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The Comparison of Antibody Responses to Natural COVID-19 Infection and Vaccination

Conflict of interest: nothing to declare.
Authors' contribution: Chmagh A.A. – conceptualization, methodology, formal analysis, investigation, resources, data curation, writing – original draft; Salman B.D. – investigation, resources, data curation, writing – original draft; Abdulridha L.A. – conceptualization, formal analysis, investigation, resources.
Ethics statement. This study was approved by Department of Medical Laboratory Techniques, College of Health and Medical Technology, Southern Technical University (No. 7/27/1225 in 7/10/2021; and No. 7/27/1368 in 1/11/2021) and assurance by College of Health and Medical Technology, Southern Technical University (No. 7/27/607 in 11/4/2023). The study takes place at Al-Sadder Teaching Hospital.
Ethical committee: Department of Medical Laboratory Techniques, Health and Medical Technical College, Southern Technical University.
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Informed consent. All participants signature written consent form when included in this study.
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Abstract

Introduction. Antibody responses are an important part of the immunity after SARS-CoV-2 vaccination.
Purpose. To investigate antibody responses and associates of protection after vaccination with the Oxford/AstraZeneca ChAdOx1, BioNTech BNT162b2, and Sinopharm vaccines.
Materials and methods. Blood samples (2 ml) were collected from 122 participants and the serum was obtained. All the immunological and enzymatic reactions were done using the mini VIDAS device.
Results. In 61 participants, the BioNTech BNT162b2 vaccine was found to produce higher antibody levels compared with the levels produced by the Oxford/AstraZeneca ChAdOx1 and Sinopharm vaccines. Females had higher antibody levels following vaccination. Prior infection substantially increased antibody levels in the vaccinated group.
Conclusion. The stated increase in the number of antibodies detected in the blood serum was accompanied by an increase in the body's immune defence against infection after vaccination.
Keywords: vaccination, comparative evolution, antigen processing, retroviral, COVID-19

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Сравнение состояний гуморального иммунного ответа на естественную инфекцию COVID-19 и проведение «противоковидной» вакцинации

Конфликт интересов: не заявлен.
Вклад авторов: Чмаг А.А. – концепция, методология, проведение исследования, ресурсы, обработка данных, анализ полученных результатов, написание исходного текста статьи, программное обеспечение; Салман Б.Д. – проведение исследования, ресурсы, обработка данных, написание исходного текста статьи; Абдулрида Л.А. – концепция, анализ полученных результатов, проведение исследования, ресурсы.
Этическое заявление. Настоящее исследование одобрено кафедрой медицинской лабораторной техники Колледжа здравоохранения и медицинских технологий Южного технического университета (№ 7/27/1225 от 10.07.2021 и № 7/27/1368 от 11.01.2021), а также заверено Колледжем здравоохранения и медицинских технологий Южного технического университета (№ 7/27/607 от 4.11.2023). Исследование проходит в клинической больнице Аль-Саддер.
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Информированное согласие. Все участники подписывают информированное согласие при включении в это исследование.
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Резюме

Введение. Гуморальный иммунный ответ является важной составляющей формирования иммунитета после вакцинации против SARS-CoV-2.
Цель. Исследовать состояние иммунного ответа и защитные свойства антител после применения вакцин Оксфорд/АстраЗенека ChAdOx1, БиоНтех BNT162b2 и Синофарм.
Материалы и методы. Для исследования были отобраны образцы крови (по 2 мл) 122 испытуемых с последующим получением сыворотки. Все иммунологические и ферментативные реакции проводили с использованием прибора mini VIDAS.
Результаты. У 61 испытуемого после применения вакцины БиоНТех BNT162b2 уровень антител превышал уровень антител после применения вакцин Оксфорд/АстраЗенека ChAdOx1 и Синофарм. У женщин уровень антител после вакцинации был выше, чем у мужчин. Ранее перенесенная инфекция вызывала значительное повышение уровня антител в группе вакцинированных.
Заключение. Констатированное увеличение количества выявленных в сыворотке крови антител сопровождалось усилением иммунной защиты организма от инфекции после проведенной вакцинации.
Ключевые слова: вакцинация, сравнительная эволюция, процессирование антигена, ретровирусный, COVID-19

■ INTRODUCTION

At the end of 2019, a series of pneumonia cases with an unknown causative agent emerged in Wuhan (Hubei, China). In January 2020, analysis of samples from the lower respiratory tract revealed the presence of a novel virus. A few weeks later, deep sequencing analysis identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as the cause for the observed viral pneumonia cases. In a preliminary report, complete viral genome analysis detected that the virus was more distant from SARS coronavirus [1]. Subsequently, in February 2020, the World Health Organization (WHO) termed the disease triggered by SARS-CoV-2 as COVID-19 [2]. By March 2020, the WHO announced the pandemic status of COVID-19 when the number of countries involved was increased with more cases and higher death rates [2].

Coronaviruses are enveloped single-stranded ribonucleic acid viruses known for their solar corona-like shape due to the presence of surface spikes [3]. Coronaviruses have four major structural proteins encoded by the coronavirus genome; these consist of spike protein (S), envelope protein (E), matrix protein (M), and unexposed nucleocapsid protein (N). Among them, spike protein (S) is a key protein that interacts with angiotensin-converting enzyme 2 receptors and mediates subsequent fusion between the envelope and the host cell membrane to facilitate viral entry into host cells [4, 5].

Vaccination against SARS-CoV-2 is an important strategy to change the course of the COVID-19 pandemic [6]. Given its role in mediating viral entry, the coronavirus S protein is the main target of neutralizing antibodies and has been the focus of therapeutic and vaccine design [5].

The global response to reduce the spread of COVID-19 pandemic resulted huge health, economic and social consequences. Based on the urgent need to respond to the calamity, clinical trials and epidemiological studies have been started, and due to the presence of different types of vaccines and their distinctive characteristics to prompt specific immunity, in this study, we aimed to investigate antibody responses and associates of protection after vaccination with the Oxford/AstraZeneca ChAdOx1, BioNTech BNT162b2, and Sinopharm vaccines.

■ PURPOSE OF THE STUDY

To investigate antibody responses and associates of protection after vaccination with the Oxford/AstraZeneca ChAdOx1, BioNTech BNT162b2, and Sinopharm vaccines.

■ MATERIALS AND METHODS

Sample collection

Blood samples (2 ml) were collected from 122 participants (74 males and 48 females). Overall, 46 males received SARS-CoV-2 vaccines, whereas only 15 females received SARS-CoV-2 vaccines and blood samples of vaccinated peoples were collected 4 weeks after the second dose. Of the 61 participants who received SARS-CoV-2 vaccines, 46 participants did not have prior infection, and 15 participants had prior infection. The samples were collected from 11st October 2021 to 11st December 2021, and the serum was obtained for antibody level measurement.

Inclusion criteria

- Patients without chronic diseases.
- Patients received with two doses of vaccines.

- Blood samples were collected 3–6 months after the second dose.
- Comfortable for the study.

Exclusion criteria

- Patients have chronic illness.
- Pregnancy.
- Patients with cancer.
- Unvaccinated subjects.
- Patients received one dose of vaccine only.
- Blood samples below three months and/or more than six months.
- Loss of follow-up patients.

The mini VIDAS device

The mini VIDAS device is one of the most advanced devices for viral analysis, characterized by accurate results and speed of performance. Although, there are other more advanced devices, the mini VIDAS device (bioMérieux S.A., France, Cat. No.: C130-3567L) and VIDAS® SARS-COV-2 IgG (9COG, bioMérieux S.A., France, Cat. No.: 124472) had been used extensively.

The basis of this device is primarily established on the interplay between two components: the coated SPR® receptacle. Including antigens or antibodies, and the Strip, which made up of a chain of wells containing the correct amount of reagent necessary for the test. In addition, all reactions happen in two key phases within the SPR: enzymatic reaction and immunological reaction.

The whole operation is totally automated from incubation to washing and the ultimate reading. The whole operation is totally automated, from incubation, to washing and final reading. The time of incubation and washing cycles number are improved to make sure of their high performance using two-step sandwich enzyme immunoassay method with a final fluorescence detection. The amount of samples needed 100µL of serum or plasma. The number of replicates 2 (can be repeat each 27 minutes). The interpretation of results according to (i=index) are if i <1 negative (no detection of IgM/ IgG anti SARS-CoV-2) or if i ≥1 positive (detection of IgM/ IgG anti SARS-CoV-2).

Action steps:

1 – The serum is placed in a tape called a strip. This strip contains 10 holes, which are as follows:

- 1 = sample well;
- 2, 3, 4, 5 = empty wells;
- 6 = conjugate well;
- 7, 8, 9 = wash buffer;
- 10 = substrate.

The second component, which is a small conical tube and it acts as a straw, that is called a SPR.

2 – The SPR includes the anti-antibody, which sucked the serum in the first hole. There is a reaction of the antigen in the serum with the antibody in hole 6, which is later on connects with the enzyme.

3 – Of course all the particles are not interacted. The washing solution from one of the holes (7, 8, 9) is absorbed by the SPR. The washing solution returned to one of the

empty holes (2, 3, 4, 5), so that the antigen and antibody attached to the SPR are kept. This process is repeated many times.

4 – A fluorinated substance is found in the last hole (Fluorescence substrate), which is developed.

5 – SPR components are interacted with the fluorinated substance. The substrate, which linked enzyme is only remained in the measuring cell and flash lamp is lit.

According to the phosphorescent light theory, these particles take 370J and emit 450J from energy, which is recorded by the device in the form of the results for analyzes, and here the analysis ends (www.biomerieux-diagnostics.com; https://5.imimg.com/data5/RW/LW/MQ/SELLER-4682066/biomeriux-minividas-blue-immunossay-analyzer.pdf).

Statistical analysis

Statistical analysis of the data done by used Statistical Package for the Social Sciences V24. (SPSS Inc., Chicago, IL, USA). All experimental data were expressed as mean± standard deviation (SD). Results were analysed by Chi-square test to compared between groups, Mann – Whitney U test to analysed relation between antibody levels and vaccine types, and Kruskal – Wallis test to compared between antibody levels and other variables. $P<0.05$ was considered statistically significant.

■ RESULTS

A total of 122 individuals were enrolled in this study. The majority of the participants were males (60.7%), and 46 of them (75.4%) received SARS-CoV-2 vaccines. On the other hand, 48 (39.3%) of the participants were females, and 15 of them (24.6%) received SARS-CoV-2 vaccines. Overall, 61 participants received SARS-CoV-2 vaccines; among them, there were 46 participants without prior infection (28.06%) and 15 participants with prior infection (9.15%). The SARS-CoV-2-specific IgG response was significantly higher in vaccinated individuals than in unvaccinated individuals (27.34±17.97 and 18.05±15.78, respectively, p=0.006) (Table 1).

Of the 61 participants, 7 of them (11.47%) received the Oxford/AstraZeneca ChAdOx1 vaccine, 17 of them (27.86%) received the Sinopharm vaccine, and 37 of them (60.65%) received the Pfizer-BioNTech BNT162b2 vaccine. Participants who received the Pfizer-BioNTech BNT162b2 vaccine had a higher antibody level (38.41 μg/ml (34.60–50.69)) compared with the levels of those who received the Oxford/AstraZeneca ChAdOx1 and Sinopharm vaccines (38.20 μg/ml (6.99–52.57) and 4.02 μg/ml (0.02–46.23),

Table 1
Gender distribution among the vaccinated and unvaccinated groups

		Vaccination status			
		Vaccinated	Unvaccinated	Total	p value
		No. (%)			
Sex	Male	46 (75.40)	28 (45.90)	74 (60.65)	0.001
	Female	15 (24.60)	33 (54.10)	48 (39.35)	
IgG response (μg/ml) (Mean±SD)		27.34±17.97	18.05±15.78	–	0.006

Table 2
Antibody levels (μg/ml) in the vaccinated group according to the type of vaccine

Category		Antibody level		
		Mean±SD	Median (Min–Max)	p value
Type of vaccine	Oxford/AstraZeneca ChAdOx1	34.50±14.65	38.20 (6.99–52.57)	0.643
	Sinopharm	8.89±11.46	4.02 (0.02–46.23)	
	Pfizer-BioNTech BNT162b2	40.53±7.16	38.41 (34.60–50.69)	

Table 3
Antibody levels (μg/ml) in the vaccinated male and female groups according to the type of vaccine

Sex	Category	Type of vaccine			p value
		Sinopharm	Pfizer	AstraZeneca	
Male	Mean±SD	9.72±11.93	33.82±15.08	21.56±16.97	0.024
	Median (Min–Max)	6.54 (0.02–46.23)	37.13 (6.99–52.27)	16.00 (2.48–63.33)	
Female	Mean±SD	2.28±0.40	36.78±13.69	40.53±7.16	
	Median (Min–Max)	2.28 (2.00–2.57)	42.75 (14.61–52.57)	38.41 (34.60–50.69)	

Table 4
Antibody levels (μg/ml) in the vaccinated group according to prior infection status

Vaccinated group	Mean±SD	Median (Min–Max)	p value
Without prior infection	25.43±18.13	23.74 (0.02–52.27)	0.16
Prior infection	33.18±16.68	36.60 (2.00–52.57)	

respectively) (Table 2). Although not statistically significant, the antibody level was higher among participants who received the Pfizer-BioNTech BNT162b2 vaccine than among those who received the Oxford/AstraZeneca ChAdOx1 vaccine (40.53±7.16 vs. 34.50±14.65, respectively, p=0.643).

In comparison with males, females had statistically significant elevated IgG levels for the Pfizer-BioNTech BNT162b2 and Oxford/AstraZeneca ChAdOx1 vaccines (36.78±13.69 and 40.53±7.16, respectively, p=0.024) (Table 3). Prior infection significantly increased the antibody level of the vaccinated group (Table 4).

■ DISCUSSION

Understanding the kinetics of the post-vaccine antibody response and vaccine-associated characteristics that determine the variability of this response is crucial for evaluating the effect of SARS-CoV-2 vaccines on patients [7].

A typical vaccination course against SARS-CoV-2 involves the delivery of a vaccine in two dosages. When the vaccines were tested for the first time, it was found that vaccination with a single dosage elicited a weak immune response. Therefore, it is necessary to deliver a second dosage for a strong antibody response. In phase one clinical trial of the Pfizer-BioNTech BNT162b2 vaccine, both dosages of the vaccine were highly effective in preventing infection following administration [8]. Furthermore, the Pfizer-BioNTech mRNA vaccine (BNT162b2) was discovered to be highly effective against COVID-19 patients who develop symptoms with an efficacy rate of 94% in a randomised clinical trial [9]. Consistent with our findings, Wei et al. [3] reported that those receiving

the Pfizer-BioNTech BNT162b2 vaccine had significantly higher antibody levels compared with the levels of those receiving the Oxford/AstraZeneca ChAdOx1 vaccine [10]. Females had elevated IgG levels for both vaccines, consistent with commonly described improved immune responses in females [10–12]. The antibody response after vaccination differed significantly according to prior infection condition. Prior infection was accompanied with a substantially elevated antibody level for both BioNTech BNT162b2 and Oxford/AstraZeneca ChAdOx1 vaccination. This is in agreement with studies involving healthcare workers showing that prior SARS-CoV-2 infection leads to higher antibody levels after the second BioNTech BNT162b2 vaccines [13, 14]. Vicenti et al. [15] similarly stated that a single dosage of the BioNTech BNT162b2 vaccine prompted post-vaccination antibody levels that were equal to or higher than the levels of those obtained two dosages who were without prior infection [14].

■ CONCLUSION

The stated increase in the number of antibodies detected in the blood serum is accompanied by an elevation in the body's immune defence against infection after vaccination. The main limitation of this study was the inadequate sample size, which could be observed as insufficient to state that prior infection may contribute to a high antibody level. Nevertheless, there is still a possibility that prior infection with SARS-CoV-2 may boost antibody levels even at the first dose of the BioNTech BNT162b2 vaccine.

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