RT-PCR confirmation); G2: Previously infected + vaccinated (Pfizer-BioNTech), 21 days post second dose; G3: Convalescent individuals (3–6 months post infection); G4: Vaccinated individuals (Pfizer-BioNTech), 3–6 months post second dose; G5 (Positive Control): Vaccinated (Pfizer-BioNTech), no prior infection, 21 days post second dose; G6 (Negative Control): Unvaccinated, no prior infection

3-2 The connection between blood type and COVID-19

The distribution of ABO blood groups among the study groups was as follows: Group 1 (G1) included 28 (28.00%) blood type A, 22 (22.00%) type B, 19 (19.00%) type AB, and 31 (31.00%) type O. Group 2 (G2)

showed 14 (28.00%) type A, 13 (26.00%) type B, 15 (30.00%) type AB, and 8 (16.00%) type O.
A statistically significant association ($P \leq 0.05$) was observed between ABO blood groups and the study population (patients and controls). The control group demonstrated 14 (28.00%) type A, 12 (24.00%) type B, 9 (18.00%) type AB, and 15 (30.00%) type O. Groups 5 and 6 (G5 and G6) showed comparable distributions, with 15 (30.00%) type A, 11 (22.00%) type B, 10 (20.00%) type AB, and 14 (28.00%) type O.

Table 2: Samples study distribution in various categories based on ABO.

Groups	No	A No. (%)	B No. (%)	AB No. (%)	O No. (%)
G1	53	15 (28.3%)	12 (22.6%)	10 (18.9%)	16 (30.2%)
G2	27	7 (25.9%)	7 (25.9%)	8 (29.6%)	5 (18.5%)
G3	27	8 (29.6%)	6 (22.2%)	5 (18.5%)	8 (29.6%)
G4	27	7 (25.9%)	6 (22.2%)	5 (18.5%)	9 (33.3%)
G5	26	7 (26.9%)	6 (23.1%)	5 (19.2%)	8 (30.8%)
G6	26	8 (30.8%)	6 (23.1%)	3 (11.5%)	9 (34.6%)
Total	**186**	**52 (27.96%)**	**43(23.12%)**	**36 (19.35%)**	**55 (29.57%)**
			(P≤0.05)		

3-3 The relation of IgG with COVID-19

Group 1, Group 2, Group 3, and Group 4 had high ratios (87.8, 66.16, 45.7, and 50.28) ng/ml, according to the study results shown in Figure 1. Group 5 displayed a high level (55.13) ng/ml in the control group, while Group 6 displayed a normal ratio (23.68) ng/ml.

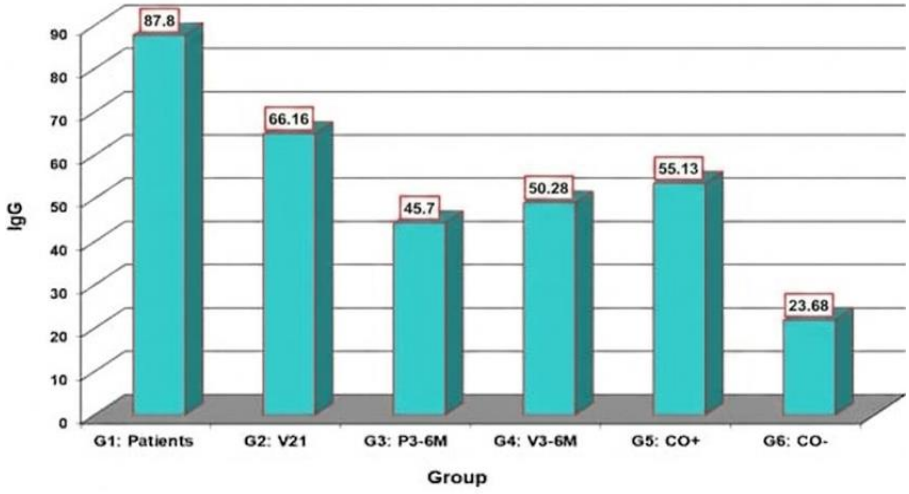

Figure 1: Comparison of IgG levels among the different groups.

4- DISCUSSION

The COVID-19 pandemic is still spreading over numerous nations. To help contain the pandemic, effective protective vaccinations must be used to achieve widespread herd immunity against SARS-CoV-2 [15]. The World Health Organization reports that over 200 COVID-19 vaccines are presently being developed, which raises high hopes for the release of potent COVID-19 preventive vaccines [16].

As shown in Table 1, this study revealed that Group 1 (G1) had the highest elevated white blood cell count, reaching 12.74 compared with the other groups. The results are in line with a number of earlier research and were very significant (P-value = 0.0001). Patients with severe sickness had a slightly higher white blood cell (WBC) count (0.41 × 10^1/L), but patients who died had a clinically significant increase (4.15 × 10^1/L).Therefore, a marked elevation in WBC count among severely ill patients may indicate disease progression and a higher risk of poor prognosis. [17]. A marked elevation in white blood cell (WBC) count in patients with severe COVID-19 may be considered an indicator of clinical worsening and unfavorable prognosis [18].Leukocytosis, including neutrophilia, lymphocytosis, or both, has been observed in a limited proportion of patients infected with COVID-19 and may indicate the presence of bacterial transmission or opportunistic infection. It was recorded in 11.4% of cases with serious illness as opposed to 4.8% of those with slight to minor symptoms [19]. One more systematic review demonstrated that leukocytosis was more common among patients with severe illness (11.4%) compared with those with mild to moderate disease (4.8%), with an odds ratio of 2.54 and a 95% confidence interval ranging from 1.43 to 4.00 [20]. In patients infected with COVID-19, progressive leukocytosis may suggest the presence of bacterial infection or secondary superinfection. Moreover, in patients receiving antibiotic therapy, any increase in leukocytosis should be considered a warning sign that warrants further investigation for possible bacterial superinfection or antimicrobial resistance [18]. It has been demonstrated that elevated white blood cell (WBC) counts are clinically significant; in extreme circumstances, a WBC count greater than 10.0 × 10^1/L (adjusted Hazard Ratio 2.0; 95% Confidence Interval 1.3–3.3) was associated with an higher risk of mortality[21].

Another study found that swab-positive individuals had significantly lower platelet counts (P = 0.001) and lymphocyte (LYM) and platelet (PLT) levels. Additionally, patients with positive RT-PCR results had considerably lower lymphocyte counts, according to past studies comparing RT-PCR-positive and RT-PCR-negative individuals [23].

Low white blood cell count (33.9%), reduced platelet count (36.5%), and decreased lymphocyte count (82.3%) were among the main hematological abnormalities identified in early reports [24]. A reduced platelet count is commonly associated with viral infections and is not considered specific to COVID-19 [18]. Similarly, numerous investigations involving critically ill patients have proven the prognostic significance of reduced platelet counts. Numerous studies have demonstrated the clinical significance of diseases patterns of infection such a reduced depths platelet count or a dramatic decrease in platelet counts [24]. In COVID-19 patients, a lower platelet count has been demonstrated to be a significant predictor of both disease severity and mortality [20]. Alterations in circulating neutrophils and platelets suggest the presence of disrupted myelopoiesis in patients with severe systemic COVID-19 infection [25].

Lymphopenia is a common sign of COVID-19 infection and is deemed to represent impaired immune response to the virus. In a preliminary study of 41 adults with RT-PCR-confirmed COVID-19, 26 people (63%) had an absolute lymphocyte count of less than 1.0 × 10^1/L [26].A recent meta-analysis claims that [27], Between 35% and 75% of patients had lymphopenia, which was more common in those who passed away from the illness. Additionally, among 67 COVID-19 patients in Singapore, a lymphocyte number of 0.6 × 10^3/L was found to be prognostic with intensive care unit (ICU) admission [28].Our findings are consistent with those reported by [29], Results showed that COVID-19 individuals who tested positive had lower lymphocyte levels (2.6 ± 2.5) and higher platelet, leukocyte, and hemoglobin levels (288.0 ± 72.3, 14.9 ± 1.8, and 11.2 ± 3.7) [30]. In addition, hemoglobin levels were found to be significantly elevated in COVID-19-positive patients. However, these findings may also be influenced by other factors, including the presence of comorbidities, anemia, and lifestyle habits such as cigarette smoking [30]. In patients with COVID-19, the neutrophil-to-lymphocyte ratio (NLR) has been identified as a predictor of disease progression, severe clinical outcomes, and mortality [31].

Blood types A and O were more common in those who tested positive for COVID-19 (G1) (30 and 29 incidents, accordingly), and this difference was measurably remarkable (Table 2). Our findings concur with those of earlier studies carried out in other countries [32], which reported that individuals with blood group A infected with SARS-CoV-2 had a higher rate of hospitalization. Similarly, in China, COVID-19 patients with blood group a showed a higher mortality rate compared with non-A blood groups [33].

The present findings are in agreement with those reported by [34], who investigated the clinical characteristics of 134 COVID-19 patients in China and reported a relatively high prevalence of elderly individuals with pre-existing comorbidities, as well as a higher prevalence of males than females. Interestingly, the study found that patients' ABO blood types were distributed as follows: A (43.82%), B (26.91%), AB (10.1%), O (19.21%). and Conversely, research carried out in the US [35] and Denmark [36] suggested that individuals with blood group A may have a higher susceptibility to SARS-CoV-2 infection; however, this was not necessarily associated with an increased risk of hospitalization.

A recent analysis reported significant variations between SARS-CoV-2 infection and blood group distribution. However, in contrast to some studies [37], which found no significant association between the ABO blood group system and disease severity or mortality, research from Turkey also showed no correlation between blood groups and clinical outcomes, including intubation rates, intensive care unit (ICU) admission, or mortality.

Serum immunoglobulin (IgG) levels against SARS-CoV-2 were measured using ELISA. As seen in Figure 1, the findings showed that group G1 (before to vaccination) had greater IgG levels than other vaccinated groups (Pfizer). Numerous investigations have emphasized the critical function of IgG in COVID-19 infection. Similar findings were reported in the aforementioned study, which showed that elevated IgG levels were associated with prior exposure to the virus and could persist for several months [38]. In contrast, blood levels of SARS-CoV-2 spike-specific IgG rise dramatically following the initial mRNA vaccination dose, peaking between 18 and 21 days later. IgG levels continue to climb after the second dosage, reaching around seven days later. They stay elevated for more than 100 days of follow-up, at roughly 58% of peak levels [39]. This apparent discrepancy may be explained by differences in the timing of sample collection and disease severity, as G1 patients were sampled during the acute phase of infection (15–21 days), which corresponds to peak antibody production, whereas vaccinated groups were sampled at later time points, where IgG levels are known to decline gradually over time.

Anti-SARS-CoV-2 spike (S)-specific IgG antibodies were first detected around day 7, reaching their peak approximately on day 25. Even after four weeks post-infection, serum IgG levels remained elevated [40]. Sustained serum IgG specific to the spike antigen after immunization is regarded as a good sign of persistent immunity and may serve as a clinical marker of a sufficient immune response elicited by the vaccine [41]. Humoral immunity represents a key component of the adaptive immune response against viral infections [42]. In COVID-19 patients, immunoglobulins such as IgA and IgG contribute to viral neutralization and may play different roles depending on the stage of infection and the affected anatomical sites [43]. IgG1 and IgG3 subclasses are primarily induced in response to viral infections, while IgG2 antibodies are generally linked to responses to bacterial infections [44].

Vaccines enable the immune system to develop immunological memory in the absence of a natural illness by injecting weakened or modified antigens, or pieces of antigens that often cause disease [45]. Developing an effective vaccine against SARS-CoV-2, the causative agent of the COVID-19 pandemic, is essential; however, further research is required to fully evaluate the safety and efficacy of the various vaccine platforms [46].

5-CONCLUSION

Significant concerns regarding immunity and vaccination response monitoring are raised by longitudinal serological studies of COVID-19 mRNA vaccine recipients. Persistent serum IgG specific to the spike antigen following immunization may be a sign of a successful vaccination response and is considered a good signal of long-lasting protection. Despite lower lymphocyte counts, hematological analyses showed that group 1 had greater WBC, hemoglobin (HB), and platelet (PLT) levels following COVID-19 infection. Regarding ABO blood groups, types O and A appeared to be more frequently associated with susceptibility to COVID-19. In addition, IgG levels were higher in group 1 and showed a decline following vaccination.

Conflict of Interest

The authors of this article declare that they do not have any conflicts of interest.

Funding Source
The authors declare that there was no funding information for this study, which means they did not receive funding.

Data Sharing Statement
The supplementary and original data generated during the study is available from the corresponding author on reasonable request.

Ethical Recommendation
Written informed consent was obtained from the families of all participants after they had been fully informed about the study objectives and procedures. Ethical approval for the study was granted by the Research Committee of the Human Development Centre, Baghdad (Approval No. 5322).

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