Original Article

Serological Studies of IgG and IgM in Response to SARS-CoV-2 Vaccination in Erbil, Kurdistan-Iraq

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ABSTRACT

The ongoing global pandemic of coronavirus disease (Covid-19) has had a considerable impact on healthcare systems and economies worldwide. The aim of vaccines against the virus is to elicit an immune response against the spike protein of the SARS-CoV-2 virus, with the objective of neutralizing the virus. Efficacy has now been demonstrated for several vaccinations, including those based on mRNA, adenoviral-vectored protein subunits, and whole-cell inactivated subunits. A comprehensive understanding of the immune responses to these vaccines, and the manner in which different antibodies are generated following vaccination, is imperative to enhance our comprehension of the pathophysiology of the disease. The present study aims to provide a comparative analysis of the humoral immune responses elicited by BNT162b2 (mRNA-based), BBIBP-CorV (inactivated virus), and ChAdOx1 (dsDNA-recombinant) vaccines against the SARS-CoV-2 virus. The study population comprised 321 individuals, with 90 individuals who had not received any vaccines (control group) and 77 individuals who had received the Pfizer and Sinopharm vaccines, respectively. Blood samples were collected 10 weeks after vaccination, and serum analysis was performed. The human SARS-CoV-2 Spike (Trimer) IgG or IgM ELISA (Thermo Fisher) was utilized to assess the quantity of IgG or IgM antibodies that had bound to the SARS-CoV-2 Spike (Trimer). The study revealed that there was no statistically significant difference between the vaccines (P value = 0.958). The investigation further demonstrated that all three vaccines (Pfizer, AstraZeneca and Sinopharm) were effective in stimulating the production of IgM and IgG. The study revealed that Sinopharm demonstrated superior efficacy in the induction of IgM and IgG. The utilization of ChAdOx1 resulted in the generation of higher levels of IgG compared to BNT162b2, as evidenced by a statistically significant difference (P value= 0.0001). The enhanced immune response observed with Sinopharm could be attributed to its nature as an inactivated subunit vaccine. The immunological reactions to the vaccines studied in the following studies have prompted a lot of issues about how they happen, and the study recommends more studies regarding the most effective vaccines among the Kurdish people in the Kurdistan Region of Iraq.

Keywords: SARS-CoV-2 Covid-19, Antibodies, IgM, IgG, Vaccines.

1. Introduction

The ongoing pandemic of coronavirus disease (Covid-19) has had a considerable impact on healthcare systems and economies worldwide. The causative agent of the pandemic is a novel strain from the coronaviridae family, designated as severe acute respiratory syndrome, coronavirus-2 (SARS-CoV-2) (1). Proinflammatory cytokine production in excess has been demonstrated to result in tissue lung injury and multi-organ failure (2,3). The structural features of SARS-CoV-2, particularly its spiked glycoproteins, aid in the pathogenesis and development of clinical symptoms ranging from mild symptoms (non-pneumonia or mild pneumonia) to severe illness and even death (4). The basic reproduction number (R0) is an epidemiological concept used to study the contagious nature of an infectious disease (5). Despite the expansion of treatment options for the disease, the emergence of new variants of the virus poses a significant challenge to existing approaches (6). Antiviral medications, such as remdesivir, lopinavir/ritonavir, and convalescent plasma therapy, as well as monoclonal antibodies including casirivimab and imdevimab, have demonstrated promising efficacy in preventing the development of symptoms (7). Vaccination is a pivotal step in reducing and eliminating SARS-CoV-2 infections (8). The human immune system responds to SARS-CoV-2 infection by producing neutralizing antibodies (IgG, IgM, and IgA), memory B and T cells (9). The purpose of vaccination is to trigger the immune system to produce SARS-CoV-2-specific functional immune memory (10).Disease severity appears to significantly affect antibody levels. Severe cases of the disease have been observed to demonstrate higher antibody levels in comparison to milder cases (11). It is evident that immune responses to vaccination vary according to age group, sex, type of vaccine, number of doses, and history of prior infection with SARS-CoV-2.The Beijing Bio-Institute of Biological Products (BBIBP) inactivated coronavirus vaccine, SinopharmCOVID-19, is the first Chinese vaccine to be granted WHO emergency approval. A glimmer of hope has been provided by Pfizer's assertion that they have developed a vaccine against the deadly virus. The interim results presented by Pfizer on November 9, 2020, demonstrated that the vaccine appeared to be 90% effective, a finding that was met with a high degree of enthusiasm (9). In addition, on April 19, 2021, the World Health Organization stated that the AstraZeneca vaccination had no fatal or seriously debilitating side effects (11).

2. Objectives

The present study is the first of its kind in the region, and its objective is to make a comparison between the antibody response revealed by the Sinopharm, Pfizer, and AstraZeneca vaccines against the disease caused by the novel severe acute respiratory syndrome (SARS-CoV-2) virus among the Kurdish people in the Kurdistan Region of Iraq.

2. Materials and Methods

2.1. Study Design

The study protocol was approved by the Research Ethics Board of Hawler Medical University (060721-477 HMU-EC).Written informed consent was obtained from all subjects for the detection of immunoglobulins and for the use of samples for other procedures. The ethical and consent implicit in providing informed consent is an assessment of the patient's understanding, rendering an actual recommendation, and documentation of the process. The study population comprised 321 individuals, with 90 of these individuals having received no vaccines (forming the control group). Moreover, 77 participants received the BNT162b2 (Pfizer-BioNTech, Pfizer Inc., NY, USA) vaccine, 77 participants received the BBIBP-CorV (Sinopharm's Beijing Institute of Biological Products, Beijing, China) vaccine, and 77 individuals received the ChAdOx1 (AstraZeneca- Oxford, Cambridge, UK) vaccine (Table 1). In the groups that received the Pfizer-BioNTech and AstraZeneca vaccines, 34% had a history of having contracted the virus, with 76% of these individuals not having a history of the disease. In the Sinopharm group, 11% had a history of contracting the virus, and 89% did not have a history of the disease.

2.2. Sampling and Data Collection

The subjects were divided into two groups: a pre-vaccination (control) group and a post-vaccination (vaccinated) group. Blood samples were collected from the subjects prior to vaccination, constituting the control group. Subsequent to this, samples were obtained from the subjects after 10 weeks of complete vaccination (Figure 1). The sampling was conducted under sterile conditions. The samples were subjected to centrifugation at 800g, 4˚C for 10 min, after which the resultant serum was stored at -80˚C for subsequent investigation. Serum levels of IgG and IgM in the samples were measured by Enzyme Linked Immunosorbent Assay (ELISA) using Quantikine kits (Thermos Fisher Scientific) at a wavelength of 450/650 nm. The manufacturer's protocol was followed.

2.3. Immunoglobulin Assay

The Human SARS-CoV-2 Spike (Trimer) IgG or IgM ELISA (Thermo Fisher) was utilized to quantify the levels of IgG or IgM antibodies that had bound to the SARS-CoV-2 Spike (Trimer).A trimerized spike protein was pre-coated in the wells of the provided microplate, after which serum samples and controls were added. The wells were then washed, and IgG antibodies conjugated to HRP were added. Subsequently, the wells were washed and a substrate solution was added. The assay was then read using an ELISA Microtiter plate reader, with the measurement taken at a wavelength of 450/620 nm.

2.4. Statistical Analysis

In order to facilitate the analysis of the data, both GraphPad Prism SAS software were utilised, as both the two-tailed t test and multivariate regression were executed. A p value of less than 0.05 was deemed to be statistically significant (*$p<0.05$; **$p<0.01$; ***$p<0.001$). The unpaired t-test and

descriptive statistical analysis were calculated using non-transformed data, and only regression analysis was carried out with transformed data.

2.5. Inclusion Criteria and Exclusion Criteria

In accordance with the manufacturer's guidelines, individuals are eligible for the Pfizer-BioNTech vaccine if they meet the following criteria:

- They are aged 18 years or older.
- They possess the capacity to comprehend the purpose and risks associated with the study.
- They have provided written informed consent.

With respect to the exclusion criteria, it is believed that individuals with a history of severe adverse reactions associated with a vaccine and/or severe allergic reactions (e.g., anaphylaxis) to any component of the study intervention (s) are not eligible for participation.

3. Results

Since the development of vaccines to combat the global pandemic, several countries have initiated vaccination programs with the objective of reducing mortality rates from the disease caused by the novel severe acute respiratory syndrome (SARS-CoV-2) virus. Despite these efforts, concerns regarding the safety and efficacy of the vaccines have persisted. The receptor-binding domain (RBD) of the virus is a viral component that facilitates the entry of the virus into host cells during the replication process. Serological testing utilizing IgG on RBD is a method by which neutralizing antibodies that can prevent the entry of SARS-CoV-2 into host cells can be detected. The study obtained blood samples from 321 participants, including 144 females and 177 males, from diverse ethnic backgrounds and age ranges from 17 to 91 years old, with a mean age of 42.58 years old (Table 1).

Table 1. Participant's characteristics.

Gender	Number of participants	Age (Mean; age range)
Pfizer		
Male	48	42.20 (18-75)
Female	29	42.96 (19-73)
Total	77	
AstraZeneca		
Male	46	39.52 (18-91)
Female	31	43.54 (19-74)
Total	77	
Sinopharm		
Male	48	39.52 (18-87)
Female	29	41.37 (21-81)
Total	77	
Control		
Male	35	45.69 (17-86)
Female	55	45.87 (17-87)
Total	90	

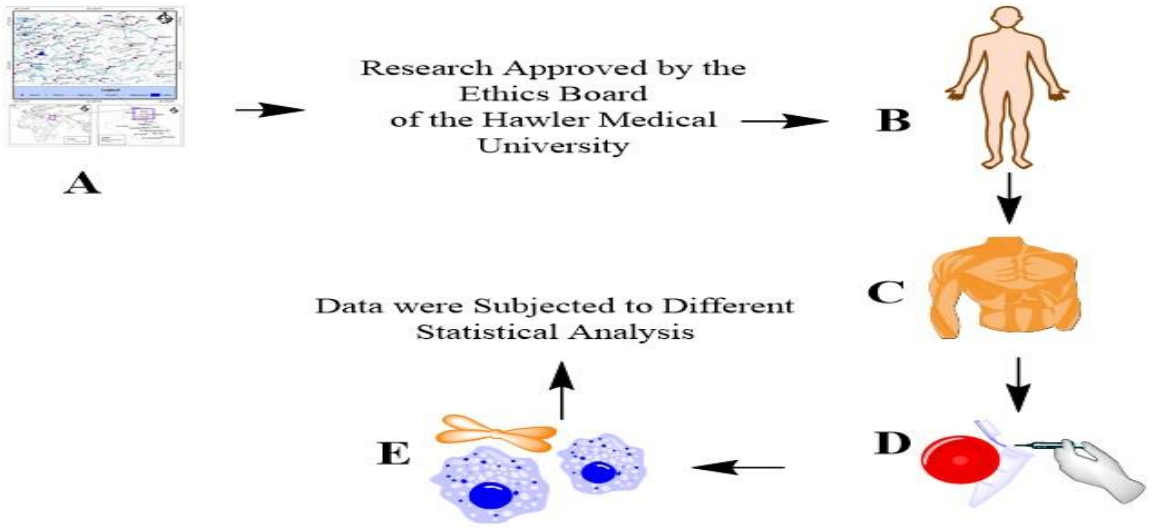

Figure 1. The following diagrammatic presentation of the research is provided: **A)** Kurdistan Region, Iraq, **B)** Participants who signed a written consent form (321), **C)** 77 participants were administered either Pfizer, Sinopharm or AstraZeneca, and 90 participants were in the control group, **D)** Blood samples were collected 10 weeks after the completion of the vaccination programme, **E)** IgG and IgM were detected using an ELISA (Thermo Fisher) and an absorption method.

As illustrated in Figure 2, the administration of vaccines by injection leads to the recognition of antigen by SARS-CoV-2, thereby initiating a series of immunological responses, including the activation of naive B cells. Activated B cells can differentiate quickly into extrafollicular, short-lived plasma cells and memory B cells (MBCs) with low somatic hypermutation rates, or they can enter the germinal centres of secondary lymphoid organs like lymph nodes, where they undergo rounds of somatic hypermutation and affinity maturation, resulting in long-lived plasma cells and MBCs. Furthermore, MBCs and plasma cells that secrete antibodies have the capacity to penetrate the bloodstream and (possibly) the mucosa, where they aid in the fight against viral infection and protect against reinfection (12).

3.1. Antibody Production Response To Vaccinations

Serum concentrations of both IgM and IgG in different groups who received different vaccines were evaluated, with the mean of IgM concentration after 10 weeks in the control group (not vaccinated) and in those vaccinated with BNT162b2, ChAdOx1, and BBIBP-CorV being 0.018, 0.269, 0.236, and 0.242 mg/dL, respectively. The IgG concentration mean after 10 weeks in the control group (not vaccinated) and in the vaccinated groups with BNT162b2, ChAdOx1, and BBIBP-CorV were 0.23, 7.39, 9.48, and 23.48 mg/dL, respectively.In addition, a comparison analysis for the production of both IgM and IgG was conducted between participants who received different vaccines. The statistical analysis of the IgM levels after 10 weeks revealed no significant differences between the various vaccines (P value = 0.958) (Figure 3). In contrast, it was observed that all participants had produced sufficient amounts of IgG against SARS-CoV-2, regardless of the vaccine type (BNT162b2, ChAdOx1, or BBIBP-CorV). Consequently, the level of IgG antibodies was found to vary among the various groups, as a statistically significant difference was identified (P<0.001) (Figure 4). Furthermore, a statistically significant increase in IgG levels was observed in participants vaccinated with BBIBP-CorV compared to those vaccinated with BNT162b2 or ChAdOx1 (P<0.001), as illustrated in Figure 4. Furthermore, the utilization of ChAdOx1 has resulted in the generation of elevated levels of IgG in comparison to BNT162b2. A statistically significant discrepancy was identified between the two (P < 0.001) (Figure 4).

3.2. Correlation Matrix Between IgM and IgG and Between 3 Different Vaccines

As illustrated in Table 2, the correlation results between the dependent variables IgM and IgG and the independent variables Pfizer, AstraZeneca, and Sinopharm are presented. The findings from Table 3 indicate a positive correlation between IgM and IgG and the aforementioned independent variables. This provides insight into the extent of the relationship between the dependent variables IgM and IgG and the independent variables in the regression model (Table 3).

3.3. Multivariate Regression of the Impact between 3 Different Vaccines on antibodies

The findings from the multivariate regression (IgM model) suggest that Sinopharm exerts a positive and significant influence on the production of IgM against SARS-CoV-2, as evidenced by the beta coefficient of 0.658540 and the probability value of 0.00. This indicates that for a given dosage of Sinopharm, approximately 66% of SARS-CoV-2 IgM is produced. Furthermore, the results of the multivariate regression analysis (IgM model) demonstrate that Pfizer has a positive significant impact on the production of IgM against SARS-CoV-2, as evidenced by the beta coefficient of 0.008510 and the probability value of 0.03. This suggests that for a complete dose of Pfizer, approximately 0.9% of SARS-CoV-2 IgM was produced. Furthermore, the multivariate regression analysis reveals that AstraZeneca has a positive and significant impact on the production of IgM against SARS-CoV-2, as evidenced by the beta coefficient of 0.100591 and the probability value of 0.00. This suggests that for every one dose of AstraZeneca, approximately 10% of SARS-CoV-2 IgM was produced even after a period of 10 weeks. This outcome indicates that the control groups demonstrated no significant development in their body's production of IgM. In a similar manner, the findings from the multivariate regression (IgG model) suggest that Sinopharm exerts a favorable and statistically significant influence on IgG antibodies against SARS-CoV-2. This conclusion can be drawn by examining the beta coefficient of 0.78152 and the probability value of 0.00 (Table 3).

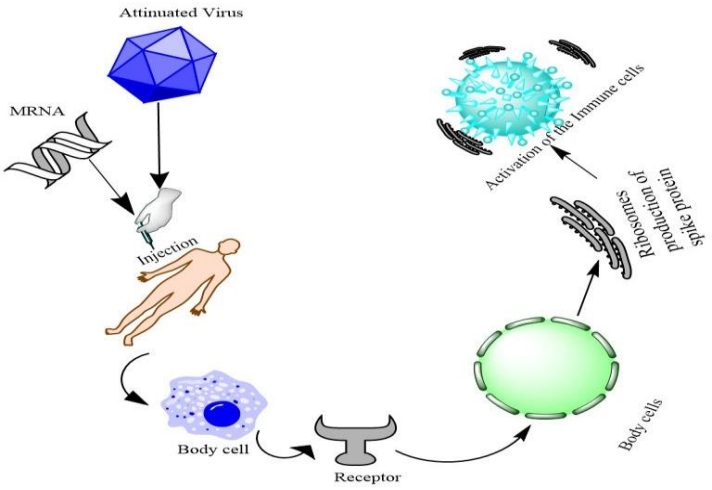

Figure 2. Overview of the body cell response to the Covid-19 vaccine.

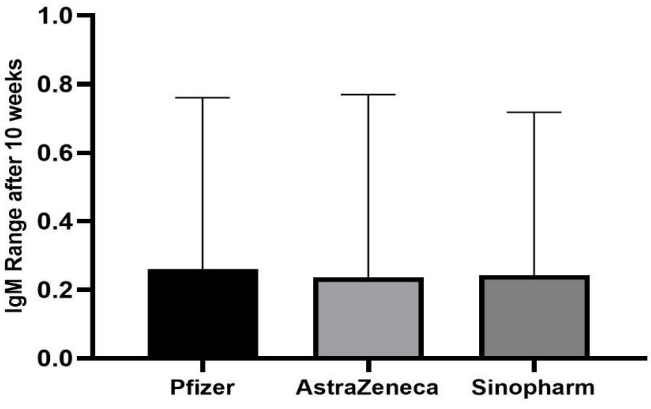

Figure 3. IgM remain compression after 10 weeks of vaccination.

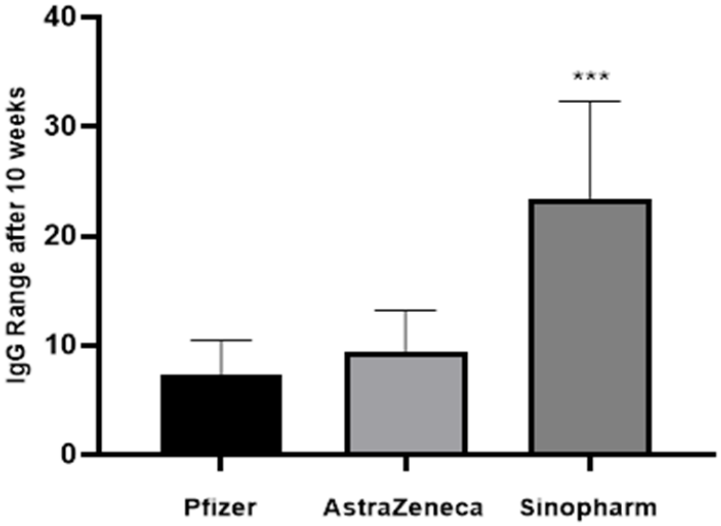

Figure 4. IgG production evaluation among different vaccines.

Table 2. Correlation matrix between IgM and IgG and between 3 different Vaccines.

Variable	IgM	IgG	IgMSi	IgG Si	IgMPf	IgGPf	IgMAs	IgGAs	IgMCo	IgGCo
IgM	1.00									
IgG	0.37*	1.00								
IgMSi	0.45**	0.27	1.00							
IgGSi	0.43***	0.57***	0.24	1.00						
IgMPf	0.54***	0.52***	0.25	0.47***	1.00					
IgGPf	0.38*	0.24	0.00	0.25	0.37*	1.00	1.00			
IgMAs	0.47**	0.18	-0.29	0.10	0.06	0.10	0.38*	1.00		
IgGAs	0.15	0.12	0.06	0.07	0.17	0.07	0.06	0.28	1.00	
IgMCo	-0.18	-0.26	0.06	-0.32	0.31	0.16	0.06	0.27	0.01	1.00
IgGCo	0.21	0.08	-0.07	0.23	0.02	0.08	0.07	0.13	0.04	0.02

Si = Sinopharm, Pf = Pfizer, As = AstraZeneca.

Table 3. Multivariate Regression of the Impact between 3 Different Vaccines against.

Variable	COEF.	STD. ERR.	T-Val.	P-Val.
IgM Model				
Sinopharm	0.658540	0.238703	5.27***	0.00
Pfizer	0.008510	0.016137	4.54**	0.03
AstraZeneca	0.100591	0.012856	5.49***	0.00
Control	-0.091710	0.151113	-1.59	0.152
_CONS	3.60303	6.344329	0.57	0.574
R2	0.3710			
F-Stats	276.899			
IgG Model				
Sinopharm	0.78152	0.363306	4.63***	0.00
Pfizer	0.139636	0.236342	4.81***	0.00
AstraZeneca	0.431848	0.269129	3.86***	0.00
Control	-0.52932	3.554608	-0.15	0.882
_CONS	519.2426	216.2988	2.4	0.019
R2	0.6010			
F-Stats	276.899			

R-sq = R square, F-Stats = F statistics, P-Val. = P values, Coef. = coefficient, Std. Err = standard errors, T-Val. = T values, all P-Val. ≤ 0.00 are significant at 1% and T values with ** and *** indicate a 5% and 10% significance level.

4. Discussion

The findings of this study imply that for every complete dose of IgG taken, approximately 78% of IgM was produced. Furthermore, it can be inferred from Table 3 that Pfizer has a positive significant impact on the production of IgG, as evidenced by the beta coefficient of 0.139636 and the probability value of 0.00. This suggests that for every dose of Pfizer taken, approximately 14% IgG is produced. Furthermore, the study indicates that AstraZeneca has a positive significant impact on IgG, as evidenced by the beta coefficient of 0.4318481 and the probability value of 0.00, suggesting that for every complete dose of AstraZeneca, approximately 43% IgG is produced. The findings of this study demonstrate that Sinopharm is more effective in producing IgG than Pfizer and AstraZeneca. Consequently, the control group exhibited no substantial development. Secretory IgA antibodies are vital in neutralizing toxins, viruses and other inflammatory agents invading the epithelial mucosa. Studies have noted that SARS-CoV-2 messenger ribonucleic acid (mRNA) vaccines elicit higher titers of anti-spike subunit 1 (S1) IgG and IgA in serum. The global emergence of the novel coronavirus SARS-CoV-2 towards the end of 2019 has precipitated a public health crisis, with over 30 million cases of infection and 1 million deaths reported worldwide (13). In response, numerous countries and regions have initiated accelerated development of SARS-CoV-2 vaccines. This endeavor is distinguished by its unprecedented pace and success (14). The majority of techniques were developed with the primary objective of protecting against the global surge in viral infections. However, as new strains of the virus emerge as it proliferates worldwide, further research into vaccine development and immune system stimulation may be necessary. The study focused on two highly efficacious vaccines, Pfizer and Sinopharm, and was conducted in the Kurdistan region of Iraq. The study examined 321 male participants. Pfizer, AstraZeneca, and Sinopharm had 77, 90, and 321 participants, respectively. The study found no significant difference in the production of immunoglobulin M (IgM) with respect to the three vaccines administered and the control group (P value = 0.958). However, a significant difference was found in the production of immunoglobulin G (IgG) among the different administered vaccines, with Sinopharm having the highest production level. The present study discovered Sinopharm to be the most active in terms of IgM and IgG production among the Kurdish people in Iraq's Kurdistan area. The present study lends support to the findings of (15), who concluded that BBIBP-CorV inactivated viral vaccination can elicit moderate anti-SARS-CoV-2 antibody after two doses. The production of a stronger immune system by the vaccine may be attributable to its live attenuated nature, in contrast to the RNA-based vaccines, which produce a weaker protective immune response against SARS-CoV-2. The immunological responses elicited by the various vaccines examined in the present study have given rise to numerous inquiries regarding the mechanisms through which these responses occur. The study calls for further research to ascertain the most efficacious vaccines among the Kurdish populace in the Kurdistan Region of Iraq. In this study, we examined the antibody responses to the generation of IgM and IgG after ten weeks of complete dosage in 321 healthy adult volunteers from either Pfizer, AstraZeneca, and Sinopharm. To the best of our knowledge, this is the first comparative study of adaptive immunity to these vaccines in Iraq's Kurdistan region. The study found that all three vaccines (Pfizer, AstraZeneca, and Sinopharm) were effective in producing IgM and IgG. The present study found Sinopharm to be more effective in producing IgM and IgG. It was also found that Sinopharm was more effective in producing IgG than Pfizer and AstraZeneca. The immunological reactions to the vaccines investigated in the following studies have raised many questions regarding how they occur. The report recommends that further research be conducted on the most effective vaccines among the Kurdish people in Iraq's Kurdistan Region.

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Authors' Contribution

MYM contributed in concept, design, acquisition of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content. Administrative, technical, and material support.

Ethics

The study protocol was approved by the Research Ethics Board of the Hawler Medical University (060721-477 HMU-EC).

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

Data included in the article/supplementary material/referenced in the article.

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