Original article

A comparative study of the effectiveness of Pfizer-BioNTech (BNT162b2), Astra Zeneca (ChAdOx1nCoV-19) and Sinopharm (BBIBP-CorV) vaccines in eliciting Humoral immunity in a sample of vaccinated population from Iraq.

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Abstract

Background: In order to tackle COVID-19 pandemic and the emerging variants, researchers around the globe have investigated many vaccine candidates from different manufacturers, however vaccine development is not an easy task but is a top priority to restore normalcy as represented a step to achieve the desired herd immunity threshold. ***Patients and methods:*** in this study we assessed and compared the level of IgG anti-RBD neutralizing antibodies triggered from each vaccine against SARS-CoV2 infection in 123 vaccinated subjects, by using isotype- and species- free competitive blocking ELISA. Blood samples were taken from vaccinated individuals 1 and 8 months after the second dose of the vaccines. ***Results:*** the findings of the current study revealed that two-dose vaccination might be effective to trigger robust humoral neutralizing immunity at 1month and even durable for as long as 8months with different sustained levels among the three studied previously mentioned vaccines. The serum level of the neutralizing IgG antibodies, Pfizer group revealed the highest level compared to AstraZeneca and Sinopharm groups ($P<0.05$); the Sinopharm showed trend of higher levels of neutralizing antibodies than AstraZeneca but without reaching statistical significance ($P>0.05$). Additionally, the serum level of neutralizing IgG antibodies, which represent the humeral immunity to SARS-CoV-2, was shown to be far higher in 1-month than in 8-month post-2nd dose vaccination groups ($P<0.0001$). ***Conclusion:*** Altogether, it is concluded that Pfizer vaccine proved to be of highest and most durable neutralizing anti-RBD IgG antibodies and followed with Sinopharm and AstraZeneca vaccines.

Keywords: effectiveness; humoral immunity; Pfizer; Sinopharm; Astra Zeneca

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Introduction:

Coronavirus disease (COVID-19) pandemic, by the etiology severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), has posed serious threats to public health, the global society and economy [1,31,32,35]. Therefore, it is imperative to develop safe and effective vaccines to defeat SARS-CoV-2 [(33)] and, most importantly, the emerging variants circulating worldwide [2].

Spike (S) proteins on the surface of SARS-CoV-2 virus mainly consist of S1 and S2 domains, which are responsible for virus-cell attachment and membrane fusion, respectively, the receptor-binding domain (RBD) in the S1 subunit is the key component that directly mediate the recognition and binding of the virus to the receptor angiotensin-converting enzyme 2 (ACE2) on host cells [1,3].

The S1 and RBD are ideal targets for evolving

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subunit vaccines against SARS-CoV-2 wild type and its variants [4,5]. However, RBD-based subunit vaccines may face some serious challenges, mostly arising from their relatively low immunogenicity, which must be combined with appropriate adjuvants or optimized for suitable protein sequences, fragment lengths, and immunization schedules[6].

As of Feb 3, 2021 the world has shown an impressive capacity for an accelerated COVID-19 vaccine development process, many COVID-19 vaccine candidates have been authorized or approved for human use and others were in experimental phases of clinical testing, only five of vaccines those developed by AstraZeneca in partnership with Oxford University, BioNTech in partnership with Pfizer, Gamaleya, Moderna, and Sinopharm in partnership with the Beijing Institute— have been authorized by stringent regulatory agencies or WHO [7].

Among the approved vaccines, different platforms have been implemented: inactivated virus, viral vectors, and mRNA-based vaccines which focus the immune response against only the key viral proteins of interest. Generally, all of them are qualified to stimulate an immune response and are efficacious against SARS-CoV-2, even at varying levels [8]. Although vaccination effectiveness against SARS-CoV-2 has been astonishing, but booster immunizations are clearly required for maintenance of effectiveness over time, they are far from perfect. Immunity wanes with time elapsed, and viral antigenic variation [9].

Vaccines induce both adaptive humoral and cellular immune responses, most of the currently accepted correlates of protection are based on neutralizing antibody responses, however, if there is no detectable antibody response after vaccination the vaccines may still offer protection through cellular immunity, since cellular responses and antibody responses are often correlate to some extent [10-12].

Three vaccines were introduced to Iraq for use namely, Pfizer, AstraZeenca, and Sinopharm. These three vaccines were introduced after being tested in controlled randomized double blind clinical trials. However, none of these trials was done in Iraq. It is well known that immune response to vaccines might be affected by race, environment, age, sex, underling health conditions and level of exposure of the population to the virus [13,34] .Hence, it was important to set off a study investigating the neutralizing humeral immune response in a sample of vaccinated Iraqi individuals with these vaccines and to test the longevity of the immune response of these vaccines for 8 months after taking the second dose of the vaccine.

Materials and methods:

Study design and subjects

The study design is a cross sectional study of 6 groups of vaccinated healthy volunteers who received full doses of vaccines in Baghdad province; each group consists of 30individual. To assess the effect of age on the immunological response to the studied vaccines, each group was equally divided into 2 halves: namely15 individuals of age less than 60 years and 15 with age more than 60 years. Both sexes were involved and from different geographical residences without any bias in selection. The study was conducted in the period between 15 December 2021 to 5 July 2022.The included groups of the study population were as follows: at (1 month and 8months) post dual vaccination with Pfizer, at (1month and 8months) post dual vaccination with Sinopharm and at (1month and 8months) post dual vaccination with AstraZeneca. Accordingly, the target of the current study was to attain a sample size of 180 individuals. The exclusion criteria of the study population are: subjects should not have history of symptomatic infection, are not on immunomodulating or immunosuppressive therapy, and have no any kind of immunosuppression-related disease.

The following data were taken into consideration and recorded for each participant by oral questionnaire: the name of the vaccinated healthy volunteer, age, sex, type of the vaccine received, number of the received vaccine doses, the duration after the second dose of each vaccine which was determined by the vaccination card for each individual, comorbidities such as diabetes, hypertension, cardiovascular diseases and others, negative PCR result if done so far, absence of COVID-19 signs and symptoms, and not being in contact with an infected individual, to assure healthy status, and having an immunosuppressive disease or taking immune-suppressive or modulating drugs.

These data were adjusted to the selection criteria at the time of sample collection, the volunteers were selected from Baghdad with the help of Al-Kadhymia vaccination regional center.

Limitation of the study

1-discontinuity of vaccine supply precisely

AstraZeneca vaccine.

2-vaccine reluctance and vaccination hesitancy.

3-the highest transmissibility Omicron variant outbreak.

4- third vaccine dose recommendation.

5- heterologous prime-boost vaccination.

6-uncertainty of healthy status and possibility of asymptomatic COVID-19 infection.

Samples collection

Up to 3 ml of non-anticoagulant whole blood were drawn into 10 ml serum separator tubes for serum isolation to determine the amount and level of anti RBD-Neutralizing antibodies by indirect competitive inhibitory ELISA kit. The blood was allowed to clot at room temperature for about two hours. Then, it was centrifuged for 10 min at 1000 g and the resultant serum was isolated and stored at -20 C in aliquots for later use in ELISA.

Isotype-free competitive ELISA for the detection and quantification of SARS-COV-2 Neutralizing antibodies in the serum of vaccinated healthy individuals.

This ELISA kit uses Competitive-ELISA as the method to quantitatively detect and quantify anti-SARS-CoV-2 neutralization antibodies in the serum. The micro ELISA plate provided in this kit (SARS-CoV-2 Neutralization Antibody ELISA Kit. Elabscience, USA. Cat No.: E-EL-E608) is pre-coated with recombinant human ACE2. During the reaction, the SARS-CoV-2 neutralization antibodies in the pretreated samples or controls competes with a fixed amount of human ACE2 on the solid phase supporter for sites on the Horseradish peroxidase (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD). After incubation at 37°C, the unbound HRP-RBD as well as any HRP-RBD bound to non-neutralization antibody will be captured on the plate and eventually form the ACE2-RBD-HRP complex, while the circulating neutralization antibodies HRP-RBD complexes remain in the supernatant and are removed during washing. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The inhibition ratio resulted will indicate the level of SARS-CoV-2 neutralization antibodies exists in the tested samples. The concentration of SARS CoV-2 neutralization antibodies in the samples is then determined by comparing the OD of the samples to the OD of the kit standard curve.

Ethical clearance:

The study was approved by the Institutional Review Board at al Nahrain University, College of medicine under number 20211047. Informed consent was obtained from all subjects to participate in the study.

Results:

Characteristics of the participants in the study

To compare the effectiveness of the elicited humoral immune responses from the used COVID-19 vaccines in Iraq namely: Pfizer, AstraZeneca and Sinopharm,123 healthy supposedly non-infected vaccinated volunteers were assessed and classified into mainly 6 groups; each group was subdivided into two groups according to the vaccine type, duration of post 2nd vaccine dose and age.

Up to 50 individuals (40.7%) were vaccinated with Pfizer, 35 (28.5%) were vaccinated with AstraZeneca and 38 (30.9%) were vaccinated with Sinopharm. And 47 individuals (38.2%) were at 1month duration post 2nd dose of vaccination and 76 (61.8%) were at 8 months duration post 2nd dose.

In regard to age, 86 individuals (69.9%) were <=60 year and 37 (30.1%) were >60 year. According to sex, 70 (56.9%) were males and 53 (43.1%) were females. And 99 (80.5%) were without comorbidities while 24 (19.5%) were with comorbidities.

Groups of the vaccinated subjects

-A total of 22 vaccinated subjects (17.9%) were at 1month duration post vaccination with the 2nd dose of Pfizer vaccine and 28 vaccinated subjects (22.8%) were at 8months, a group of 30 subjects (34.9%) were <=60 year and 20 subjects (54.1%) were >60, and 26 subjects (37.1%) were males and 24 subjects (45.3%) were females.

-A total of 8 vaccinated subjects (6.5%) were at 1month post vaccination with the 2nd dose of AstraZeneca and 27 vaccinated subjects (22%) were at 8months post vaccination, a group of 27 subjects (31.4%) were <=60 year and 8 subjects (21.6%) were >60, and 24 subjects (34.3%) were males and 11 subjects (20.8%) were females.

-A total of 17 vaccinated subjects (13.8%) were at 1month post vaccination with the 2nd dose of Sinopharm vaccine and a total of 21 vaccinated subjects (17.1%) were at 8months post vaccination,

a group of 29 subjects (33.7%) were <=60 and 9 vaccinated subjects (24.3%) were >60, and 20 subjects (28.6%) were males and 18 subjects (34%) were females.

Vaccine induced humoral immunity with age, sex and comorbidity

It was found that there was no association between the age of vaccinated participants and the type of vaccine received (P>0.05), as shown in table 1.

Table 1: Count and percentages of the age groups according to the vaccine type.

			Qui square			
P=0.14			vaccine_type			Total
			Pfizer	Astrazeneca	Sinopharm	
Age_group	<=60 year	Count	30	27	29	86
		% within Age_group	34.9%	31.4%	33.7%	100.0%
		% within vaccine_type	60.0%	77.1%	76.3%	69.9%
		% of Total	24.4%	22.0%	23.6%	69.9%
	>60 year	Count	20	8	9	37
		% within Age_group	54.1%	21.6%	24.3%	100.0%
		% within vaccine_type	40.0%	22.9%	23.7%	30.1%
		% of Total	16.3%	6.5%	7.3%	30.1%
Total		Count	50	35	38	123
		% within Age_group	40.7%	28.5%	30.9%	100.0%
		% within vaccine_type	100.0%	100.0%	100.0%	100.0%
		% of Total	40.7%	28.5%	30.9%	100.0%

In addition, sex of participants was shown not to be associated with the type of vaccine taken (P>0.05) as shown in table 2.

Table 2: The count and percentage of male and female according to vaccine type.

P=0.25			vaccine_type			Total
			Pfizer	Astrazeneca	Sinopharm	
Sex	Males	Count	26	24	20	70
		% within Sex	37.1%	34.3%	28.6%	100.0%
		% within vaccine_type	52.0%	68.6%	52.6%	56.9%
		% of Total	21.1%	19.5%	16.3%	56.9%
	Females	Count	24	11	18	53
		% within Sex	45.3%	20.8%	34.0%	100.0%
		% within vaccine_type	48.0%	31.4%	47.4%	43.1%
		% of Total	19.5%	8.9%	14.6%	43.1%
Total		Count	50	35	38	123
		% within Sex	40.7%	28.5%	30.9%	100.0%
		% within vaccine_type	100.0%	100.0%	100.0%	100.0%
		% of Total	40.7%	28.5%	30.9%	100.0%

Observantly, the concentration of neutralizing IgG antibodies ug/ml was shown to be borderline higher in younger age group (<=60 year) than in older age group (>60 year) (P=0.053), as shown in table 3, figure 1, 2.

Table 3: The mean rank and median values along with the P values of concentration of neutralizing antibodies in age group <=60 versus >60 year.

	Age group	N	Mean Rank	Median	P value
nAb_ concentration_ ug_ml	<=60 year	86	57.33	4	
	>60 year	37	72.85	3.85	0.056
	Total	123			

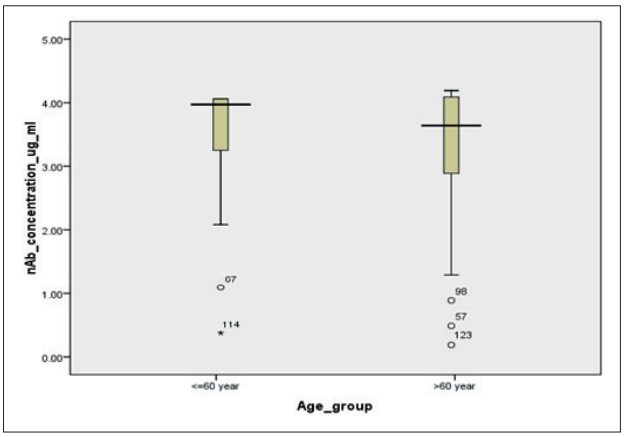

Figure 1: A box-plot shows the median, upper and lower quartiles of the neutralizing antibody concentration in age group =<60 versus >60.

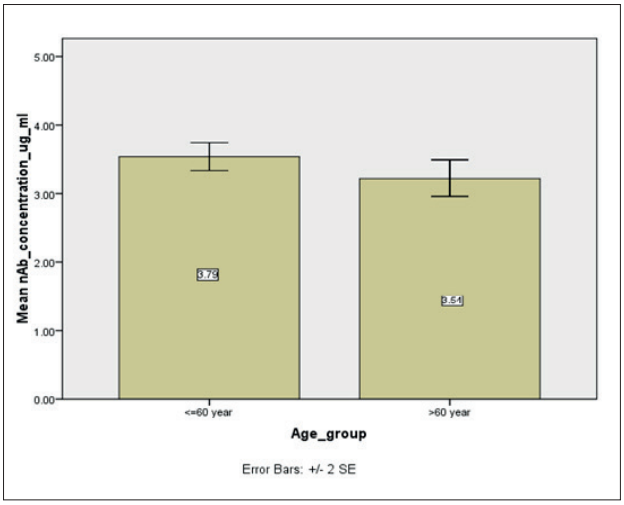

Figure 2: The mean±2SE values of neutralizing antibody concentration in age group =<60 versus >60 years.

Regarding sex, neutralizing antibodies concentration in plasma was shown to be not significantly different between male versus female sex groups (P >0.05), as shown in table 4, figure 3.

Table 4: The mean rank and median values along with the P values of concentration of neutralizing antibodies in male versus female sex.

Mann Whitney test					
	Sex	N	Mean Rank	Median	P value
nAb_concentration_ ug_ml	Males	70	62.24	4.1	
	Females	53	61.68	4	0.93
	Total	123			

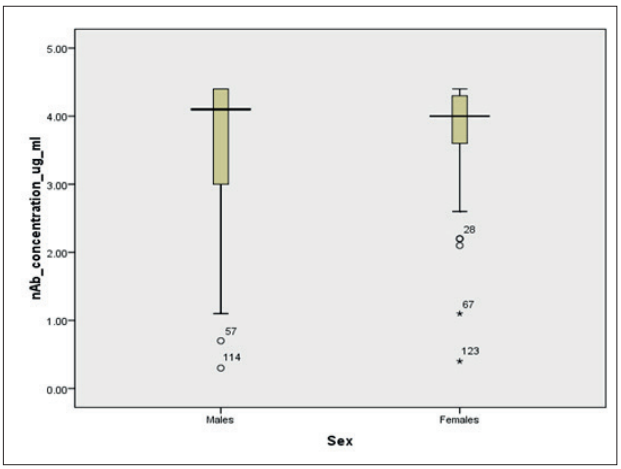

Figure 3: A box-plot shows the median, upper and lower quartiles of the neutralizing antibody concentration in male versus female sex.

As expected, the group of participants with comorbidities was with higher age median, than those without comorbidities (P<0.05). This study findings did not show any significant difference in the serum level of neutralizing IgG antibodies between participants with and without comorbidities (P>0.05), as shown in table 5.

Vaccine induced humeral immunity at different time interval

Additionally, the serum level of neutralizing IgG antibodies, which represent the humeral immunity to SARS-CoV-2, was shown to be far higher in 1-month than in 8-month post-2nd dose vaccination groups (P<0.0001), as shown in table 6, figure 4, 5.

Table 5: The mean rank and median values along with the P values of concentration of neutralizing antibodies in participants with and without comorbidities.

Comorbidity		N	Mean Rank	Median	P value
Age(years)	No	99	55.47	41	
	Yes	24	88.92	61	<0.0001
	Total	123			
nAb_ concentration_ ug_ml	No	99	61.44	4	
	Yes	24	64.29	4.1	0.72
	Total	123			

Table 6: The mean rank and median values along with the P values of concentration of neutralizing antibodies in 1month versus 8months duration post 2nd vaccine dose.

Mann-Whitney					
		N	Mean Rank	Median	P value
nAb_ concentration_ ug_ml	1 month	47	80.16	4.3	
	8 months	76	50.77	3.6	<0.001
	Total	123			

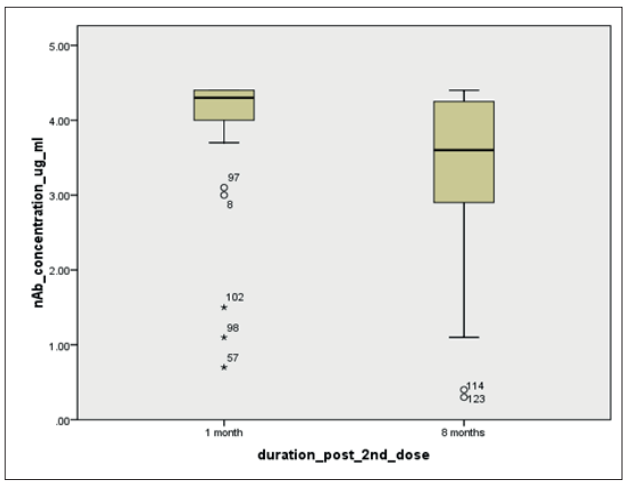

Figure 4: A box-plot shows the median, upper and lower quartiles of the n Ab concentration in 1month versus 8months duration post 2nd vaccine dose.

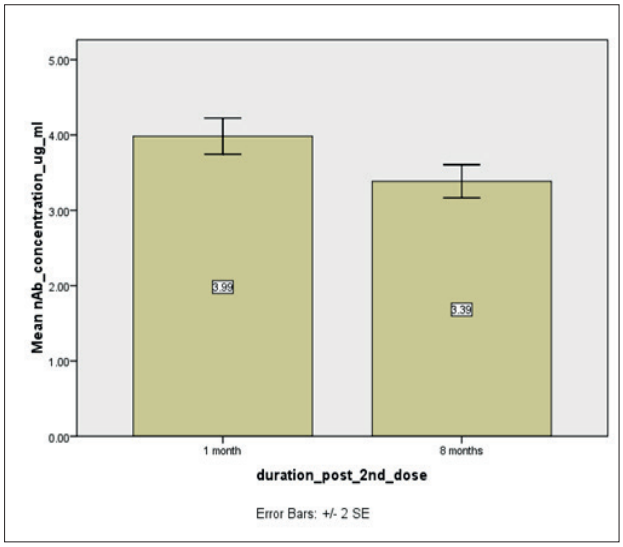

Figure 5: The mean±2SE values of neutralizing antibody concentration in 1month versus 8months duration post 2nd vaccine dose.

Vaccine induced humoral neutralizing immunity considering the vaccine type

The serum level of neutralizing IgG antibodies, Pfizer group revealed the highest level compared to AstraZeneca and Sinopharm groups (P<0.05); the Sinopharm showed trend of higher levels of neutralizing antibodies than AstraZeneca but without reaching statistical significance (P>0.05), as shown in table 7, figure 6, 7.

Table7: The mean rank and median values along with the P values of concentration of neutralizing antibodies in Pfizer versus AstraZeneca versus Sinopharm vaccines.

Kruskal- Wallis test					
Vaccine type		N	Mean Rank	Median	P value
	Pfizer	50	66.76	53	
	AstraZeneca	35	59.31	45	0.46
	Sinopharm	38	58.21	44.5	
	Total	123			
nAb_ concentration_ ug_ml	Pfizer	50	72.83	4.3	
	Astrazeneca	35	54.97	3.7	0.019
	Sinopharm	38	54.22	3.95	
	Total	123			

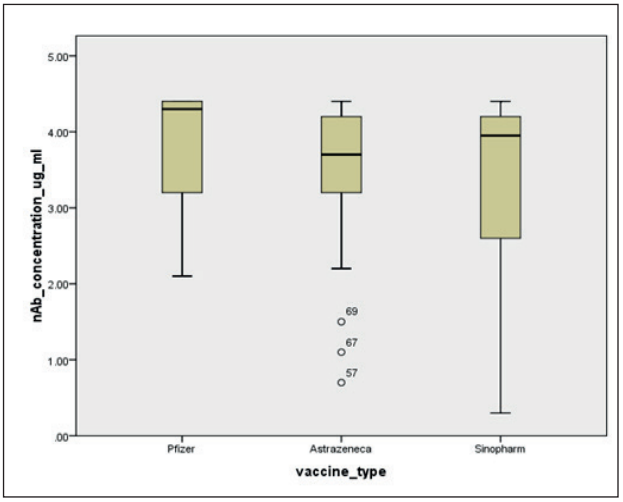

Figure 6: A box-plot shows the median, upper and lower quartiles of the n Ab concentration in Pfizer versus AstraZeneca versus Sinopharm vaccines.

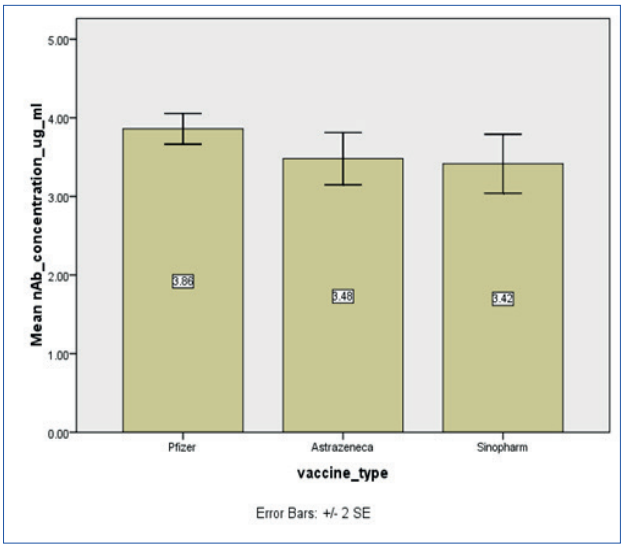

Figure 7: The mean±2SE values of neutralizing antibody concentration in Pfizer versus AstraZeneca versus Sinopharm vaccines.

Vaccine induced humoral immunity considering study group

By using Kruskal Wallis test, for IgG anti-RBD neutralizing antibodies concentration ug/ml in 1month and 8months post vaccination, it was shown that the median levels were significantly different among the study groups (P<0.01). It was found that Pfizer then AstraZeneca, then Sinopharm induced the highest median levels of neutralizing antibodies 1month post vaccination, respectively (P<0.05); by contrary, for 8 months post vaccination, Sinopharm, then, Pfizer, and AstraZeneca induced highest levels of neutralizing antibodies, respectively (P<0.05). Altogether, the current findings reveal that Pfizer vaccine, then AstraZeneca, then Sinopharm are the best ones for inducing high neutralizing antibodies shortly after the vaccination; nevertheless, AstraZeneca proved to be short in preserving good level of neutralizing antibodies after 8 months of vaccination while the best vaccine found to preserve highest levels of neutralizing antibodies by month 8 was Sinopharm then Pfizer. As shown in table 8, figure 8, 9.

Table 8: The mean rank and median values along with the P values of concentration of neutralizing antibodies in Pfizer (1and 8 months) versus Astrazeneca (1and 8months) versus Sinopharm (1and 8 months).

Study_group		N	Mean Rank	Median	P value
nAb_concentration_ug_ml	Pfizer 1 month post vaccination	22	95.36	4.4	
	Pfizer 8 months post vaccination	28	55.12	3.6	
	Astrazeneca 1 month post vaccination	8	82.69	4.3	
	Astrazeneca 8 months post vaccination	27	46.76	3.4	<0.001
	Sinopharm 1 month post vaccination	17	59.29	4	
	Sinopharm 8 months post vaccination	21	50.12	3.7	
	Total	123			

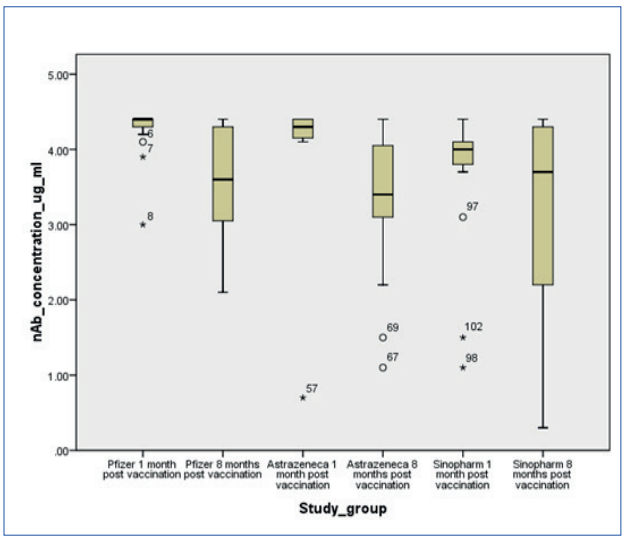

Figure 8: A box-plot shows the median, upper and lower quartiles of the n Ab concentration in Pfizer (1and 8 months) versus Astrazeneca (1and 8months) versus Sinopharm (1and 8 months).

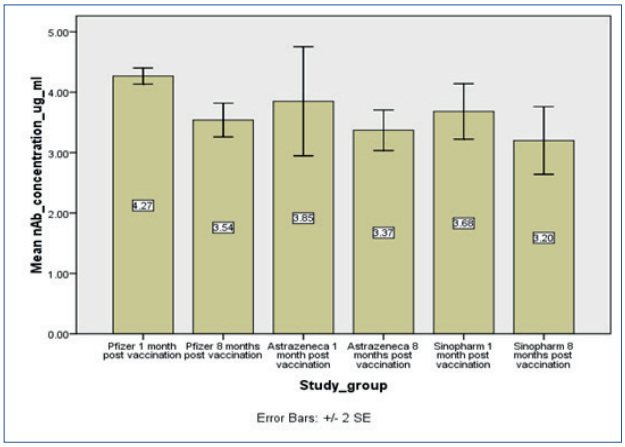

Figure 9: The mean±2SE values of nAb concentration in Pfizer (1and 8 months) versus Astrazeneca (1and 8months) versus Sinopharm (1and 8 months).

Discussion:

In contrary to the disparity in COVID-19 infection clinical outcomes based on sex as a biological variable as females tend to experience less severe disease than males [14]; In similarity with other studies our findings showed that COVID-19 vaccine responses and efficacy rates were almost comparable between the two sexes [15].

As age significantly determines the clinical features and prognosis of COVID-19 which was worse in patients older than 60 years, revealing that age is not just a number. Hence; the concept of immune senescence is particularly relevant within the context of the declared pandemic [(16)]. Several studies have provided evidence that antibody level and antibody quality are both diminished in older adults as compared to younger adults, well, but this is not true for all vaccines; vaccines that are more effective in older adults utilize several strategies including: 1) altering administration route, 2) increasing vaccine dose and 3) using vaccine adjuvants [17], as such our findings showed that vaccination potential might be insignificantly associated with age.

A study was done in Italy focused on the tremendous impact of comorbidities precisely on the elderly people since older adults confounding higher rates of underlying health conditions [(18)], which lead to decreasing of vaccine immunogenicity particularly poor antibody response; however, the current study did not show a clear association between comorbidities and vaccine-induced humeral response; this might be attributed to the fact that the vaccines trialed in this study are tailored particularly for elderly, or maybe the sample of size of this study was not sufficient to detect divergence in response to vaccines between elder and younger subjects.

Dual vaccination with Pfizer resulted in an observed maximum neutralizing antibody response at one month followed by a sharp decline by month 8; Evangelos, et al., found that there was sustained humoral immunity with a statistically significant decline thereafter up to 9 months [(19)]. For vaccination with AstraZeneca, there were an initial substantially lower specific nAb responses at month 1 than in Pfizer, but these responses were more durable and persisted at month 8. Our findings indicated that Sinopharm vaccine at 1month of vaccination elicited moderate antibody levels compared to very high levels following two doses of Pfizer then decay gradually with time.

The three vaccines studied behaved in some aspects quite differently and in other aspects behaved similarly. All of them revealed a clear decline in the humeral immunity over 8 months post-vaccination. This was in harmony with several previous studies [20-22]. this is explained by the fact that Coronaviridae family have the tendency to induce short-to midterm memory B cells and SARS-CoV-s is not an exception. As known, humeral immunity is the only arm considered as protective immunity [(23)]. Nevertheless, the current study found that Pfizer vaccine elicit nAbs more efficiently than AstraZeneca and Sinpharm did. This is can be attributed to the novel platform design of this vaccine which help translate mRNA of RBD domain in a robust and quick manner [24]. Anyway, AstraZeneca and Sinopharm performed similarly well in eliciting nAbs and they generated quite enough level of nAbs. In fact, Pfizer and AstraZeneca elicited nAbs at quite close levels in both 1 and 8months interval while Sinopharm lagged behind in eliciting nAbs in 1month interval but Sinopharm compensated that shortage at 8month interval where nAbs level of Sinopharm became comparable to that of Pfizer and Astrazeneca. This indicated several notions: First, Pfizer and Astrazeneca vaccine are potently inducing humeral immunity weeks after the second dose while Sinopharm lags behind in this completion indicating long-time production process. Second, the rate of decline of of nAbs level by Sinopharm was shown to be significantly slower than Pfizer and Astrazeneca vaccines. This might be explained when comparing vaccine designs and platforms, a potential advantage of inactivated vaccines over other vaccine types is that they comprise all viral structural proteins

which may induce a broader spectrum of immunity in addition to NAbs against RBD, this means more epitopes, especially those conserved epitopes in proteins other than spike engaged [25], typically, make the vaccine more durable trigger. This was seen as well by other studies [26,27], while other studies contradicted this observation [28,29]. Taken together, we observed that better sustained levels of neutralizing response at month8 might be elicited with Sinpharm than in Pfizer and AstraZeneca. As such, neutralizing humoral immunity were shown to be significantly different among the study groups.

It is quite known that cellular immunity of Coronaviruses do not fade easily and persist for maybe decades [30]; However, a question might be laid then why the humeral immunity is not augmented as well? The answer might be because of the resurgence of variants of concern that show some level of changes in epitopes recognized by nAbs but not quite same variations in the epitopes recognized by cell mediated immunity.

Conclusions and Recommendations:

The societal value of safe and effective COVID-19 vaccines is enormous. We can conclude from the current study that Pfizer, AstraZeneca and Sinopharm vaccines were shown to be quite effective in eliciting humoral immunity and was robustly activated against SARS-CoV-2 from two doses as early as 1 month. The neutralizing humeral immune response induced by the studied vaccines was shown to last up to 8 months after the second dose but at significantly reduced level.

The level of immune response by the vaccines studied did not correlate with age, sex and comorbidities of the vaccinated individuals.

Vaccine design platforms seem to play a crucial role in vaccine effectiveness and how far this effectiveness can be sustained.

We recommend that COVID-19 vaccines with high immune response should be encouraged in Iraqi vaccination campaigns, and further studies are recommended for more follow up of the vaccine effectiveness and protection against the variants of concern of SARS-CoV-2 in Iraq.

Further studies are recommended for the detection and quantification of the IgA neutralizing antibodies in Iraqi vaccinated subjects. It is recommended to conduct studies to monitor COVID-19 vaccines effectiveness in age younger than 18 years and even in children.

There is no conflict of interest

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Authors contributions: Prof. Dr. Ahmed Sahib and Furqan Mohammed.

Data gathering: Furqan Mohammed

Data gathering and idea owner of the study: Furqan Mohammed and Prof. Dr.Ahmed Sahib

Writing and submitting manuscript: Furqan Mohammed

Editing and approval of final draft: Furqan Mohammed and Prof. Dr. Ahmed Sahib.

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