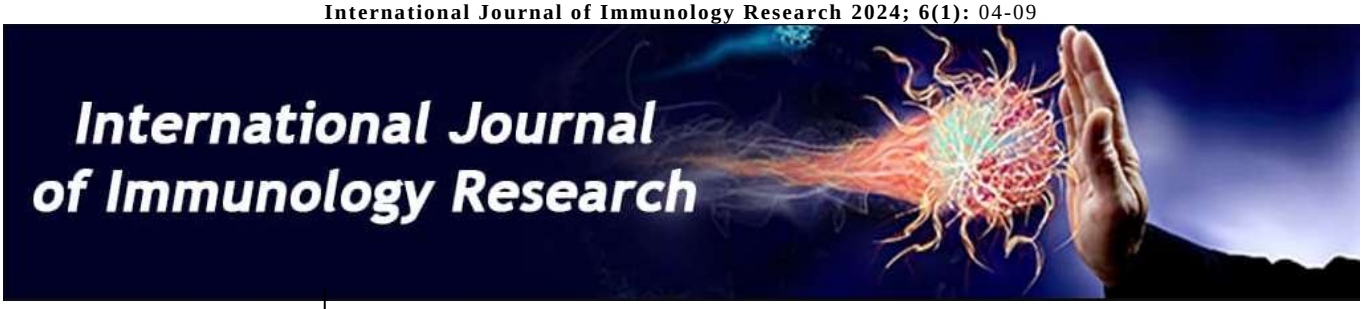

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Evaluation of human vaccination Pfizer, Astrazeneca and Sinopharm vaccine in Iraq / Najaf

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Abstract
Background: Studying the immune response to different COVID-19 vaccines is crucial for refining vaccine selection and dosage worldwide. The aim of the study is to measure the concentration IgG antibody of the human vaccinated, which include (Pfizer, AstraZeneca, and Sinopharm vaccinations) and to find out which vaccine is more efficient by RT-qPCR technology. It highlights risk factors like age and sex. The cross-sectional study took place between November 25, 2021, and January 25, 2022.
Methods: Ethical approval was secured, and consent was obtained from participants. Serum samples from eighty-six vaccinated individuals were analyzed using RT-qPCR to measure IgG antibodies.
Results: In our study of eighty-six cases, fifty tested positive for COVID-19, comprising thirty-eight females and twelve males. Monitoring occurred from the second to the seventh-month post-vaccination, with IgG titers measured via RT-qPCR. We observed higher vaccine efficacy in females compared to males. Age groups were categorized as follows: 19-29, 30-40, 41-51, and 52-62 years old. The 19-29 age group showed the highest proportion vaccinated with Pfizer, AstraZeneca, and Sinopharm vaccines. IgG concentration after 7 months was significantly higher than in earlier months. Further, IgG levels were lower in participants vaccinated with Sinopharm ($p<0.0001$) and AstraZeneca ($p<0.01$) compared to Pfizer.
Conclusion: Participants who received the Pfizer vaccine demonstrated the highest antibody concentration relative to other vaccine recipients. There was observed higher vaccine efficacy among females. Notably, the 19-29 age group represented the largest proportion of individuals vaccinated with various vaccines. Consequently, this study holds significant potential for optimizing vaccine selection and dosage. Further investigations are warranted to delve deeper into these initial findings.

Keywords: SARS-CoV-2, Pfizer, AstraZeneca, sinopharm, IgG

Introduction
The emergence of SARS-CoV-2 was first reported in China towards the end of 2019, rapidly evolving into a global pandemic that affected numerous countries. On March 11, 2020, the World Health Organization (WHO) officially declared the COVID-19 pandemic. In the months following this declaration, several candidate vaccines, including those developed by Pfizer, AstraZeneca, and Sinopharm, emerged [1]. Each of these vaccines targets specific components of the coronavirus. SARS-CoV-2, like other coronaviruses, is an enveloped virus with a single-stranded, positive-sense ribonucleic acid (RNA) genome (~30,000 nucleotides), and featuring spike proteins on its surface [1]. The Pfizer-BioNTech vaccine (COMIRNATY) is an mRNA vaccine encoding the viral spike S glycoprotein of SARS-CoV-2. It induces a protective immune response, reducing the likelihood of future COVID-19 cases [2, 3]. Vaxzevria (formerly COVID-19 Vaccine AstraZeneca) consists of a chimpanzee adenovirus modified to carry the spike S1 gene (ChAdOx1-S). It is produced using genetically engineered human embryonic kidney (HEK) 293 cells and recombinant DNA technology [4]. Sinopharm, developed in China, is an inactivated vaccine composed of viruses treated using physicochemical methods to reduce their pathogenicity [5].
The primary objective was to develop a vaccine effective in preventing SARS-CoV-2 infection [6], with the expectation that COVID-19 vaccination would decrease hospitalizations and deaths attributed to SARS-CoV-2. Clinical trials of mRNA vaccines demonstrated efficacy rates of 92-95% in preventing COVID-19 infection [6, 7]. Following vaccination, the immune system produces antibodies against the inactivated viruses, priming itself for future encounters. However, vaccine development can lead to side effects [8],

highlighting the importance of identifying and reporting adverse reactions. Common side effects associated with vaccination include fever, redness, swelling at the injection site, and, in rare instances, anaphylaxis [8].

Iraq was severely affected by the pandemic, with over one million confirmed cases and more than fifteen thousand deaths attributed to COVID-19. Vaccination efforts in Iraq commenced in March 2021, with individuals receiving vaccines through electronic registration at hospitals [9]. The available vaccines included Sinopharm, Oxford-AstraZeneca, and Pfizer-BioNTech. Despite the promising results of COVID-19 vaccines, uncertainty persists regarding optimal immunization strategies tailored to specific communities [9].

In human SARS-CoV-2 serological assessments, the primary focus has been on three antibody classes: IgM, IgG, and IgA [10]. However, our research specifically targets IgG, the antibody that predominates in both serum and plasma. IgG demonstrates heightened specificity compared to assays targeting IgM and IgA and becomes prominent roughly 2-3 weeks after acute infection, playing a crucial role in establishing long-lasting immune memory that may persist for several months or even years [11, 12].

Monitoring immunoglobulin G (IgG) levels post-vaccination among individuals receiving various types of vaccines can offer valuable insights into updating vaccine development, as the humoral immune response to vaccines varies significantly among individuals. Therefore, this study aims to evaluate the humoral immune response in individuals receiving different vaccinations, with a particular focus on risk variables such as age and sex. This study represents a significant advancement in Iraq. It utilizes the real-time qPCR method to analyze IgG levels in response to three vaccines. Previous research relied on diagnostic methods such as ELISA, indirect chemiluminescence immunoassay, and other serology techniques.

Further, our study contributes novel insights into COVID-19 vaccine effectiveness, immune response dynamics, and factors influencing vaccine efficacy, paving the way for informed decision-making in vaccination strategies and further research endeavours.

Materials and method ethics approval and consent to participate

The research ethical approval for the study was granted by the Ethical Committee at the University of Kufa, and informed consent obtained from the participants prior to data collection. Written informed consent form was received from each study participant. All methods were carried out in accordance with relevant guidelines and regulations.

Serum Sample

Eighty-six serum samples were collected from healthy individuals who had received the second dose of the Pfizer, AstraZeneca, and Sinopharm vaccines. Samples were collected according to the form within the ethical issues of the attached persons and with their consent. The time for measuring antibodies was determined after taking the vaccine for two doses, as is our practice in the health centers / Najaf Health Department, where a vaccine card is given to each person after taking the first dose and the date for taking the second dose is determined, so the concentration (IgG) is measured approximately after taking the second dose 45 days and more. These clear serum samples, collected in sterile, suitable containers, were labeled and stored at -20 °C. They were obtained from five age groups ranging from 19 to 62 years old. Subsequently, these samples were utilized to diagnose the IgG antibody response to the different human vaccines using real-time qPCRMethods: Primers: PCR primers are short pieces of IgG primer, usually around 20 nucleotides in length. Two primers are used in each RT-qPCR reaction, and they are designed to flank the target region (the region that should be copied).In the current study, the housekeeping gene (HKG) U6 detected in the samples increases the accuracy of the positive or negative results obtained.

Primer design

The primer design by NCBI and optimase this primers use to detect IgG antibody in Table 1.

Table 1: Primer design of IGg depending of NCBI

Type	Primer Name	Sequence	Bases	Product size
IgG	Primer F	ACTTAGGCCTGTCTGCCTGA	20	203 bp
	Primer R	GTGGAAGGATCCGTCACTGT	20	203 bp
SFRP-U6	Primer F	GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT	30 bp	135 bp
	Primer R	CTCAACCTACAATCAAAAACAACACAAACA	30 bp	

RNA Extraction

The using of kit RNA extraction [RNAsimple total RNA kit, Cat.no: 4992858.ID:DP419, TIANGEN Biotech (Beijing)].

Protocol

Real-Time qPCR Technique

Principle of RT-qPCR: The progress of DNA amplification during a Polymerase Chain Reaction (PCR) can be monitored in "real-time" (RT-qPCR) by measuring the release of fluorescent "flashes" during amplification. This technique was used to amplify IgG antibodies specific to the different human vaccines. The mixture was prepared with a final volume of 20 µl by mixing all the contents mentioned in Table 2.

PCR Master Mix Preparation

PCR master mix was prepared using Cat. No. Lot.0234845744001, Addbio, Lot 2001A, Korea, as detailed in Table 2.

Table 2: Contents of Real time (RT-qPCR) mixture

Items	Volume
Bright Green 2X qPCR	10 gl
Forward Primer (20 pmol)	1 µl
Reverse Primer (20 pmol) 1.0 gl	1 µl
Template DNA	5g l
DNase free Water	3gl
Total Volume	20

Thermo Cycler Conditions

Table 3: The thermo cycler conditions

Step	Condition
Pre-Denaturation	95 ºC, 2 min.
Denaturation	95C/30sec
Annealing	59.3 ºC, 30 sec.
Extension	72 ºC, 60.0 sec.
Step 5	Repeat steps 2-4 29 more times
Final extension	72 ºC, 5 min.
Hold	4 ºC, forever

Delta Ct equations the threshold cycle (Ct) value was determined using the following equations

ΔCt Value (Control) (ΔCtC) = Average control Ct Value (test gene) - Average experimental Ct value (housekeeping gene)

ΔCt value experimental (ΔCtE) = Average experimental Ct value (test gene) - Average experimental Ct value (housekeeping gene)

Delta Delta Ct value (ΔΔCt) = ΔCtE - ΔCtC

The fold change (ΔΔCq) = 2-ΔΔCt

Statistical Analysis

The Kruskal-Wallis test was used to assess the statistical significance of differences in IgG levels among individuals who received the Sinopharm, AstraZeneca, and Pfizer vaccines. Corrections for multiple comparisons were made using the Benjamini and Hochberg False Discovery Rate (FDR) method. GraphPad 8 software facilitated the analysis, with a significance threshold of $p< 0.05$ considered applicable in all cases.

Results

Detection of Human Vaccination with different human Vaccines

Analysis was conducted on population groups vaccinated with Pfizer, AstraZeneca, and Sinopharm vaccines, examining correlations with gender and age. Eighty-six samples were collected from individuals who had received their second dose of the vaccines three months earlier, ranging in age from 19 to 62 years. Among the samples, 50 tested positive, with thirty-eight belonging to females and twelve to males The cases were distributed across four age groups: 19-29 (19 cases), 30-40 (15 cases), 41-51 (10 cases), and 52-62 (6 cases) years. Notably, the 19-29 age group showed the highest proportion of individuals vaccinated with the different human vaccines in Table 4. Furthermore, IgG concentration after 7 months post-vaccination was notably higher compared to other months, as measured by RT-qPCR in Figure 1.

Table 4: The appearance results of age groups, sex, and type of vaccine

Interviewer	Details	Results number
Age group	19-29 years	19
	30-40 years	15
	41-51 years	10
	52-62 years	6
Sex	Female	38
	Male	12
Type vaccine	Pfizer	19
	AstraZeneca	19
	Sinopharm	12

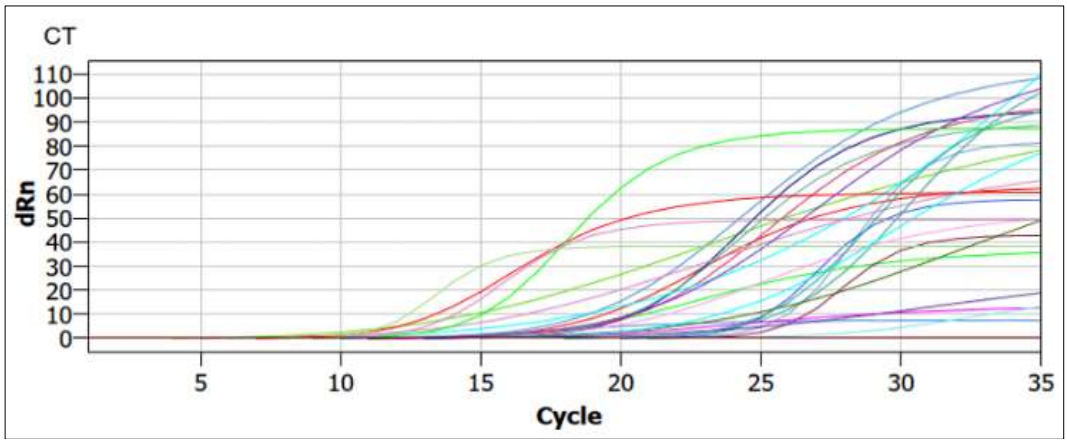

Fig 1: Diagnosis IgG concentration of human vaccinated by RT-qPCR

The comparison of immunoglobulin G concentration among the three types of vaccines reveals significant differences. As indicated in Figure 2, Sinopharm and AstraZeneca vaccines exhibited lower IgG concentrations compared to Pfizer's vaccine.

The Pfizer, AstraZeneca, and Sinopharm vaccines were evaluated for their immunoglobulin G antibody levels using Real-time qPCR six months post the second dose. An analysis of the median antibody titers following the second dose for each vaccine through a box plot showed notable statistical variances among them. The median IgG concentration was lower in participants who received the Sinopharm (35.3) and AstraZeneca (601) vaccines than in those who received the Pfizer (6417) vaccine. The dataset refers to the median levels of immunoglobulin G antibodies. The statistical significance between groups was determined using the Kruskal-Wallis test, with adjustments made for multiple testing using the Benjamini and Hochberg False Discovery Rate (FDR) method. The significance levels were denoted as follows: *$p< 0.05$, **$p< 0.01$, ****$p< 0.0001$.

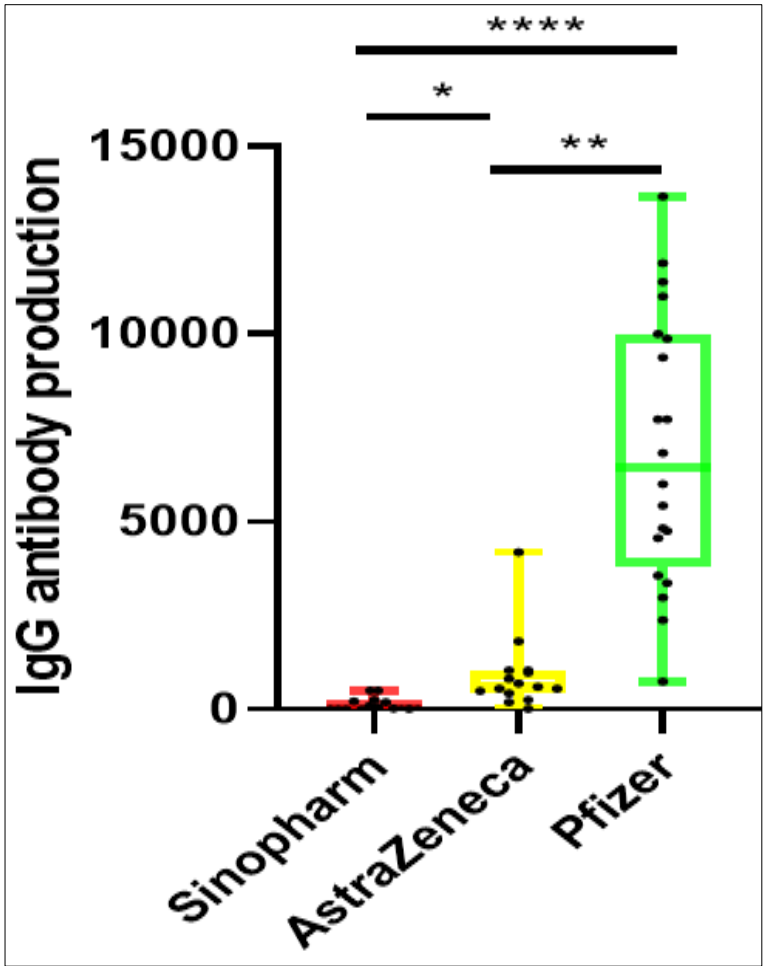

Fig 2: IgG antibody production of three vaccines including Pfizer, AstraZeneca, and Sinopharm

The concentration of IgG antibodies against the S protein was analyzed, considering factors such as the time elapsed since full vaccination, previous COVID-19 infection, age, gender, and blood type of participants. Comparative analyses revealed significant differences in post-vaccination antibody levels, primarily influenced by the type of vaccine, duration since vaccination completion, and prior SARS-CoV-2 infection.

Discussion

Our research findings indicated a higher prevalence of positive results among females, with a higher antibody concentration compared to males. This difference can be attributed to various physiological and genetic factors, which confer females with stronger acquired and innate immunity. Studies have shown that females generally exhibit stronger innate and adaptive immune responses compared to males [13].

Several factors contribute to this stronger immune response in females, including sex differences and immune response variations. Notably, research by Engler RJ and colleagues demonstrated that young women receiving half doses of the seasonal influenza vaccine generated a stronger antibody response equivalent to that of men receiving full doses. Additionally, despite higher morbidity and mortality rates during influenza pandemics, women exhibited better responses to influenza vaccination, displaying higher levels of neutralizing antibodies [14].

Sex-related differences in immunogenicity and effectiveness have also been observed for other vaccines, including Hepatitis A and B, measles, and yellow fever. Notably, the yellow fever vaccine has been reported to elicit a stronger response in females [14]. Regarding our study, the number of females is greater than the males due to the number of attached people, more than males during the research. In addition, a specific number was not determined for each gender, but rather the attached people were relied upon only according to their consent according to ethical issues.

The age group under 25 years old comprised 62.6% of the study by Hassan *et al*., 2022 [15], while those over 25 years old accounted for 37.3%. Bruna *et al*., 2021 [16], studied the COVID-19 mRNA BNT162b2 vaccine and found that the age group 31-40 years had a higher titer compared to other groups.

In the study by Peter *et al*., 2023 [17], the majority of participants were females, constituting 56.2%. The mean age of patients was 39.8±9.3 years, ranging from 23 to 60 years. Reyhaneh *et al*., 2023, reported that 72.6% of participants were female. Conversely, Hassan *et al*., 2022, showed that male cases (90) accounted for 51.7%, while female cases (84) comprised 48.2%. Bruna *et al*., 2021 [16], also observed a higher percentage of females in their study.Firstly, mRNA vaccines stimulated more robust IgG antibody production compared to vector vaccines. Serum samples from individuals vaccinated with Moderna consistently exhibited the highest antibody levels, regardless of the measurement time. mRNA vaccines demonstrated significant advantages over conventional vectored vaccines in terms of efficacy, safety, and activation of a broad spectrum of immune response components. This advantage

stems from factors such as higher immunogenicity due to improved translation efficiency and the ability to produce elevated levels of neutralizing antibodies by activating CD4+ and CD8+ T cells at relatively low doses.

Scientists have confirmed that antibody levels are several times higher after mRNA vaccination compared to vector vaccination in the initial weeks. For Pfizer, antibody levels decreased from a median of 7506 U/mL at 21-41 days to 3320 U/mL at 70 or more days, while for the AstraZeneca vaccine, levels reduced from a median of 1201 U/mL at 0-20 days to 190 U/mL at 70 or more days. According to information from the leaflet, the efficacy range of the AstraZeneca vaccine is similar to Johnson & Johnson but lower than Pfizer and Moderna.

Comparing antibody levels between mRNA vaccines, researchers observed a more robust immune response with the Moderna vaccine. Their studies indicated that participants vaccinated with two doses of mRNA-1273 had a mean titer of neutralizing antibodies of 3836 U/ml, whereas those vaccinated with BNT162b2 had a mean titer of neutralizing antibodies of 1444 U/ml. A potential difference in immunogenicity between the compared mRNA vaccines was attributed to higher mRNA content in mRNA-1273 compared to BNT162b2 and a longer interval between priming and boosting doses for the Moderna vaccine (4 weeks vs. 3 weeks for Pfizer/BioNTech vaccine) [18].

In addition, most studies have demonstrated the weakening of the immune system in antibody formation with advancing age. The effects of aging on the immune system manifest at multiple levels, including reduced production of B and T cells in the bone marrow and thymus, as well as diminished function of mature lymphocytes in secondary lymphoid tissues. Consequently, elderly individuals do not respond to immune challenges as robustly as the young. The immune risk profile also encompasses B cells with impaired function. While the number of B cells in mice remains unchanged with aging, the absolute number in human peripheral blood is reduced. This decline is likely attributed to decreased numbers of IgM memory and switched memory B cells, as the total number of naive B cells remains unchanged with aging [19].

In our study, 30% of individuals received the Sinopharm or AstraZeneca vaccines, while 40% received the Pfizer vaccine. Additionally, 60.3%, 33.9%, and 5.7% had received the Pfizer, Sinopharm, and AstraZeneca vaccines, respectively, as reported by Hassan *et al*., 2022. In total, 356 students participated, of whom 219 (61.5%) had received a primary vaccine series of Pfizer-BioNTech or Moderna mRNA vaccines, and 85 (23.9%) had received vaccines from Sinovac or Sinopharm. Median anti-S levels were significantly higher for mRNA primary vaccine series recipients (2.90 and 2.86 log [BAU/mL], respectively), compared with those who received Sinopharm or Sinovac vaccines (1.63 and 1.95 log [BAU/mL], respectively), as reported by Peter *et al*., 2023 [17]. By reviewing the published research [20], the efficiency of the Pfizer vaccine is more efficient than the rest of the vaccines, and this is due to its modernity. This vaccine was manufactured in a modern way that differs from the rest of the other vaccines. This is the first reason, and secondly, age has a role, according to our study, as ages vary between groups, and one group differs from another, with different immune defenses compared to the middle and elderly ages. In addition, the nature of the work has a role; if the person is a worker in the health sector, he will be exposed to contact with infected people compared to workers in the health sector-other sectors and non-employees.

As for future expectations about the effectiveness of the vaccine, it depends on the method of preparing the vaccine, which has a role in terms of modernity, in addition to the method of taking the vaccine and the instructions followed before taking the vaccine, and following up on the people enrolled in taking the vaccine between the first and second dose for six months or more. We suggest conducting other studies on the same people enrolled. Previously, was the vaccine taken in the coming years, the same vaccine, or was the vaccine changed, and the efficiency of the vaccine was measured through (IgG) antibody and conducting other studies on mixing two vaccines and studying them? This depends on the official approvals according to the people attached to it, according to ethical issues. Frankly, we face difficulty in every research regarding People and their approval, so we delay preparing the research. Reyhaneh *et al*., 2023 [21], reported a mean titer of anti-spike IgG of 4.3±2.29 units. The percentage of positive cases of the antibody was estimated to be 96.4% as measured by ELISA, with three types of vaccines administered to healthcare workers: Sputnik V, Sinopharm, and AstraZeneca. The titer of anti-spike IgG antibody was dependent on both the occupational area and a positive history of Covid-19 disease.

Through our study, the Pfizer vaccine has shown greater efficiency based on IgG concentration. Additionally, our next study will focus on individuals vaccinated between 2023-2024, allowing for a comparison between the results of this study and the subsequent one. It is preferable to measure the concentration of (IgG) using the RT-qPCR method for several reasons accuracy in results to determine the concentration of antibodies and modernity in technology, the sensitivities of other tests including RT-qPCR and ELISA test were 88.24% and 95%, respectively. RT-qPCR PCR showed the best specificity more (95%), and the speed of work [22-23].

Limitation of the study

It's important to acknowledge several limitations in the study. Specifically, the IgG levels of individual participants need to be monitored post the administration of both the initial and subsequent vaccine doses. Furthermore, conducting extensive studies will be essential to determine the variation in protection against infection according to antibody levels.

Conclusion

The study underscores the importance of understanding sex-related differences, age-related variations, and vaccine efficacy profiles to optimize immunization strategies and enhance public health outcomes. Continued research efforts, particularly longitudinal studies and methodological advancements, are essential for addressing evolving challenges and refining vaccination approaches

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We extend our appreciation to all individuals who willingly participated in this study.

Ethics Approval and Consent: Participants were briefed on the research process and objectives, and their informed

consent was obtained before the project commenced. Questionnaires were anonymized, and participants were assured that their data would be kept confidential.

Authors' Contributions

DH and FA conducted the original research, with DH responsible for the final statistical analysis of the Real-Time qPCR data. Data analysis was a joint effort, and both DH and FA contributed to drafting the manuscript. All authors have given their approval for the final version.

Conflict of Interests

The authors confirm that they do not have any competing interests.

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