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

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

ABSTRACT

The current pandemic of coronavirus disease (COVID-19) has brought significant challenges to healthcare systems and economies globally. COVID-19 vaccines aim to generate immune responses against the SARS-CoV-2 spike protein, ideally neutralizing antibodies. Efficacy has now been documented for several vaccinations, including mRNA, adenoviral-vectored protein subunit, and whole-cell inactivated subunit vaccines. Understanding the immune responses to COVID-19 vaccines and how different antibodies are created after vaccination is critical to better understanding the pathophysiology of COVID-19. In the current study, humoral immune responses that elicited by BNT162b2 (mRNA-based), BBIBP-CorV (inactivated virus), and ChAdOx1 (dsDNA-recombinant based) vaccines against SARS-CoV-2 virus were compared. A total of three hundred and twenty-one individuals were included in this study, in which 90 individuals have taken no vaccines (control group) and post vaccinated with Pfizer, and Sinopharm each 77 participants, respectively. Blood samples were collected after 10 weeks vaccination and serum was analysed. The human SARS-CoV-2 Spike (Trimer) IgG or IgM ELISA (Thermo Fisher) were used to measure the amount of IgG or IgM antibodies bound to SARS-CoV-2 Spike (Trimer). it was found that statistically there is no significant difference between the vaccines (P value =0.958). The study found all three vaccines (Pfizer, AstraZeneca and Sinopharm) were effective in the production of IgM and IgG. This study showed that Sinopharm is more effective in the production of IgM and IgG. the usage of ChAdOx1 has led to the production of more IgG compared to BNT162b2 as statistically it was found to be significantly different (P value= 0.0001). Sinopharm's generation of a stronger immune system may result from the fact that it's 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. The study recommends more studies regarding the most effective vaccines among Kurdish people in Kurdistan Region of Iraq.

1. Introduction

The current pandemic of coronavirus disease (COVID-19) has brought significant challenges to healthcare systems and economies globally. The ongoing pandemic is caused by a new strain from the coronaviridae family, known as severe acute respiratory syndrome, coronavirus-2 (SARS-CoV-2) (1). Excessive proinflammatory cytokine production leads to tissue lung injury and eventually leading to 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). Although treatment options for COVID-19 continue to expand, the global emergence of new SARS-CoV-2 variants brings more challenges to existing treatment approaches (6). Antiviral drugs such as remdesivir, lopinavir/ritonavir, as well as convalescent plasma therapy and monoclonal antibodies such as casirivimab and imdevimab, have shown promising protection against COVID-19 (7). Immunization through vaccination is a crucial step to reduce and eliminate SARS-CoV-2 infections. (8). The human immune system responds to SARS-CoV-2 infection by producing neutralizing antibodies IgG, IgM, and IgA, and memory B and T cells (9). Vaccination aims at triggering the immune system to produce SARS-CoV-2-specific functional immune memory (10). Disease severity appears to significantly affect antibody levels. Severe COVID-19 cases showed higher antibody levels than milder cases (11).

Hence, immune responses through vaccination vary by age group, sex, type of vaccine, number of doses, as well as history of prior infection with SARS-CoV-2.

The Beijing Bio-Institute of Biological Products (BBIBP) inactivated coronavirus vaccine, Sinopharm COVID-19, is the first Chinese COVID-19 vaccine to get WHO emergency approval. A glimpse of hope has been supplied by Pfizer's statement that they have found a vaccine against the deadly COVID-19 virus. The interim results presented by Pfizer on November 9, 2020, showed that the vaccine appeared to be 90% effective, which was the source of the most enthusiasm (9) The World Health Organization said on April 19, 2021, that the AstraZeneca vaccination had no fatal or seriously debilitating side effects (11).

2. Objectives

The current study, which is the first of its kind in the region, aims to compare the antibody response revealed by Sinopharm, Pfizer, and AstraZeneca vaccines against COVID-19 disease among Kurdish people in the Kurdistan Region of Iraq.

3. Materials and methods

3.1. Study design

The study protocol was approved by the Research Ethics Board of the Hawler Medical University (060721-477 HMU-EC). All subjects signed a written informed consent for the detection of immunoglobulins and allowed the use of the samples for other procedures. Our 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. A total of three hundred and twenty-one individuals were included in this study in order to evaluate the antibodies IgG and IgM response to the vaccines, in which 90 individuals have taken no vaccines (control group). Moreover, 77 participants have received 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 Pfizer-BioNTech- and AstraZeneca vaccinated group, 34% had a history of COVID-19 with 76% were without history of Covid In Sinopharm, 11 % had a history with COVID-19 and 89 % were without history of Covid.

3.2. Sampling and data collection

The individuals were grouped into pre (control) and post (vaccinated) vaccinations. Blood samples were collected to obtain serum from individuals before vaccination as a control group. Following this, individuals' samples were collected after 10 weeks of complete vaccination (Figure 1), the sampling was done under sterile conditions. The samples were centrifuged at 800g, 4˚C for 10 min and the supernatant was stored at -80˚C for further 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. Manufacturer's protocol was followed.

3.3. Immunoglobulin Assay

The Human SARS-CoV-2 Spike (Trimer) IgG or IgM ELISA (Thermo Fisher) were used to measure the amount of IgG or IgM antibodies bound to SARS-CoV-2 Spike (Trimer). A trimerized spike protein was precoated in the wells of the supplied microplate. Serum samples and controls were added to wells. The wells were washed and IgG antibodies conjugated to HRP were added. The wells were washed and a substrate solution were added, then the absorbance at 450/620 nm was read using an ELISA Microtiter plate reader.

3.4. Statistical analysis

For the purpose of data analysis, both GraphPad Prism SAS software were used, as applying both two-tailed t test as well as multivariate regression were run. P value < 0.05 was statistically significant as ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$). Unpaired t test and descriptive statistical analysis were calculated using non-transformed data, and only regression analysis was carried out with transformed data.

3.5. Inclusion criteria and Exclusion Criteria: Eligible for COVID-19 vaccination as described by the instructions of the manufacturer, Age of 18 years or older, Capable of understanding purpose and risks of the study, given written informed consent. About the exclusion criteria, it believed the history of severe adverse reaction associated with a vaccine and/or severe allergic reaction (eg,anaphylaxis) to any component of the study intervention(s).

Figure 1. Diagrammatic Presentation of the Research: A= Kurdistan Region, Iraq, B = Participants Signed a Written Consent Form (321), C = 77 Participants each were Administered either Pfizer, Sinopharm or AstraZeneca and 90 as (control group), D = Blood Samples were Collected 10 Weeks After Complete Dosage Vaccination, E = IgG and IgM were detected using ELISA (Thermo Fisher) and Absorbance were detected.

4. Results

Several countries have launched vaccination initiatives to reduce the death toll from Covid19 since vaccinations were developed. Concerns over the safety and efficacy of vaccines have remained despite a worldwide push to vaccinate as many people as quickly as possible. The RBD is a viral

component that aids in pathogen entry by attaching to a receptor during viral replication in host cells. Serological testing can use IgG on RBD because this is where neutralizing antibodies can prevent the entry of SARS-CoV-2 into host cells.

Blood samples we obtained from 321 participants, 144 females and 177 males from different ethnic backgrounds and different ages ranged 17 to 91 years old with a mean of 42.58 years old, Table 1.

Table 1. Participant's characteristics

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

Note: Figure 2 diagrammatic presentation of how vaccines were administered to through injection, whereby antigen recognition by SARS-CoV-2 triggers 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 centers 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. MBCs and plasma cells that secrete antibodies can penetrate the bloodstream and (possibly) the mucosa, where they aid in the fight against viral infection and protect against reinfection (12).

4.1. Antibody production response to vaccinations:

Serum concentrations of both IgM and IgG in different groups who received different vaccines were evaluated. The mean of IgM concentration after 10 weeks in control group (not vaccinated), and vaccinated with BNT162b2, ChAdOx1, and BBIBP-CorV were 0.018, 0.269, 0.236, and 0.242 mg/dL respectively. The IgG concentration mean after 10 weeks in control (not vaccinated), and vaccinated with BNT162b2, ChAdOx1, and BBIBP-CorV were 0.23, 7.39, 9.48, and 23.48 mg/dL, respectively.

In addition, the comparison analysis for the production of both IgM and IgG was conducted between the participants who have got different vaccines. As for IgM after 10 weeks, it was found

that statistically there is no significant difference between the vaccines (P value =0.958) (Figure 3)

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

On the other hand, it was found that all participants have produced sufficient amount of IgG against SARS-CoV-2 as the impact of different (BNT162b2, ChAdOx1, and BBIBP-CorV) vaccine types. Consequently, the level of IgG antibodies was found to be different amongst the various groups as a statistically significant difference was found ($P < 0.001$) (Figure 4). In addition, the IgG level was significantly higher in BBIBP-CorV vaccinated participants compared to BNT162b2, ChAdOx1 by which $P < 0.001$, as indicated in Figure 4.

Figure 4. IgG production evaluation among different vaccines.

Moreover, the usage of ChAdOx1 has led to the production of more IgG compared to BNT162b2 as statistically it was found to be significantly different ($P < 0.001$) (Figure 4).

4.2. Correlation matrix between IgM and IgG and between 3 different Vaccines

Table 2 Depicts the correlation results between the dependent variables IgM and IgG and the independent variables Pfizer, AstraZeneca, and Sinopharm from the Table 3, it revealed that IgM and IgG have positive corrections with Pfizer, AstraZeneca and Sinopharm. This provides a clue about the degree of relationship between the dependent variables IgM and IgG and the independent variables in the regression model (Table 3).

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

Note: Si = Sinopharm, Pf = Pfizer, As = AstraZeneca

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

The results from the multivariate regression (IgM model) indicate that Sinopharm has a positive significant impact on the production of IgM against SARS-CoV-2. This can be deduced from the beta coefficient of 0.658540 and the probability value of 0.00. The result implies that for the dose of Sinopharm taken, approximately 66% of SARS-CoV-2 IgM was produced. It is also observed from Table 3 that Pfizer has a positive significant impact on the production of IgM against SARS-CoV-2. This can be inferred from the beta coefficient of 0.008510 and the probability value of 0.03. The result implies that the complete dose of Pfizer was taken, approximately 0.9% of SARS-CoV-2 IgM was produced. Moreover, the multivariate regression reveals that AstraZeneca has also a positive significant impact on the production of IgM against SARS-CoV-2. This can be observed from the beta coefficient of 0.100591and the probability value of 0.00. The result suggests that for every one dose of AstraZeneca, approximately 10% of SARS-CoV-2 IgM was produced even after 10 weeks. This means that the control groups have no significant development on the production of IgM in their body.

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

Note: 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.

Similarly, results from the multivariate regression (IgG model) also indicate that Sinopharm has a positive significant impact on IgG against SARS-CoV-2. As it can be deduced from the beta coefficient of 0.78152 and the probability value of 0.00 (Table 3).

5. Discussion

In this study, the result implies that for every complete dose of IgG taken, approximately 78% of IgM was produced. It is also perceived from Table 3 that Pfizer has also positive significant impact on the production of IgG. This can be inferred from the beta coefficient of 0.139636 and the probability value of 0.00. The result implies that for every dose of Pfizer taken, approximately

14% IgG was produced. Moreover, it is revealed that AstraZeneca has a positive significant impact on IgG. This can be observed from the beta coefficient of 0.4318481 and the probability value of 0.00. The result suggests that for every complete dose of AstraZeneca, approximately 43% IgG was produced. Overall, the findings during this study show that Sinopharm is more effective in the production of IgG than Pfizer and AstraZeneca. This means the control group has no significant development. Secretory IgA antibodies are vital to neutralize toxins, viruses, and other inflammatory agents invading the epithelial mucosa. Studies noted that SARS-CoV-2 messenger ribonucleic acid (mRNA) vaccines elicit higher titers of anti-spike subunit 1 (S1) IgG and IgA in serum

An outbreak of the novel coronavirus SARS-CoV-2, which surfaced around the end of 2019, has led to a public health disaster that has resulted in more than 30 million cases of infection and 1 million deaths worldwide (13). Many countries and regions around the world have seen an increase in the development of SARS-CoV-2 vaccines. SARS-CoV-2 vaccine development and deployment is unique in terms of speed and success (14). Most techniques were developed only to protect against the viral increase all over the world. There have been new strains of the virus discovered as it spreads around the globe, and these may necessitate further research into vaccine development and immune system stimulation. Study of the highly efficacious vaccines; Pfizer, and Sinopharm were carried out in the Kurdistan region of Iraq. Three hundred and twenty-one (321) participants were examined in the following study on dominance of men. Pfizer, AstraZeneca, Sinopharm 77 participants, respectively, and 90 participants as control. The production of immunoglobulin M (IgM) with respect to the three vaccines administered and the control group were found to have no significance difference at (P value = 0.958). There was a significance difference 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 Kurdish people in Iraq's Kurdistan area. The study is in support of (15) where they concluded 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 might be as a result it’s a live attenuated vaccine, while the RNA-based vaccines, on the other hand, produce a weaker protective immune response against SARS-CoV-2. The immunological responses of the various vaccines examine in the following study have raised many questions regarding how these responses occur. The study

recommends more studies regarding the most effective vaccines among Kurdish people in 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. All three vaccines (Pfizer, AstraZeneca, and Sinopharm) were found to be effective in producing IgM and IgG, according to the study.

4. Conclusion

Overall, Sinopharm is more effective in producing IgM and IgG, according to the findings and it has been indicated that the findings during this study show that Sinopharm is more effective in the production of IgG than Pfizer and AstraZeneca. The immunological reactions to the vaccines investigated in the following studies have raised many questions regarding how they occur. More research on the most effective vaccines among Kurdish people in Iraq's Kurdistan Region is recommended, according to the report.

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Authors' contributions

All authors contributed equally

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.

References

1. Shen L, Wang C, Zhao J, Tang X, Shen Y, Lu M, et al. Delayed specific IgM antibody responses observed among COVID-19 patients with severe progression. Emerg Microbes Infect. 2020;9(1):1096–101. https://doi.org/10.1080/22221751.2020.1766382
2. Merza MY, Hwaiz RA, Hamad BK, Mohammad KA, Hama HA, Karim AY. Analysis of cytokines in SARS-CoV-2 or COVID-19 patients in Erbil city, Kurdistan Region of Iraq. PLoS One. 2021;16(4 April):e0250330. https://doi.org/10.1371/journal.pone.0250330
3. Hwaiz R, Merza M, Hamad B, HamaSalih S, Mohammed M, Hama H. Evaluation of hepatic enzymes activities in COVID-19 patients. Int Immunopharmacol. 2021;97:107701. https://doi.org/%2010.1016/j.intimp.2021.107701
4. Hu B, Guo H, Zhou P, Shi Z-L. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021;19(3):141–54. https://doi.org/10.1038/s41579-020-00459-7
5. Najafimehr H, Mohamed Ali K, Safari S, Yousefifard M, Hosseini M. Estimation of basic reproduction number for COVID-19 and the reasons for its differences. Int J Clin Pract. 2020;74(8). https://doi.org/10.1111/ijcp.13518
6. Gong W, Aspatwar A, Wang S, Parkkila S, Wu X. COVID-19 pandemic: SARS-CoV-2 specific vaccines and challenges, protection via BCG trained immunity, and clinical trials. Expert Rev Vaccines. 2021;20(7):857–80. https://doi.org/10.1080/14760584.2021.1938550
7. Hurt AC, Wheatley AK. Neutralizing antibody therapeutics for covid-19. Viruses. 2021;13(4):628. https://doi.org/10.3390/v13040628
8. Awadasseid A, Wu Y, Tanaka Y, Zhang W. Current advances in the development of sars-cov-2 vaccines. Int J Biol Sci. 2021;17(1):8–19. https://doi.org/10.7150/ijbs.52569
9. Pang NYL, Pang ASR, Chow VT, Wang DY. Understanding neutralising antibodies against SARS-CoV-2 and their implications in clinical practice. Mil Med Res. 2021;8(1):47. https://doi.org/10.1186/s40779-021-00342-3
10. Shah VK, Firmal P, Alam A, Ganguly D, Chattopadhyay S. Overview of Immune Response During SARS-CoV-2 Infection: Lessons From the Past. Front Immunol. 2020;11:553450. https://doi.org/10.3389/fimmu.2020.01949
11. Hellerstein M. What are the roles of antibodies versus a durable, high quality T-cell response in protective immunity against SARS-CoV-2? Vaccine X. 2020;6:100076.

https://doi.org/10.1016/j.jvacx.2020.100076

12. Röltgen K, Boyd SD. Antibody and B Cell Responses to SARS-CoV-2 Infection and Vaccination: The End of the Beginning. Annu Rev Pathol Mech Dis. 2024;19(7):69–97. https://doi.org/10.1146/annurev-pathmechdis-031521-042754
13. Zhang Y, Li D, Zhao H, Wang L, Liao Y, Li X, et al. The role of multiple SARS-CoV-2 viral antigens in a vaccine-induced integrated immune response. Vaccine. 2021;39(18):2500–3. https://doi.org/10.1016/j.vaccine.2021.03.067
14. Matchett WE, Joag V, Stolley JM, Shepherd FK, Quarnstrom CF, Mickelson CK, et al. Cutting Edge: Nucleocapsid Vaccine Elicits Spike-Independent SARS-CoV-2 Protective Immunity. J Immunol. 2021;207(2):376–9. https://doi.org/10.4049/jimmunol.2100421
15. Vályi-Nagy I, Matula Z, Gönczi M, Tasnády S, Bekő G, Réti M, et al. Comparison of antibody and T cell responses elicited by BBIBP-CorV (Sinopharm) and BNT162b2 (Pfizer-BioNTech) vaccines against SARS-CoV-2 in healthy adult humans. Geroscience. 2021;43(5):2321–31. https://doi.org/10.1007/s11357-021-00471-6

Legends

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

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

Figure 4. IgG production evaluation among different vaccines.

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Table 1

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		

Table 2

VARIABLE	IgM	IgG	IgM Si	IgG Si	IgM Pf	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

Table 3

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			