Evaluation of the Efficacy of COVID-19 Vaccines (Pfizer, Astra Zeneca, Sinopharm) Using Iraqi Local Samples

Drgam Flah Ismael Al-Khazrajy1*, Qussay Nouri Raddam2

Abstract

Subject: The current study was aimed to evaluate the efficacy of the three approved vaccines in Iraq (Pfizer, AstraZeneca, and Sinopharm). By detecting the IgG and IgM antibody to the first subunit of the structural spike protein (S1), which is responsible for binding the virus to the angiotensin-converting enzyme receptor (ACE-2) on the surfaces of most cells of the body. Object: Study include 44 samples which were divided into four groups of samples. Each group had (11) samples. The first group of people consisted of those who had received both the first and second dose of Pfizer vaccine after 30 or more days the second group of people who had received the first and second dose of AstraZeneca vaccine after 30 or more days, the third group of people who had received the first and second dose of Sinopharm vaccine after 30 days or more, and the control group Which included people recovering from COVID-19 disease, all samples were collected from of Baghdad city-Iraq. Result: The results indicated the superiority of the Pfizer vaccine over the AstraZeneca and Sinopharm vaccines when compared with the control group, The average Pfizer vaccine concentration was (106.8 ± 16.2), while the AstraZeneca vaccine showed a weak superiority with an average concentration of (79.65 ± 14.33) compared to the control group, for which the average concentration of IgG-S1 was (64.55 ± 14.6). While the concentration of the Sinopharm vaccine was (70.94 ± 8.77), it did not show a significant difference compared to the control group. Conclusion: Our result showed that all vaccines (Pfizer, AstraZeneca, and Sinopharm) used in this study were able to stimulate the production of antibodies against (SARA0COV2), and the Pfizer vaccine was the most effective and immunization.

Key Words: COVID-19 Vaccines, AstraZeneca, Pfizer and Sinopharm, IgM-S1, IgG-S1 Protein.

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Introduction

The new coronavirus, SARS-CoV-2, the deadly virus that causes the Covid-19 epidemic [1] as it was called by the World Health Organization, which was subsequently declared by the World Health Organization as a global pandemic [2] for what it caused of injuries and deaths in large numbers, causing more than 3.7 million deaths [3] and as of the date of this writing, the SARS-CoV-2 virus is still claiming the lives of many people around the world. In addition to the loss of lives caused by the Covid-19 virus, it also destroyed all aspects of life, starting with the economy, livelihoods, and the social situation that became paralyzed, passing through health institutions that suffered from the huge numbers of cases that exceeded their absorptive capacities [4] [5]. The appeared of Coronavirus in China in Wuhan, specifically [6]. This virus has its origins and roots in the history of epidemics and diseases.

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It is from the Coronaviridae family of the order Nidovirales, which has caused similar epidemic cases through the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and the Corona Virus Middle East Respiratory Syndrome (MERS-CoV), which previously caused by taking many human lives [7] [8]. However, this virus was characterized as being more dangerous and severe in terms of symptoms [9] [10]. After revealing the complete genetic sequence of the genetic material of the SARS-CoV-2 virus by Chinese scientists [11], vaccine manufacturers took the initiative to stand up to this deadly virus and took bold and unprecedented steps in producing many types of vaccines in a short period [12]. It was helped by modern technologies and the technological development that took place in the field of vaccine manufacturing. More than 200 vaccines were candidates in the research phase. However, only 60 vaccines have succeeded due to the expensive and laborious vaccine development programs, starting with manufacturing, pre-clinical trials, and then intensive clinical trials on humans in three phases [13] [14]. After that, it was announced that some types of vaccines would be allowed to be used as an emergency [15] [16] and among these vaccines was Pfizer / BioNTech (BNT162b2) based on a previously unused modern technology based on RNA. The messenger (mRNA), was manufactured by the American company Pfizer in cooperation with the German company BioNTech [17] [18]. And the AZD1222 (ChAdOx1) vaccine, manufactured by the British company AstraZeneca, is one of a group of vaccines that depend on viral vectors [19]. Such as Ebola and malaria [20] [21] and the BBIBP-CoV vaccine manufactured by the Chinese company Sinopharm, which is one of the vaccines based on the traditional method, are dead viruses [22] [23]. All vaccines manufactured against Covid-19 targeted the structural spike protein, which studies have proven to be directly related to virus penetration into the host cell, especially the first subunit (S1) responsible for binding to the ACE-2 receptor found on the surfaces of most cells of the body [24] [25] [26]. Thus, inducing the production of antibodies to this protein and preventing it from binding to the receptor of the angiotensin-converting enzyme 2 (ACE-2) and thus preventing the process of virus penetration into the cell [27].

The main common aim of all vaccines manufactured against COVID-19 although they are designed in different ways is to induce the immune system to produce antibodies against SARS-CoV-2. Although the immune response after vaccination and antibody levels differ between individuals [27]. Various tests have been developed that can detect IgM and IgG immune antibodies from blood samples of people infected with the SARS-CoV-2 virus, or people who have recovered from an infection, as well as people who have received a vaccine against the emerging coronavirus. These serological tests are performed using various viral antigens and recombinant proteins to capture specific SARS-CoV-2 antibodies. The spike (S) protein expressed by SARS-CoV-2 is known to be the optimal target for antibodies [24] [25]. The spike protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell surface that mediates virus entry into the cell via the receptor-binding domain (RBD) in the protein structure (S). Since the spike protein plays an important role in the entry of the virus into the cell, it is an important target in impeding the activity of the virus and the immune response after the vaccine [26]. It is considered a highly immunogenic protein and is used as a core protein in the SARS-CoV-2 virus test [28].

Materials and Methods
Blood samples were collected from the target persons In the period between September 29, 2021, and until November 2 of the same year, the number of samples was (44), including (11) of the females 25% and (33). of the males 75%, divided into (4) groups, each consisting of (11) samples. The first group included people who had received both first and second dose of Pfizer (Pfiz.), Vaccine and the second group included people who had received both doses of AstraZeneca (Astra.) Vaccine. And the third group included people who had received both doses of Sinopharm (Sino.) Vaccine. And the fourth and last group included people who had recovered from infection with COVID-19 disease (disappearance of symptoms of the disease) and did not receive any type of COVID-19 vaccines, which was considered a control group in the study. Each group also included (5) people (45%) who suffer from chronic diseases, most notably diabetes, vascular diseases, and chronic high blood pressure. And (6) 55% healthy people who do not suffer from comorbidities. Where the serum was isolated and the necessary Tests were made.
IgM antibody to S1 protein was detected using an assay kit produced by CUSABIO of the US Company that follows the working principle of Indirect
Enzyme-Linked ImmunoSorbent Assay (ELISA). After following the work steps mentioned in the instructions attached to the test kit, the test was carried out using a device provided by the German company HumaReader HS at the wavelength OD: 450. After obtaining the results, the cut-off value was extracted by applying the equation mentioned in the attached instructions, which states: (Cut-Off Value (CO) = 0.2 x Positive control (PC): OD450), then the positive and negative values of the test were inferred That is, the presence or absence of IgM antibodies specific to the S1 protein (Covid-19) by calculating the value of the sample obtained from the device to the ratio of the cut-off value C.O. as follows:

- If S/C.O. ≥ 1, the sample is classified as positive for Test.
- If S/C.O. < 1 then the sample is classified as negative for Test.

IgG antibody to S1 protein was also detected using an assay kit produced by the British company MYBioSource that follows the principle of action of Direct Enzyme-Linked ImmunoSorbent Assay (ELISA). By following the work steps mentioned in the instructions attached to the Test kit, the test was examined with the same device mentioned above and at the same wavelength. Where the concentration of IgG-S1 was obtained for each sample from each group.

The data were statistically processed using the statistical program Graph pad prism version 9 (Graph pad Software Inc., Lajolla, CA), where significant differences were revealed between groups of different vaccines compared to the control group through Dunnett’s multiple comparisons test, and significant differences were also revealed. Among the same different vaccine groups (Pfiz., Astra, and Sino.), through Tukey’s multiple comparisons test.

**Results**

**IgM-S1 Protein**

Data in the table (4-1) revealed the results of examining samples whose values that were greater than or equal to 1 were positive for the IgM Covid-19 antibody, and samples whose values that were less than 1 were negative for the IgM COVID-19 antibody, after extracting the cut-off value (CO) and calculating the (S/CO) value for each sample. Thus, all samples for the four groups (Control group, Pfizer vaccine group, AstraZeneca vaccine group, and Sinopharm vaccine group) were negative for the IgM COVID-19 test.

**Table 4-1.** Shows the results of the IgM-S1 protein assay for the different vaccine groups (Pfiz., Astra, Sino.) and the control group (Cont.)

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.255</td>
<td>0.405</td>
<td>0.401</td>
<td>0.535</td>
</tr>
<tr>
<td>2</td>
<td>0.267</td>
<td>0.496</td>
<td>0.731</td>
<td>0.387</td>
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<td>0.391</td>
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<td>4</td>
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<td>0.673</td>
<td>0.631</td>
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<td>0.382</td>
<td>0.434</td>
<td>0.496</td>
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<td>0.27</td>
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<td>0.277</td>
<td>0.305</td>
</tr>
<tr>
<td>8</td>
<td>0.269</td>
<td>0.444</td>
<td>0.684</td>
<td>0.205</td>
</tr>
<tr>
<td>9</td>
<td>0.356</td>
<td>0.365</td>
<td>0.359</td>
<td>0.348</td>
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<tr>
<td>11</td>
<td>0.495</td>
<td>0.469</td>
<td>0.685</td>
<td>0.334</td>
</tr>
</tbody>
</table>

Mean 0.30009 0.350454 0.48545 0.363818

**IgG-S1 Protein**

Results in Tables (4-2- A, B) and Figure (4-1) show a significant difference at the probability level (P≤0.05) between the Pfizer vaccine group (P<0.0001) and the AstraZeneca vaccine group (P<0.0383). Compared to the control group (people who recovered from COVID-19 and were not vaccinated), the results also showed that there was no significant difference between the Sinopharm group (P<0.5576) compared to the control group. With a clear significant difference between the Pfizer vaccine group and the AstraZeneca vaccine group (P<0.0002) and between Pfizer and Sinopharm (P<0.0001). The results also showed that there was no significant difference between the AstraZeneca vaccine group and the Sinopharm group (P<0.4441).

**Table 4(2-A).** The significant difference (Signf.) between the results of IgG-S1 protein assay for the control group and groups of different vaccines (Pfiz., Astra, Sino.) at the probability level

<table>
<thead>
<tr>
<th>A Mean IgG-S1protein U/ml (± SD)</th>
<th>Vaccine Vs. Cont.</th>
</tr>
</thead>
<tbody>
<tr>
<td>64.55 ± 14.6</td>
<td><strong>P-value</strong></td>
</tr>
<tr>
<td>Pfizer 106.8 ± 16.2</td>
<td>****</td>
</tr>
<tr>
<td>Astra. 79.65 ± 14.33</td>
<td>*</td>
</tr>
<tr>
<td>Sin. 70.94 ± 8.77</td>
<td>ns</td>
</tr>
</tbody>
</table>

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Table (4-2-B). Shows the significant difference between the results of IgG-S1 protein assay among the different vaccine groups at a level of probability (< 0.05).

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Mean ± SD</th>
<th>signf.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfiz. Vs.</td>
<td>106.8 ± 16.2</td>
<td>***</td>
<td>0.0002</td>
</tr>
<tr>
<td>Astra.</td>
<td>79.65 ± 14.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pfiz. Vs.</td>
<td>106.8 ± 16.2</td>
<td>****</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sino.</td>
<td>70.94 ± 8.77</td>
<td></td>
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</tr>
<tr>
<td>Astra. Vs.</td>
<td>79.65 ± 14.33</td>
<td>ns</td>
<td>0.4441</td>
</tr>
<tr>
<td>Sino.</td>
<td>70.94 ± 8.77</td>
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</tbody>
</table>

Figure (4-1). The significant differences between groups of different vaccines (Pfiz., Astra., Sino.,) for the Test of IgG-S1 compared to the control group and the significant differences between groups of the same vaccines at the probability level (P < 0.05).

Discussion

IgM-S1 Protein

The results of the current study showed that all the people targeted in the study of the four groups (control group, Pfizer vaccine group, AstraZeneca vaccine group, and Sinopharm vaccine group), were free of infection or viral load of Covid-19 disease during serum samples collection. Through the use of this test, antibodies can be detected in infected people in the first weeks of infection and before symptoms appear (incubation period). As well as to detect cases of infection in people who do not suffer from the emergence of symptoms of the disease [29] [30] [31]. This is based on the detection of an IgM antibody that targets the subunit of the structural spike protein (S1) responsible for the binding process of the virus to the angiotensin-converting enzyme 2 (ACE2) receptor through which the virus penetrates to the host cell [11] [32] [33]. Protein S is the antigen that can provide accurate diagnostic results for COVID-19 disease [5]. This Test is one of the important approved methods for the detection of COVID-19 disease [34] [35] [36] [37] and the results of this study are consistent with several studies that indicate a low level of IgM for COVID-19 infection in recovering people, which may be undetectable sometimes [22] [38] [39] [40] [41].

IgG-S1 Protein

Thus, the above results showed the presence of IgG antibodies in the serum of all subjects targeted in the study and of the four groups (control group, Pfizer vaccine group, AstraZeneca vaccine group, and Sinopharm vaccine group) against the Spike structural protein of the Covid-19 virus, which is considered the most important protein in terms of immunogenicity against the virus, because it is the main cause of the virus entering the host cell after its binding with the angiotensin-converting enzyme 2 (ACE2) receptor [42] [43] [44]. Where the mean concentration of antibodies in the control group (people who recovered from COVID-19 and were not vaccinated) was 64.55 ± 14.05 U/ml, which was consistent with the results of the study of Zhou et al. (2021) [45], which indicated the stability of the rate of IgG antibodies in serum People recovering from infection for more than three months. In agreement with the results of the study of Zhang et al. (2021) [46], Hotez et al. (2020) [47], and Grifoni et al. (2020) [48], which showed that antibodies were retained in people who recovered from COVID-19 until six months after infection. It also agreed with Wu et al., (2020) [49], study, which indicated the presence of antibodies in the serum of people recovered from Covid-19 disease.

The results of the Pfizer vaccine group concerning the IgG-S1 assay showed a highly significant difference at the probability level (P≤0.05) compared to the control group (P<0.0001), with an average concentration of 106.8 ± 16.02 U/ml, and also showed a highly significant difference compared to the Sinopharm vaccine group (P<0.0001) and the less significant difference compared to the AstraZeneca vaccine group (P<0.0002). The results also showed a low significant difference between the AstraZeneca vaccine group compared to the control group (P<0.0338), with an average concentration of 79.65 ± 14.33 U/ml, while the results did not show a significant difference between the AstraZeneca vaccine group. Compared to the Sinopharm vaccine group (P<0.4441). The
results also showed that there was no significant difference between the Sinopharm vaccine group compared to the control group (P<0.5576), with an average concentration of 70.94 ± 8.778.

The confirmed results show that the Pfizer vaccine shows a higher level of immune stimulation compared to AstraZeneca and Sinopharm. The reason for the high level of immunization shown by this vaccine may be due to the mechanism by which the Pfizer vaccine was produced and is based on presenting the messenger RNA (mRNA) manufactured by selective transcription, to the cell to be translated by the cellular ribosomes in the cytoplasm directly into the full-length structural spike protein. In turn, the cell presents it to the immune system through the MHC I in a concentrated form, without causing any distraction of the immune system with other antigens [50] [51]. The Spike protein is the most important target of the immune system, which mediates the entry of the virus into the host cell after it binds to the angiotensin-converting enzyme 2 (ACE2) receptor through which the host cell is penetrated by the virus [24] [33]. In vitro mRNA synthesis, consists of the following elements: Cap5' conjugate with UTR5' followed by SARS-CoV-2 Spike sequence, UTR3 region and a long tail of poly- Adenine [50] [51]. All of these elements gave the messenger RNA stability and efficiency in the translation process and reduced the unwanted side effects due to the activation of the innate immune system [52]. Also, the lipid nanoparticles (LNP) lipid nanoparticles were used as a coating for the messenger RNA it contains. The vaccine is characterized by its ability to biodegrade quickly in the injected tissues, it does not induce inflammation and the immune system does not attack it before it integrates with the cell membrane and delivers the messenger RNA to the cell [53]. It also maintains appropriate levels of translation of the mRNA for the expression of the target protein [54].

As for the AstraZeneca vaccine, which shows less efficacy than the Pfizer vaccine, as the results showed, it is a vaccine based on the use of adenovirus vectors to deliver genetically modified DNA into cells that encodes the structural spike protein instead of the original nuclear material of the vector virus [55]. The lower efficacy of this vaccine compared to the Pfizer vaccine may be due to several reasons, one of which is that this vaccine uses adenovirus as a vector for the modified nuclear material that expresses the structural spike protein of SARS-CoV-2 [56]. Since the adenovirus is a widespread virus, the human immune system possesses memory cells and antibodies against this virus [57] [58]. Although the manufacturer has taken the necessary measures to overcome these obstacles by modifying the epitopes on the viral capsid to block the viral vectors from the immune system [59], the virus has also been genetically modified to prevent it from replicating when given to humans to prevent effects Undesirable side effects of the vaccine [60]. It may retain some of the immunological properties that enable the immune system to identify it, which leads to its attack and thus prevents the delivery of the transferred nuclear material to the nucleus by adenoviruses that fail to escape from the immune system and weaken the effectiveness of the vaccine [61] [62]. The difference may also be due to the immune stimulation between the Pfizer and AstraZeneca vaccines because the latter depends on genetically modified DNA, which expresses the structural Spike protein after it enters the nucleus of the host cell, and then a transcription process of the messenger RNA, and then a process Translation the Spike protein into the host cells, so this process takes a longer period and involves more complex steps than simply translating the messenger RNA (mRNA) into the cytoplasm for the Pfizer vaccine [55].

Sinopharm did not show any significant difference in stimulating the production of antibodies to spike protein when compared to the control group (recovered and unvaccinated persons), and the AstraZeneca vaccine group. While it showed a significant and clear decrease in the stimulation of IgG-S1 antibody production when compared to the Pfizer vaccine group. The Sinopharm vaccine is based on the traditional method of producing vaccines and is based on dead viruses [23] [24]. Therefore, its behaviour in immune stimulation similar to infection with the virus is not surprising. These results were in agreement with many previous studies that dealt with the issue of the efficiency of Covid-19 vaccines, where the results of the study of Vályi-Nagy et al. (2021) [63], indicated that there is a difference in the concentration of IgG in favour of the Pfizer vaccine in the production of antibodies compared to the Sinopharm vaccine. The results of the study by Voysey et al. (2021) [64], also demonstrated a difference in the effectiveness of the Pfizer immunization vaccine, which according to the study amounted to (95%) compared to the AstraZeneca vaccine, which amounted to 70%. The effectiveness of the three
vaccines was also studied on vaccinated people in Mongolia, where the Pfizer vaccine ranked first in terms of immunization and antibody stimulation, followed by the AstraZeneca vaccine, and then Sinopharm vaccine [65]. And another study conducted by Moghnieh et al. (2021) [66], whose results indicate the efficacy of Pfizer and AstraZeneca immunization vaccine compared to Sinopharm showed a decrease in immune stimulation. A comparative study between the effectiveness of different vaccines for Covid-19 disease showed a significant difference between the effectiveness of the Pfizer vaccine, which according to the study amounted to 95% compared to the effectiveness of the AstraZeneca vaccine, which amounted to 66-70% [55], which is in agreement with the results of the current study regarding immune stimulation. Vaccines from Pfizer and AstraZeneca. The current study results also agreed with the results of Kim et al. (2021) [67], which indicated the immunization efficacy of the three vaccines, Pfizer 95%, AstraZeneca 70%, Sinopharm 79%. Another study showed that the interim analysis of the third phase of clinical trials of the Sinopharm vaccine showed that the vaccine is 72.8% effective against symptomatic Covid-19 cases and 100% against severe diseases [68].

Conclusions
The four target groups in the study (the control group, the Pfizer vaccine group, the AstraZeneca vaccine group, and the Sinopharm vaccine group) showed high levels of IgG concentration for the first subunit of the structural spike protein (S1) of the SARS-CoV-2 virus. However, with a difference, the Pfizer vaccine topped the list of immunostimulants by a large margin, followed by the AstraZeneca vaccine, and then the Sinopharm vaccine with a small difference between them. While the latter did not show any significant difference from the people who recovered from the injury. Therefore, recommend taking the full doses of the above vaccines (especially the Pfizer vaccine) to prevent infection with Covid-19 or reduce acute infection symptoms.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Because the research samples were collected through the National Blood Donation Center. No patient privacy data such as the patient’s name or identification number and address are collected. So there was no need for informed consent in this study.

Conflicts of Interest: The authors declare no conflict of interest.

Contribution of researchers: Preparing and writing the research, collecting samples and conducting tests by Drgam Flah Ismael Al-Khazrajy, supervision and follow-up by Qussay Nouri Raddam.

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