Investigation The Effect of Pfizer , AstraZeneca , and Sinopharm Vaccines Against SARS-CoV-2 in Iraq Using Cluster of Differentiation 4 (CD4) and Vitamin D3

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Abstract

   The research aimed to study the vaccination efficiency against the emerging virus SARS-CoV-2, which includes Pfizer, AstraZeneca and Sinopharm vaccines that are used exclusively in Iraq, by measuring the level of immune cells CD4$^+$ as well as vitamin D3 using ELISA technology. Taking serums were taken from vaccinated individuals: 20 uninfected before individuals who got a vaccination and 20 people with prior infections who had the vaccine (for each type of the three vaccines).

Samples were collected 21 days following the second dose as well as 3 to 6 months t hereafter.   The results showed that the most effective vaccine was AstraZeneca and the results of T-cells were the highest compared to Pfizer and Sinopharm.

Keywords: COVID-19, ELISA, CD4, Vitamin D3, Iraq .

التحقيق في تأثير لقاحات فايزر وأسترازينيكا وسينوفأرم ضد فيروس كورونا في العراق باستخدام مجموعة التمايز 4 (CD4) وفيتامين دال.

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الخلاصة

   استهدف البحث دراسة فعالية اللقاحات المستخدمة ضد فيرس كورونا المستجد والتي تشمل لقاح فايزر و لقاح استرازنيكا ولقاح سينوفأرم المستخدمة في العراق حصرا وذلك من خلال قياس مستوى الخلايا المناعية التائيه و كذلك فيتامين د والاجسام المضادة باستخدام تقنيه الأليزا (تم ذلك من خلال اخذ عينات مصل من افراد ملقحين ٢٠ فرد غير مصاب سابقا واخذ اللقاح ٢٠ فرد مصاب سابقا واخذ اللقاح) لكل نوع من اللقاحات الثلاثة وتم جمع العينات بعد ٢١ يوم من الجرعة الثانية و كذلك بعد مرور من ٣الى ٦اشهر بعد الجرعة الثانية واظهرت النتائج ان اللقاح الاكثر فعالية هو لقاح الاسترازنيكا حيث كانت نتائج الخلايا التائية والاجسام المضادة هي الاعلى مقارنه بالفايزر و السينوفأرم.

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Introduction

In late December 2019, the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) was identified for the first time as the main cause of a pandemic that primarily, though not exclusively, affects the respiratory system [1]. Located in Hubei Province, China, Wuhan, and has since rapidly expanded throughout the rest of the world and developing into a global pandemic that impacts the most potent nations on the globe , poses a threat to the entire world [2] . A number of vaccines developed in various technologies are permitted to aid in preventing infection with the SARS-CoV-2 virus. The COVID-19 vaccinations that are now approved have demonstrated good safety and effectiveness characteristics in a variety of contexts. The majority of these immunizations, including the Pfizer-BioNTech COVID-19 vaccine (tozinameran), the Moderna COVID-19 vaccine, the Oxford-AstraZeneca COVID-19 vaccine, and the Sinopharm COVID-19 vaccine contributed to a decrease in terms of frequency and severity of SARS-CoV-2 illness. Uneven distribution worldwide and the ongoing generation of SARS-CoV-2 genotype variations that allow immune escape potential could pose obstacles to the success of COVID-19 immunization efforts [3]. Coronavirus spike protein (S protein), which can significantly elicit B-cell and T-cell immune responses, was recognized early on in the development of vaccination efforts as a viable target. As a result of their capacity to elicit a potent T-cell response and the fact that their genes are typically conserved and less susceptible to recombination than spike proteins, other coronavirus proteins, such as the nucleocapsid (N) proteins, are also being researched for vaccine development .Viral entry into cells is facilitated by attachment to cells that express certain receptors. The class I major histocompatibility complex presents viral peptides to CD8+ cytotoxic T lymphocytes proteins to fight these viruses [4]. By using CD8+ cytotoxic T lymphocyte , the virus-infected tissue cells are killed. Viral particles are delivered to CD4+ T lymphocytes by other antigen-presenting cells, including dendritic cells and macrophages, using MHC-Class-II. These CD4+ T cells, also known as helper T cells, can collaborate with B cells to directly recognize viruses and generate virus-specific antibodies. Also the Vitamin D receptor (VDR) and the vitamin D-activating enzyme CYP27B1 (1-hydroxylase) are expressed by circulating T cells, B cells, and dendritic cells, which use intracranial conversion of circulating 25D to bioactive 1,25D. Dendritic cell maturation is constrained and CD4+ T cell activity is regulated by increased intracellular 1,25D. Generally speaking, vitamin D promotes the switch from TH1 to THαβ cells, which affects adaptive immunity. Vitamin D essentially prevents activation of type 1 T helper cell and TH1 immunological responses. The link of THαβ cells with antiviral immunity is further promoted by vitamin D, which promotes interleukin-10 and antiviral IgG1 production from B-cell lineages. Appropriate vitamin D intake could decrease pro-inflammatory responses while boosting COVID-19's anti-inflammatory effects [5] .

Materials and Methods

Study Samples

A case study was conducted during September 2021–February 2022 to determine the levels of CD4 and Vitamin D3 in Iraqi patients with SARS-COV-2, vaccinated people with Pfizer and AstraZeneca and Sinopharm vaccines, and control subjects who were not infected and not vaccinated. The Ethics Committee at the Department of Biology (University of Baghdad) approved the study protocol by (Reference: CSEC/0921/0096) on September 15,2021.

Patients

Consecutive 100 cases (62 males and 38 females) with positive PCR for SARS- COV-2 were randomly selected and recruited from Medical City/ Al-Shifa Hospital (Corona patients isolation center) that were diagnosed by PCR technique and most of patients have sever and

acute symptoms about (70%) and (30%) have mild symptoms. As well as all cases were selected without any chronic disease.

Normal Control Group:

   Thirty healthy individuals, male and female, without SARS-CoV-2 infection (not vaccinated) and other apparent diseases were randomly selected as the normal control groups during the period of this study.

 Vaccinated Group included :

1-Previous Infected Individuals including :

• (20 previous infected individuals (males and females) and vaccinated with Pfizer Vaccine , the serum was collected in a period from 14 – 21 day after the second dose .

• 20 previous infected individuals (males and females) vaccinated with   Pfizer Vaccine, the serum was collected in a period from 3-6 month after the second dose.

• 20 previous infected individuals (males and females) and vaccinated with Sinopharm Vaccine ,the serum was collected in a period from 14 – 21 day after the second dose.

• 20 previous infected individuals (males and females) vaccinated with Sinopharm Vaccine, the serum was collected in a period from 3-6   month after the second dose.

• 20 previous infected individuals (males and females) and vaccinated with AstraZeneca Vaccine ,the serum was collected in a period from 14 – 21 day after the second dose .

• 20 previous infected individuals (males and females) vaccinated with AstraZeneca Vaccine, the serum was collected in a period from 3-6   month after the second dose.

2-   Not-infected individuals includes :

• 20 not-infected individuals (males and females) and vaccinated with   Pfizer Vaccine , the serum was collected in a period from 14 – 21 day after the second dose .

• 20 not-infected individuals (males and females) vaccinated with   Pfizer Vaccine, the serum was collected in a period from 3-6 month after the second dose .

• 20 not-infected individuals (males and females) and vaccinated with Sinopharm Vaccine , the serum was collected in a period from 14 – 21 day after the second dose .

• 20 not-infected individuals (males and females) vaccinated with   Sinopharm Vaccine , the serum was collected in a period from 3-6 month after the second dose .

• 20 not-infected individuals (males and females)and vaccinated with AstraZeneca Vaccine , the serum was collected in a period from 14 – 21 day after the second dose .

• 20 not-infected individuals (males and females) vaccinated with   AstraZeneca Vaccine , the serum was collected in a period from 3-6 month after the second dose.

 Laboratory methods

   The Enzyme-linked Immunosorbent Assay (ELISA) kit was used for qualitative assessments of CD4 and Vitamin D3 in sera of patients and control. The kit was product of My BioSource Company (USA) and was based on a sandwich ELISA technology. Standard procedures recommended by the manufacturers were followed in these assessments.

Statistical Analysis

   The Statistical Analysis System-SAS (2012) application was used to determine how various study parameters were impacted by various circumstances. The Chi-square test was used to compare percentages in a significant way (0.05 and 0.01 probability).

 Results and Discussion:
Comparison between different groups in CD4

The level of cellular immunity (CD4) is increased in patients and individuals that have been vaccinated, but there is a highly significant increase in people that have been vaccinated with the AstraZeneca vaccine, as shown in table (1).

Table1: CD4 levels in study groups

Group	Mean ± SE
	CD4: normal value 2.7-4.3 (ng/ml)
G1:control	3.69 ±0.07 g
G2: Patients(without vaccination)	6.91 ±0.17 bcd
G3: Non inf.+ Pfizer after 21 day	5.58 ±0.25
G4: Infected +Pfizer after 21 day	3.80 ±0.14 g
G5: Infected Astra 21 d	6.94 ±0.36 bc
G6: Non inf.+ Astra. after 21 day	5.15 ±0.25 ef
G7: Inf.+ Sinoph. after 21 d	6.92 ±0.82 bc
G8: Non inf.+ Sinoph. after 21day	6.04 ±0.65 cde
G9: Inf. +Pfizer after (3-6) months	6.19 ±0.28 cde
G10: Non inf.+ Pfizer after (3-6) months	5.08 ±0.24 ef
G11: Inf. Astra months	8.08 ±0.65 ab
G12: Non inf. +Astra. after (3-6) months	8.87 ±0.69 a
G13: Inf. +Sinoph after (3-6) months	4.62 ±0.20 fg
G14: Non inf. + Sinoph. after (3-6) months	5.72 ±0.45 def
LSD value	1.192 **
P-value	0.0001 **
Means having with the different letters in the same column differed significantly. ** (P≤0.01).	

The CD4+ T helper cells play a variety of activities that are essential for directing and coordinating antiviral immunity. By providing soluble mediators and co-stimulating these processes, CD4+ T follicular helper (TFH) cells regulate the development of high-affinity neutralizing antibodies and the differentiation of germinal center B cells into memory and long-lived antibody-secreting cells [6]. Fast CD4+ T cell responses in acute COVID-19 have been proven to be associated with mild illness and faster viral clearance [7] . In a separate study that examined SARS-CoV-2-specific antibodies, CD4+ and CD8+ T cells in people with a variety of COVID-19 illness severities, they were shown to be connected to poor or nonexistent SARS-CoV-2-specific CD4+ and CD8+ T cell responses [8]. The age-specific AstraZeneca-primed T cell responses may be crucial in protecting against severe illness brought on by COVID-19. The T cells are crucial for preventing SARS-CoV-2 disease [9] . For the generation of pathogen-specific antibody responses, B cells rely on CD4+ T cells. Plasmacytoid dendritic cells and macrophages have the capacity to take up DNA molecules that have been ingested. These antigen-presenting cells then transfer the antigen to CD4 T cells to begin adaptive immunity. DNA vaccines, as opposed to RNA vaccinations, induce cellular signaling that enables IRF7 to activate type 1 interferons such as IFN and IFN by using the Toll-like receptor 9 (TLR9) as the cellular receptor. The Toll-like receptors 3 and 7 are also employed to detect double-or single-

stranded RNA molecules during the transcription of DNA into RNA. The procedure for producing antiviral immunity is comparable to that of mRNA vaccines [10]. The most important antigen-presenting cells for the development of TH immunity remain plasmacytoid dendritic cells. Innate lymphoid cells (IL-10-producing cells), which secrete type 1 interferon and IL-10, help the antigen-presenting cells transmit viral antigens to CD4 T cells. As a result, interleukin 10 produced from CD4 T cells increases as they mature into TH CD4 T cells. For long-term immunity, memory T cells and memory B cells are created [11].Anti-spike protein CD4+ T cell responses and antibody responses with an anti-spike protein receptor-binding domain were linked in COVID-19-recovered individuals [12]. Circulating Tfh cells have been seen in individuals who have recovered from COVID-19 [13] . Vaccination against ChAdOx1 nCoV-19 significantly increased effector T-cell responses specific for SARS-CoV-2, which surged as early as day 7, peaked at day 14, and was sustained up to day 56, as is typical with adenoviral vectors as shown in Figure(1). High cellular immunity is produced by vaccinations with adenovirus vectors as known . Adenovirus-vectored vaccines may generate potent humeral reactions, including RBD-binding IgG, IgA, and SARS-CoV-2 neutralizing antibodies, as well as cellular immunological reactions. ChAdOx1 nCoV-19 recognizes both S antigen subunits, leading to a potent and widespread T cell response. Similar to previous replication-deficient adenoviral vectors, the T cell response described here functions similarly in that responses are dominated by individual T cells secreting a single cytokine rather than several [14] . Through analysis of cytokine secretion after peptide stimulation of PBMCs, it was discovered that IFN- and IL-2 secretion were higher in ChAdOx1 vaccine recipients compared to controls; IL-4 and IL-13 levels were not elevated, and CD4+ T cells secreted more Th1 cytokines (IFN-, IL-2, and TNF-) than Th2 cytokines (IL-5 and IL-13) [15]. Vaccination with ChAdOx1 nCoV-19 primarily induces a Th1 response . An important safety factor for vaccinations against respiratory infections is strong TH1 responses. Because they are less likely to result in immunopathology ,the targeted COVID-19 immunization outcomes include TH1-predominant responses and balanced CD4+ and CD8+ T cell responses. The spike protein-specific TH2 responses after AZD1222 immunization was modest in all age groups [16].A vaccination against COVID-19 with the ChAdOx1 vector has shown high immunogenicity in people over the age of 50. TNF, interleukin-2 (IL-2), and interferon-gamma (IFN) were the three cytokines that Th1 cells mostly produced. According to the research, the AZD1222 vaccine strongly induces polyfunctional Th1-dominated T cell responses to the SARS-CoV-2 spike protein, which could provide long-lasting protection against COVID-19. However, no Th2 response was seen in those who had received the vaccine. Additionally, the vaccine results in a sizable proliferation of CD4+ and CD8+ T cells that are specific for spikes and have distinct T cell receptor sequences that map to several spike epitopes [17]. Long-term protection against SARS-CoV-2 infection is believed to require a combination of antibody and T-cell immunity. As has been previously demonstrated in all age groups, vaccination with AZD1222 also induces a polyfunctional Th1-dominated T-cell response to the SARS-CoV-2 spike protein. SARS-CoV-2 spike-specific CD4+ and CD8+ T cells have increased significantly as a result of this response [18] .
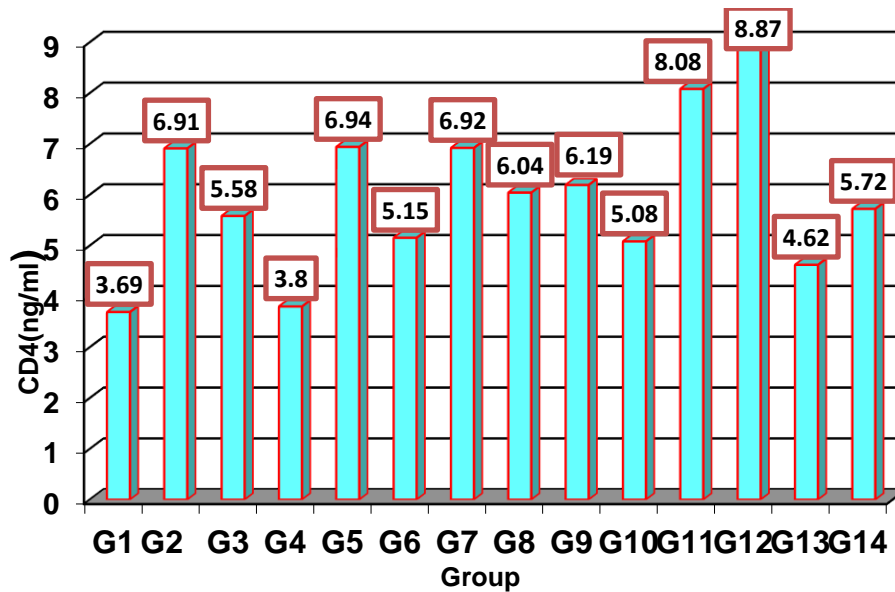
Figure 1: Comparison between difference groups in CD4.

G1:Control which not infected and not vaccinated ,G2 :Patients with SARS CoV-2 (without vaccination), G3: not infected + vaccinated with Pfizer after 14 – 21 day ,G4: infected + vaccinated with Pfizer after 14 – 21 day ,G5: infected + vaccinated with Astrazeneca after 14 – 21 day ,G6: not infected + vaccinated with Astrazeneca after 14 – 21 day ,G7 : infected + vaccinated with Sinopharm after 14 – 21 day ,G8: not infected + vaccinated with Sinopharmafter 14 – 21 day ,G9: infected + vaccinated with Pfizerafter (3-6) months , G10: not infected + vaccinated with Pfizerafter (3-6) months ,G11: infected + vaccinated with Astrazeneca after (3-6) months ,G12: not infected + vaccinated with Astrazeneca after (3-6) months ,G13: infected + vaccinated with Sinopharm, after (3-6) months ,G14: not infected + vaccinated with Sinopharm, after (3-6) months.

Comparison between difference groups in vitamin D3

The result of the current study showed that normal levels of vitamin D3 in study groups but there were highly significant increase in people that were vaccinated with the AstraZeneca vaccine ,as appear in table (2)

Table 2: Comparison between difference groups in vitamin D3

Group	Mean ± SE
	D3: normal value (30-100 ng/ml)
G1:control	38.66 ±4.08 gh
G2: Patients(without vaccination)	41.90 ±1.72 fg
G3: Non inf.+ Pfizer after 21 day	55.70 ±4.46 cd
G4: Infected+ Pfizer after 21 day	30.20 ±2.97 h
G5: Infected+ Astra. after 21 day	76.33 ±2.93 a
G6: Non inf. +Astra. after 21 day	49.57 ±4.60 defg
G7: Inf.+ Sinoph. after 21 day	53.77 ±6.14 cde

G8: Non inf. +Sinoph. after 21day	54.70 ±5.56 cd
G9: Inf. +Pfizer after (3-6) months	52.17 ±2.61 cdef
G10: Non inf.+ Pfizer after (3-6)months	5.08 ±0.24 defg
G11: Inf.+ Astra. after (3-6) months	61.60 ±3.85 bc
G12: Non inf.+ Astra. after (3-6) months	67.42 ±3.88 ab
G13: Inf. + Sinoph. after (3-6) months	42.41 ±3.08 efg
G14: Non inf.+ Sinoph. after (3-6) months	40.81 ±4.59 fgh
LSD value	11.42 **
P-value	0.0001
Means having with the different letters in the same column differed significantly. ** (P≤0.01).	

An important immune system modulator is vitamin D. It is   well known that vitamin D deficiency results in lower numbers of CD4+ and CD8+ T lymphocytes, whereas vitamin D supplementation results in higher numbers of CD+4 cells [19]. As showed in Figure (2), numerous immune cells. The vitamin D receptor is expressed by a variety of cell types, including macrophages, monocyte dendritic cells, neutrophils, and lymphocytes . The VDR and the vitamin D-activating enzyme CYP27B1 (1-hydroxylase) are present in circulating T cells, B cells, and dendritic cells. These cells utilize the circulating 25D through intracranial conversion to bioactive 1,25D. Vitamin D promotes the shift from TH1 to THαβ cells, which affects adaptive immunity. Vitamin D increases the antimicrobial activity of these immune cells and has anti-inflammatory effects because it increases T regulatory cells while reducing the synthesis of cytokines that promote inflammation and the immune response to T helper-17 [20].The vitamin D receptor is the mechanism by which vitamin D works. This receptor is highly expressed in the immune system and is located throughout the body. The innate and adaptive immune systems are regulated by the vitamin D receptor. Patients with low vitamin D levels are more likely to be PCR positive and to contract COVID-19 infection , according to a number of recent studies. and that they have greater fatality rates and a poor outlook for the illness [21]. However, compared to patients with COVID-19 infection, COVID-19 severity, and death who did not receive vitamin D supplementation, patients' chances of contracting COVID-19 were shown to be lower when their serum 25 (OH) D concentrations reached 30 ng/ml [22]. T-cell migration activity and development are controlled by 1,25 (OH)2D3. VDR expression is essentially undetectable in dormant T cells, but it increases five - fold following activation. Active vitamin D directly targets both Th1 and Th2 cells. Direct activities on T cells represent an additional or alternative pathway for 1,25(OH) 2D3 to modulate T-cell antigen receptor signaling and shape T-cell responses. ChAdOx1 nCoV-19 (AZD1222) is a candidate. A replication-deficient simian adenovirus produces the whole SARS-CoV-2 spiking SARS-CoV-2 protein [23]. Experience with the creation of SARS-CoV-2 vaccines has raised concerns concerning the link between pulmonary histopathology and immune responses to Th2 cytokines [24]. Th2 cells have the ability to release a variety of cytokines, including IL-4, IL-5, IL-10, and IL-13. The Th2 cytokine concentrations that are abnormally high can trigger eosinophilic infiltrations via inducing immunological reactions. The development of Th2-type immunopathology with increased eosinophilic infiltration was caused by four distinct SARS-CoV-2 vaccinations [25] .The COVID-19 vaccine's T cells are required for the production of antibodies by B cells, which can be accelerated and collaborated with by appropriate vitamin D supplementation, which can boost immunity [26] .T cells and antibodies that specifically target

the antigen and neutralize the SARS-CoV-2 spike protein were created after the vaccination, which was well tolerated. Adults produced S-protein-reactive CD4+ T cells with a T helper (THαβ)-type cytokine bias and showed CD8+ T cells with a cytotoxic profile. These findings are important because THαβ-type immunity supports protective antiviral immunity. Strong B-cell proliferation and activation were also seen, and IgG from anti-S proteins, especially the IgG1 isotype, was identified from days 14 to 56. In addition to raising antibody titers, ChAdOx1 nCoV-19 vaccination markedly improved IgG antibody avidity to offer seroprotection. Sufficient vitamin D intake can support THαβ-type immunity and promote the activation of B cells that produce more IgG-neutralizing antibodies. A second vaccination raises anti-S antibody titers and neutralizing activity, which enhances THαβ-type T-cell responses, according to a study. The booster dose enhances anti-S antibodies' capacity for complement deposition, activation of natural killer cells, and antibody-dependent cellular cytotoxicity [27] . In a prospective study conducted in the USA, higher vitamin D status was also linked to lower COVID-19 risks . A simple, safe, and inexpensive method of lowering the risk of COVID-19 could be to increase vitamin D intake to lower the rate of deficiency. Supplementing with vitamin D may increase immune responses to several COVID-19 vaccinations. Antigen-presenting cells (APCs)**,** treat the vaccination as an antigen and subsequently present it to CD8+ T and CD4+ T cells. TH**αβ** cytokines have the ability to activate CD8+ T lymphocytes, giving them the ability to attack infected cells. Appropriate vitamin D supplementation could be used in combination with this process. The B cell differentiation may benefit from TH**αβ** cytokines . The activated B cells can make Nabs . In a T-cell-dependent B-cell manner, vitamin D can also enhance antibody production [28] .
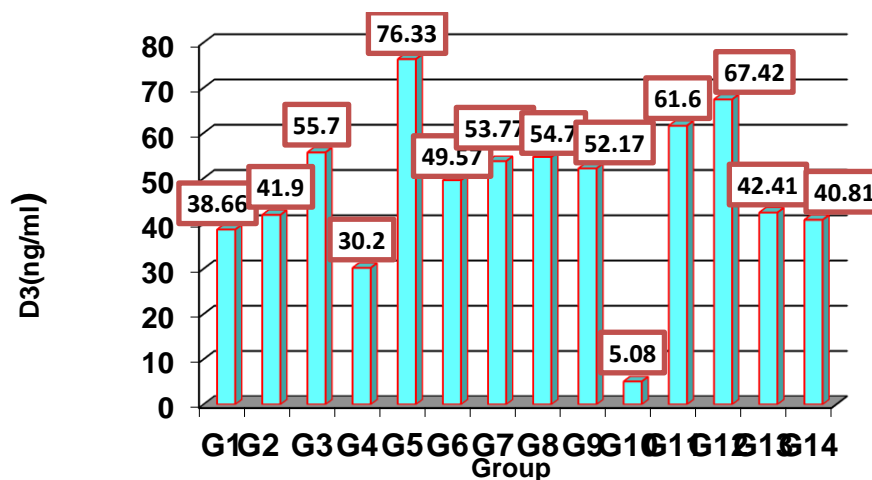

Figure 2: Comparison between difference groups in D3.

G1:Control which not infected and not vaccinated ,G2 :Patients with SARS CoV-2 (without vaccination), G3: not infected + vaccinated with Pfizer after 14 – 21 day ,G4: infected + vaccinated with Pfizer after 14 – 21 day ,G5: infected + vaccinated with Astrazeneca after 14 – 21 day ,G6: not infected + vaccinated with Astrazeneca after 14 – 21 day ,G7 : infected + vaccinated with Sinopharm after 14 – 21 day ,G8: not infected + vaccinated with Sinopharmafter 14 – 21 day ,G9: infected + vaccinated with Pfizerafter (3-6) months , G10: not infected + vaccinated with Pfizerafter (3-6) months ,G11: infected + vaccinated with Astrazeneca after (3-6) months ,G12: not infected + vaccinated with Astrazeneca after (3-6) months ,G13: infected + vaccinated with Sinopharm, after (3-6) months ,G14: not infected + vaccinated with Sinopharm, after (3-6) months.

Conclusions

In previously infected people, CD4 T-cells increased after two doses of vaccine, leading to an increase in IgG antibodies. The AstraZeneca vaccine has been shown to be the most effective vaccine in raising the immune system against COVID-19 in Iraqi individuals. A sufficient amount of vitamin D3 plays a key role in controlling the functions of immune cells, as has been observed as a small amount of vitamin D reduces the activity of immune cells, and a sufficient amount leads to an increase in the activity of immune cells. The inactivated vaccine is the vaccine that is least effective in protecting against infection with COVID-19, and it is the vaccine with the fewest side effects compared to other vaccines.

Conflict of interest

The authors have no conflicts of interest to declare.

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