

## Estimation the concentrations of Immunoglobulin (IgM and IgG), C3, C4, and Total protein in serum of Covid -19 Vaccinated person

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### Abstract

Immunoglobulin and complement proteins play a key role in immune system health and function, orchestrating a range of innate and acquired responses against pathogens such as viruses. It may work to enhance the process by which antibodies neutralize viruses, as well as to promote immune cell phagocytosis of viruses and result in virus lysis. Vaccination has created a great step toward improving global health. The innovation and application of vaccinations have contributed significantly to the control of infectious illnesses. A study was conducted randomized on vaccinated people in Iraq/Babylon governorate for both sexes. The number of vaccinated people was 695 people, including 561 people taking the Pfizer vaccine, 120 people taking Sinovac, and 14 people taking AstraZeneca. The results appeared to investigate the levels of IgG, IgM, C3, C4, and total protein concentrations in the serum of the vaccinated people and compare them with unvaccinated people. The study found elevated levels of C3 in the vaccinated people with AstraZeneca 328.900029.01293 than in control 282.02846.187, while C4 concentrations were lower in the vaccinated group (77.550021.40537, 74.966715.95755, 73500027.36110) than control 89.006014.18771. The total protein, IgG, and IgM concentrations were higher in the vaccinated people than in the control group.

**Keywords:** *Immunoglobulins, IgG, IgM, C3, C4, vaccine.*

### Introduction

A pneumonia pandemic caused by a novel coronavirus occurred in Wuhan, China, in Dec 2019. On February 11, Tedros Adhanom Ghebreyesus, the head of the WHO, declared that the sickness brought on by the novel coronavirus was known as "COVID-19" {1}. The coronavirus which is responsible for the

severe acute respiratory syndrome (SARS-CoV-2) belongs to the subgenus Sarbecovirus. On March 11, 2020, the World Health Organization (WHO) declared the SARS-CoV-2 outbreak a pandemic. {2}.

Given the disease's fast spread, understanding the work of the immune system and how it reacts to the vaccines of coronavirus

and SARS-CoV-2 is crucial. In response to SARS-CoV-2 infection, humans create specific antibodies, including CD4+ T cells that activate high-affinity antibodies that are produced by B cells and CD8+ T cells that kill infected cells. Antibodies, commonly known as immunoglobulin, are glycoproteins produced by plasma cells (white blood cells). By specifically detecting and adhering to particular antigens, such as viruses or bacteria, and assisting in their eradication, they serve a critical role in the immune response. The antibody response of the immune system is exceedingly complex. Different classes and subclasses (isotypes) of immunoglobulin have different biological characteristics, such as target specificities, distribution, and structures. As a result, understanding the immunoglobulin isotype can be helpful in understanding intricate humoral immune responses. for the selection and manufacture of antibodies as tools for immunoassays and other detection applications.

A variety of methods are used to create a vaccination that is both safe and highly effective, including attenuation, inactivation, viral vector, nucleic acid, subunit, and viral particle-based approaches. In the history of science, the development of a SARS-CoV2 vaccine has advanced significantly more quickly. Although there are many restrictions, ongoing work has been done to create a highly competent and successful vaccination for numerous diseases linked to humans and animals.

The COVID-19 vaccination could be helpful. prevents from getting COVID-19, limiting the spread of COVID-19 to other people. If more people in the community receive the COVID-19 vaccine, It hinders the COVID-19 virus's ability to replicate and propagate. After receiving the first or second

dose of the COVID-19 vaccine, some people may experience mild side effects such as discomfort, redness, or swelling where the vaccine was injected; fever, exhaustion; headache; swollen lymph nodes; chills; nausea, joint pain, vomiting; and pain of muscle. The aim of this study to investigate the effect of vaccine on concentrations of immunoglobulin and complement and total protein compared with unvaccinated.

### Methods:

Blood samples were collected from Babylon governorate people for both sexes, (males and females), for the period from {14-11 to 14-12} for the year 2021-2022. A blood sample of 5 ml was withdrawn from the humeral vein using a special one-time syringe. The drawn blood was placed in tubes containing a gel that helps to separate the serum. Then the blood was separated using a centrifuge at a power of 4500 for 5 minutes, then the serum was separated using a Pasteur pipette and the sera were kept in Eppendorf tubes and kept by freezing at a temperature of 5 ° C.

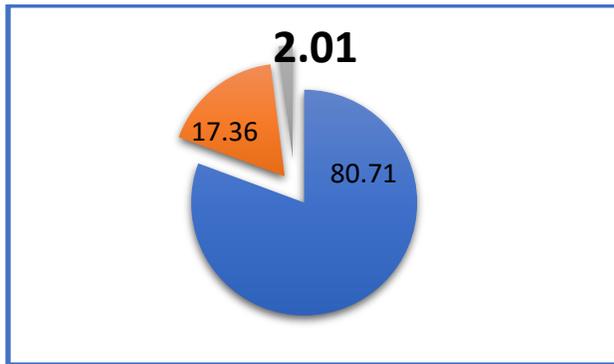
The concentration of immunoglobulins ( IgG, IgM) and complement levels (C3, C4) were estimated using the radial immune diffusion method (Easy RID/Italy) that contains a plate with 12 wells for the quantitative determination of human plasma proteins in serum and plasma, and an estimate of the total protein concentration was done to detect total protein depending on the kit procedure (Colorimetric total protein kit/France).

### Result and Discussion

A study was conducted randomized on vaccinated people in Babylon governorate for both sexes, the number of vaccinated people were 695 people, including 561 person taken

Pfizer vaccine, 120 people taken Sinovac, and 14 people taken AstraZeneca, The study report shows that the highest rate of vaccinated people was Pfizer in the rate of (80.71%) and followed by the Sinovac vaccine in the rate of (17.36%), While less percentage was AstraZeneca vaccine in the rate of (2.01%) as shown in figure (1).

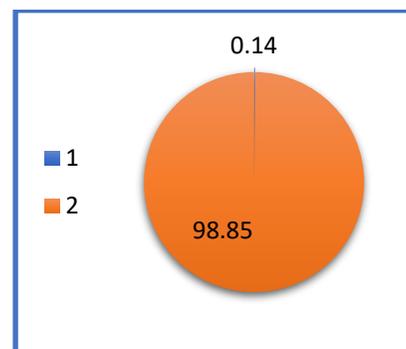
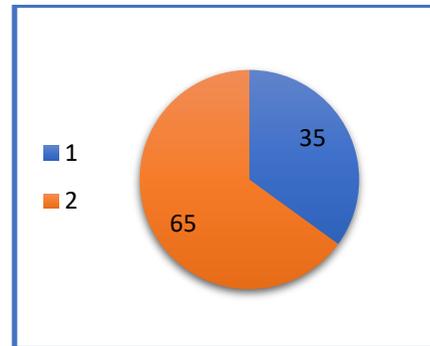
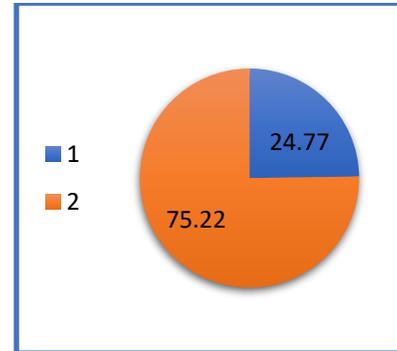
**Figure (1): Percentage of vaccination groups 1-Pfizer vaccine, 2-Sinovac, 3- AstraZeneca.**



The highest rate of vaccinated people was Pfizer, with a rate of 80.71%. This result agrees with (Halim et al., 2021) They said that, according to the research, the Pfizer vaccine has a 95% efficiency rate, demonstrating that it is an effective vaccination, compared to 78% for Sinovacc and 70.4% for AstraZeneca, respectively {7}.

A total of (695) people (males and females) were distributed into two groups according to Symptom and without symptoms associated with Pfizer vaccine, Sinovac, and AstraZeneca where the symptoms were fever and pain. The results showed that the highest rate of symptoms was in the AstraZeneca vaccines, where the percentage of symptoms of AstraZeneca(98.85%), followed by the percentage of symptoms of Pfizer vaccine(75.22%), and the percentage of symptoms of Sinovac (65%) as shown in figure (2).

**Figure (2): Percentage Symptom and without symptom associated with a-Pfizer vaccine, b-Sinovac c-AstraZeneca 1- without symptom 2- symptom such as fever and pain.**



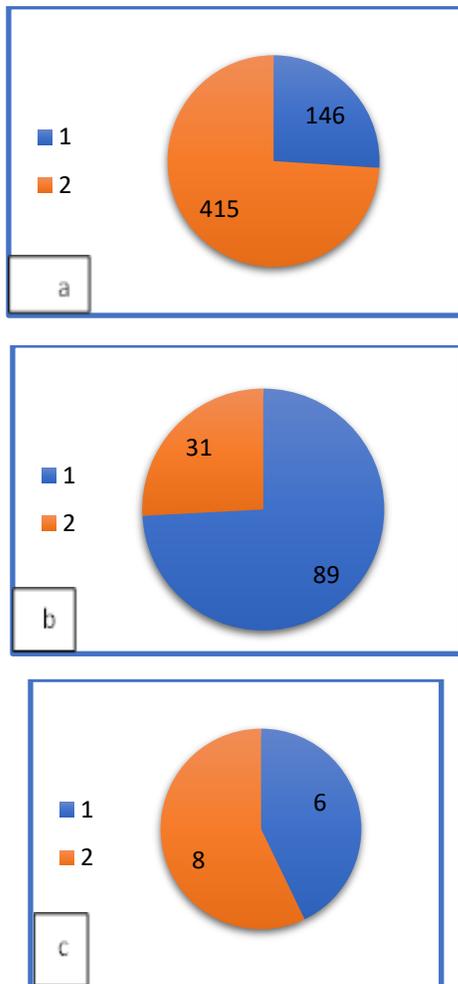
This is consistent with (Meo et al., 2021) who reported that the vaccines for COVID-19 can have moderate side effects after the first or second doses. Pain, fever; redness, or swelling at the injection site; headache, muscle pain, fatigue; joint pain, chills; itching, nausea, and vomiting are all possible side effects {8}.

Additionally, this agrees with (Malayala, et al., 2021) who stated in the trials related to the vaccine, there appeared mild to moderate

intensity side effects like myalgia, fever, malaise, and chills after being taken whereas the "Moderna vaccine." {9}.

All vaccinated people in this study were distributed according to gender (males and females), were found the number of vaccinated females more than males, the highest number of vaccinated females with Pfizer vaccine (415), followed by the Sinovac vaccine (31), While less number of vaccinated females with the AstraZeneca vaccine (8) shown in figure(3).

**Figure (3): Number of a-Pfizer vaccine, b-Sinovac, c-AstraZeneca, groups according gender 1-male 2 –female.**



The study included the recognition of the Concentrations of C3, C4, IgG, IgM, and Total protein concentrations, by using the special kit.

In this study, we investigated the levels of Total protein, C3, C4, IgG, and IgM concentrations in the serum of the vaccinated people and compared them with non-vaccinated people.

The study has shown elevated levels of C3, IgG, IgM, and Total protein concentrations serum of the vaccinated people compared with the control, while the concentration of C4 is low compared with the control as shown in tables (1,2,3,4,5).

The mean of C3 concentration in serum of vaccinated people of Pfizer vaccine was  $256.2250 \pm 78.56689$  pg/ml, Sinovac vaccine was  $291.3333 \pm 43.16901$  pg/ml and AstraZeneca vaccine was  $328.9000 \pm 29.01293$  pg/ml while control was  $282.028 \pm 46.18720$  pg/ml, there significantly as show in table (1).

**Table (1): Concentrations of C3 among immunization groups**

groups	Mean ±Sd
Control	282.028±46.18720
F	256.2250±78.56689
S	291.3333±43.16901
A	328.9000±29.01293

LSD  $\geq 0.05$  sig=0.27

The central molecule of the complement system is complement protein C3, and activation of this protein is necessary for all of the system's crucial functions, such as phagocytosis, localized inflammatory

responses against pathogens, and instructing the using the adaptive immune system to choose the right antigens for humoral responses{10}.

Kurtovic and Beeson (2021) stated that our hypothesis is that complement may also play a protective role, enhancing virus neutralization by antibodies, promoting phagocytosis of the virus by immune cells, and causing virus destruction. These features may be used in the creation of effective SARS-CoV-2 therapies and vaccines.

In one study, researchers looked at the link between complement and mortality in COVID-19 disease in humans and discovered that higher C3 levels in the blood were linked to a lower risk of death compared to those with lower levels {11}.

The mean of C4 concentration in serum of vaccinated people of Pfizer vaccine was  $77.5500 \pm 21.40537$  pg/ml, Sinovac vaccine was  $74.9667 \pm 15.95755$  pg/ml and AstraZeneca vaccine was  $73.5000 \pm 27.36110$  pg/ml while control was  $89.0060 \pm 14.18771$  pg/ml there significantly as show in table (2).

**Table (2): Concentrations of C4 among immunization groups**

groups	Mean $\pm$ Sd
Control	$89.0060 \pm 14.18771$
F	$77.5500 \pm 21.40537$
S	$74.9667 \pm 15.95755$
A	$73.5000 \pm 27.36110$

LSD  $\geq 0.05$  sig=0.34

The complement system is essential for the protection against microbes. In the classical

pathway, the C1 complex is stimulated by pathogen-related antibodies, and the C1 complex initiates a cleavage cascade that includes C2, C3, C4, and C5 and triggers microbial clearance. Human genetics indicates that C4 has significant immunological functions. Because the C4-dependent antiviral mechanism does not depend on downstream complement components, C4 is crucial for defense against viral processes{12}..

The study has shown elevated levels of Immunoglobulin G (IgG), and Immunoglobulin M (IgM) in serum of vaccinated people to all vaccines (Pfizer vaccine, Sinovac vaccine, and AstraZeneca vaccine), and showed there a significance of (IgG), and (IgM) in serum of vaccinated people compared with control, as shown in the tables (3).

The mean of IgG concentration in serum of vaccinated people of Pfizer vaccine was  $3115.0000 \pm 620.38648$  pg/ml, Sinovac vaccine was  $3115.0000 \pm 620.38648$  pg/ml and AstraZeneca vaccine was  $3582.9667 \pm 459.37998$  pg/ml while control was  $2836.4000 \pm 943.85630$  pg/ml, there significantly as show in table (3).

**Table (3): Concentrations of IgG among immunization groups**

groups	Mean $\pm$ Sd
Control	$2836.4000 \pm 943.85630$
F	$3115.0000 \pm 620.38648$
S	$3582.9667 \pm 459.37998$
A	$3485.8333 \pm 307.69131$

LSD  $\geq 0.05$  sig=0.20

Immunoglobulin G (IgG) is an antibody that interacts with a range of antigens and is essential for protection against invading pathogens. It is one of the most abundant proteins in human serum, IgG playing a central role in systemic antiviral immunity. Initial results suggested that antibodies ( IgM ) to SARS-Cov-2 appeared earlier than IgG antibodies, and the testing of both IgG and IgM antibodies might enhance the detection of infection by SARS-Cov-2. {13}.

The study's findings showing IgG concentrations were higher than those of the control group are in agreement with those of Wisniewski et al. (2021), who observed that IgG levels increased exponentially until plateauing 21 days following the initial dosage of the vaccine. IgG levels rose after the second dose of the vaccination and peaked about 7 to 10 days later. They remained high (an average of 58% of peak levels) throughout the following >100 days of follow-up. {14}.

The mean of IgM concentration in serum of vaccinated people of Pfizer vaccine was  $439.0500 \pm 57.44635$  pg/ml, Sinovac vaccine was  $459.1333 \pm 120.95488$  pg/ml and AstraZeneca vaccine was  $525.4667 \pm 112.06770$  pg/ml while control was  $402.3500 \pm 172.40591$  pg/ml, there significantly as show in table (4).

**Table (4): Concentrations of IgM among immunization groups**

groups	Mean $\pm$ Sd
Control	$402.3500 \pm 172.40591$
F	$439.0500 \pm 57.44635$
S	$459.1333 \pm 120.95488$
A	$525.4667 \pm 112.06770$

LSD  $\geq 0.05$  sig=0.22

These data revealed some potential differences in the nature of the humoral response induced by the three vaccines, these results may be agree with (Parry et al., 2021) who reported antibody titers by Roche ELISA were 691-fold higher in vaccinated donors with a previous infection compared to vaccinated people without a previous infection {15}.

Correlation between IgG and IgM, Correlations between C3 and C4 in serum of vaccinated people:

Correlation between IgG and IgM was positive correlation and Show in figure (5), while the correlation between C3 and C4 was negative correlation Show in figure (6).

The antibody tests such as Immunoglobulin M (IgM) and Immunoglobulin G (IgG) II are clinically significant because they allow the assessment of humoral immunity following infection and vaccination {16}.

**Table (5): Correlations between IgG and IgM**

		IgG	IgM
IgG	Pearson Correlation	1	.711**
	Sig. (2-tailed)		.010
IgM	Pearson Correlation	.711**	1
	Sig. (2-tailed)	.010	

\*\* . Correlation is significant at the 0.01 level (2-tailed).

The levels of IgG and IgM antibodies vary over time caused by infection with COVID-19, which is expected because antibodies are produced during the lag period after infection, the early immunoglobulin (IgM) are later replaced by IgG antibodies, and the levels of

antibodies in the blood generally decrease over time following infection{ 17}.

This result agrees with (Shah, et al., 2021) who proved the detection of antibodies such as IgM or IgG is preferable for assessment.

**Table (6) : Correlations between C3 and C4**

		C3	C4
C3	Pearson Correlation	1	-.298-
	Sig. (2-tailed)		.281
C4	Pearson Correlation	-.298-	1
	Sig. (2-tailed)	.281	

The system of complement has been observed to be activated in (COVID-19) illness. commonly Immunoassays are utilized in clinical practice to detect and monitor the activation of complement by using the complement proteins C3 and C4. C3 and C4 testing in COVID-19 patients may provide benefits in determining the balance of 'physiological' vs. 'abnormal' complement activation and overall clinical risk, often the level of C3 is decreased during consumption during infections, whereas in immune complex diseases observed a combined reduction in C3 and C4{ 18,19,20}.

**Table (7): Total protein concentrations**

groups	Mean ±Sd
Control	5.7780±.16574
F	6.1660±.16562
S	5.5880±.18913
A	5.3980±.15482

The total protein test counts the total amount of two types of proteins in human blood's fluid component. Albumin and globulin are these. Between 6 and 8.3 (g/dL) is considered to be the typical range for total protein. Each laboratory may have a somewhat different range. These ranges are also influenced by other elements like test design, population, gender, and age. During pregnancy, the total protein measurement may rise. Before a diagnosis can be made if a total protein is abnormal, additional tests must be run to determine whether a specific protein is deficient or excessive. Increased total protein can be a sign of infection, inflammation, HIV, viral hepatitis B or C, or diseases of the bone marrow, including multiple myeloma or Waldenstrom's disease{ 21,22}.

**Conclusions:**

The most common type of vaccine taken was Pfizer followed by Sinopharm and less with AstraZeneca and the Vaccines it Enhanced humoral immune response

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