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Elevated Serum Levels of SARS-CoV-2 Anti-Spike S1 RBD IgM, IgG, and IgA in Pfizer-BioNTech Vaccinated, COVID-19 Infected, and COVID-19 Infected after Pfizer-BioNTech Vaccinated Individuals after One Month

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Abstract **Background:** While protective immunity against some viruses, such as coronaviruses, is relatively short-lived, healing from acute infections of many different viruses, such as those caused by yellow fever, polio, measles, and smallpox, can give cell-mediated and humoral immunity for a lifetime. The main element in the long-term prevention of reinfection by most viruses might be due to the specific antibodies generated by plasma cells. **Aim:** This study aims to estimate the levels of SARS-CoV-2 Anti-Spike S1 RBD IgM, IgA, and IgG in serum among people in Baghdad after one month of receiving the Pfizer-BioNTech vaccine, infected with the SARS-CoV-2 virus after vaccination and COVID-19 infected patients respectively. **Methods:** A total of 120 volunteers were enrolled in this study, which was conducted between the 1st of November 2022 and the 13th of January 2023, and they were divided into four groups, each group containing 30 individuals. The study groups were categorized after one month into vaccinated with the Pfizer-BioNTech vaccine (BNT162b2), infected with SARS-CoV-2 after Pfizer vaccination, COVID-19 patients, and control. **Results:** The study presented a significant difference where (P value < 0.05) in the serum levels of Anti-Spike S1 RBD IgM, IgA, and IgG for all groups compared to the control. Serum levels of S1 RBD IgM Anti-Spike of SARS-CoV-2 in all groups were significantly increased (P value >0.05) compared to each other. For Anti-Spike S1 RBD IgA and IgG, there was no significant difference (P value >0.05) between the COVID-19-infected patients group and those infected by SARS-CoV-2 after the Pfizer vaccination group. Positive correlations were found among Anti-Spike S1 RBD IgM, IgA, and IgG levels in the serum. **Conclusions:** Natural infection by SARS-CoV-2 or vaccination with Pfizer-BioNTech (BNT162b2) provides significant humoral Immunological protection.

Key Words COVID-19, SARS-CoV-2, Pfizer-BioNTech vaccine, Anti-Spike IgG, Anti-Spike IgA, Anti-Spike IgM

1. Introduction

One of the coronaviruses known as the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is the source of highly contagious disease identified as COVID-19. In late December 2019, this virus initially broke out in Wuhan, China, and rapidly spread to other countries worldwide [1].

From infection without symptoms to mild, moderate, and severe pneumonia with failure in many organs, which can be deadly to the afflicted persons, it presents a broad spectrum of clinical manifestations [2]. Concerns about the safety of newly developed and marketed vaccines, as well as possible long-term side effects, have significantly increased among the public. It is essential to evaluate the acceptability rate, perspectives, and worries regarding receiving COVID-19 vaccinations to look into the frequency side effects of vaccines among the Iraqi population in Baghdad province [3].

COVID-19 infection and vaccinations both cause immunological responses. By carefully managing the damage caused by SARS-CoV-2, a coordinated immune response may be generated using inactivated viruses or mRNA-based adenoviral vectors [4]. Antigen-specific antibody responses are monitored following SARS-CoV-2 vaccination as markers of protective immunity. Immunoglobulin (IgM) antibodies are created shortly after the humoral immune system's response to viral infections, offering prompt protective protection [5]. After that, Higher affinity immunoglobulin G antibodies are

produced together with isotype class shift and maturation. The substantial IgG responses to SARS-CoV-2 elicited via the current mRNA vaccines have been extensively studied. However, there needs to be more consensus about the timing of vaccine-induced IgM responses and the role of previous immunity. The importance of IgM in protective immunity against COVID-19 was highlighted by the robust association seen between reducing levels of IgM against spike (S) protein and the receptor binding domain (RBD) and declining responses of neutralizing antibodies [6]. Following the infection, IgA plays a vital role in the early neutralization of the SARS-CoV-2 virus. In individuals with no measurable IgG and without symptoms recorded for a medical history, serosurveys have revealed positive results for IgA. A prior study found that individuals with mild or asymptomatic illnesses might have both transient or non-existent IgG positive and IgA positivity. Although there was no detectable IgA or IgG in their serum, a few individuals had mucosal IgA discharges [5].

This study aims to estimate the levels of SARS-CoV-2 Anti-Spike S1 RBD IgM, IgA, and IgG in serum among people in Baghdad after one month of receiving the Pfizer-BioNTech vaccine, COVID-19 infected and infected with the SARS-CoV-2 virus after vaccination patients respectively.

2. Materials and Methods

A. Sampling

This study, a case-control analysis, was carried out between November 1, 2022 and January 13, 2023. Following thirty (30) days of Pfizer-BioNTech (BNT162b2) second dose vaccination, COVID-19 infection, and COVID-19 infection following Pfizer-BioNTech (BNT162b2) full vaccination before six months to one-year (6 months to one year) with a control group, one hundred twenty (120) volunteers were chosen for this study. Each group had thirty individuals, with a 50:50 male-to-female ratio, age ranging from 20 to 45 on average, and from various regions of Baghdad. In addition, research volunteers with a history of gastrointestinal illnesses, pharyngitis, urinary tract infections, or immune response issues during the previous year were not allowed to participate.

The study groups' individuals were divided into:

- The Control group (C) included persons not previously infected by COVID-19 or vaccinated with any vaccine related to the Coronavirus and did not suffer from any type of chronic diseases (DM, IHD, HT, or Asthma).
- The Pfizer-BioNTech (BNT162b2) vaccinated group (V) included persons who did not have previous infection with COVID-19 but were vaccinated with the Pfizer-BioNTech vaccine and were selected one (30) days after the second dose.
- The COVID-19 infected group (CoV) included individuals who were selected after one month of COVID-19 infection and were not vaccinated.
- The Pfizer-COVID-19 group (V-COV) included individuals who were vaccinated with the Pfizer-BioNTech (BNT162b2) vaccine six months to one year after the date of the second dose, and then after one month, they got COVID-19.

B. Blood Sample Collection

By the research's protocol, each person in the study group had their vein punctured to get five milliliters of blood, which were then placed into gel tubes and left for around twenty minutes to allow the blood to clot at room temperature. The serum was extracted from the tubes and centrifuged for 10 minutes at 5000 RPM. The serum was then transferred into Eppendorf tubes to prevent contamination, freezing, and melting. The following Humoral immunological markers were measured using the ELISA technique: SARS-CoV-2 Anti-Spike S1 RBD IgM, IgA, and IgG.

C. ELISA Protocol

By the manufacturer's protocol, serum levels of the COVID-19 S1 RBD IgG Kit for ELISA (catalog number IEQ-CoVS1RBD-IgG), SARS-CoV-2 S1 IgA Kit for ELISA (catalog number MBS398093), and SARS-CoV-2 S1 IgM Kit for ELISA (catalog number MBS7612291) were measured by using an indirect ELISA (enzyme-linked immunosorbent assay) test. The results were computed using a microplate reader (Paramedical/Italy ELISA system). Six standards were created for the sandwich, Anti-Spike S1 IgA, Anti-Spike S1 RBD IgG, and Anti-Spike S1 IgM, according to the manufacturing company's instructions. These standards will be utilized for serum Anti-Spike S1 IgM titers (ng/ml), Anti-Spike S1 RBD IgG titers (Unit/ml), and Anti-Spike S1 IgA titers (ng/ml) to be quantified and analyzed. The concentration serum Anti-Spike S1 IgM titers, Anti-Spike S1 RBD IgG titers, and Anti-Spike S1 IgA titers were plotted for each calibrator regarding the serum Anti-Spike S1 IgM titers, Anti-Spike S1 RBD IgG titers, and Anti-Spike S1 IgA titers ELISA Kit to obtain the mean calculation. Data were counted by determining the mean absorbance for each duplicated measurement.

D. Ethical Approval

This study was given ethical approval by the Aliraqia Medical College Review Board / the Medical College / Al-Iraqia University.

E. Statistical analysis

The statistical program SPSS software for statistics v.26.0 (SPSS Inc., Chicago, IL, USA) was used to run the investigations. To determine if there are statistically significant differences, do a one-way ANOVA. Furthermore, a chi-square analysis was performed to determine the significance of the percentage differences. Statistics are deemed significant when P is less than 0.05.

3. Results

The anti-spike S1 RBD IgM serum levels mean were significantly increased (P value <0.05) in the control group compared to all groups Figure 1 and also the same results

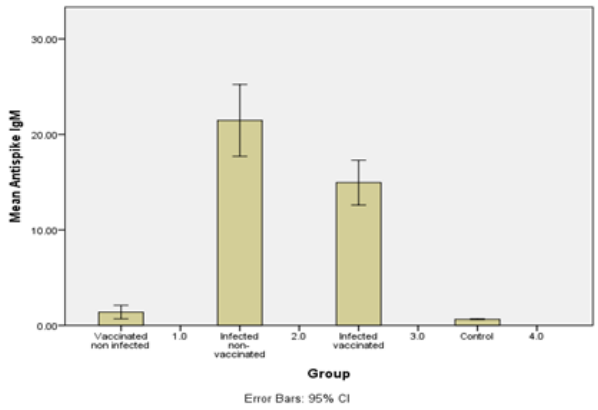

Figure 1: Mean of Anti-Spike S1 RBD IgM serum level for all groups in this study

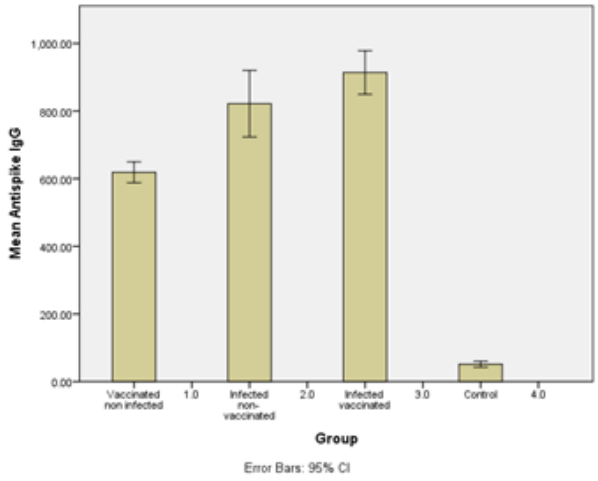

Figure 2: Mean of Anti-Spike S1 RBD IgG level in the serum for all groups in this study

were observed between each group with each other's Table 1.

The immunoglobulin G and immunoglobulin A serum titers were significantly increased (P value <0.05) in all groups which compared to the control group Figures 2 and 3. There was a significant difference (P value <0.05) among the (V) group and both the (CoV) and (V-CoV) groups while there were non-significant difference (P value <0.05) between the (CoV) group and the (V-CoV) group Table 2 and 3.

4. Discussion

Following vaccination or natural infection, antibody responses specific to an antigen are tracked and observed as measures of protective immunity [5] because antibody levels can indirectly reflect the effectiveness of immune responses [7]. Both T-cell mediated and humoral immune responses are induced by SARS-CoV-2 vaccines and natural infection, which act mainly through targeting the S protein of the virus, in particular the S1 domain, which in turn results in the pro-

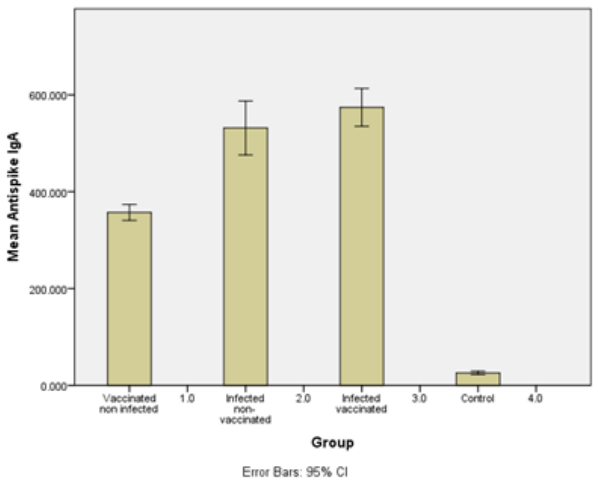

Figure 3: Mean of Anti-Spike S1 RBD IgA serum level for all groups in this study

duction of immunoglobulins A, G, and M antibodies against the spike protein [8]. Although vaccination will reduce the severity of COVID-19 infection, antibodies induced during vaccination will eventually fade away [9].

IgM is crucial for defensive immunity against COVID-19 because there is a clear correlation between decreasing anti-S IgM titers and decreasing neutralizing antibody responses [6], [10].

In the current study, we observed that, in terms of titters, the IgM response in the (CoV) and (V-CoV) groups was higher than in the (V) group and control persons, which agrees with previous research investigations [11]. It is interesting to note that Ruggiero et al. [10] found that those who had not been exposed to COVID-19 and had received the Pfizer mRNA vaccination had unusual response patterns of anti-S IgM, which included either no immunoglobulins M, IgM developing after IgG, or IgM and IgG present at the same time. It is challenging to hypothesize the cause of vaccine-induced aberrant responses of IgM in possibly individuals who received naive COVID-19 vaccine.

An earlier response of primary immune against asymptomatic virus infection, an earlier booster vaccination with increased IgM decay, or an IgM memory response from an earlier immunity to cross-reactive human corona-viruses may all be contributing factors to the absence of IgM entirely after two weeks of the entire vaccination. Due to the reported Th1-polarized responses, the accessory action of the vaccine's lipid components in promoting early and widespread IgG class-switching is another likely explanation [12]. The endurance of non-class-switched immunoglobulin M+ memory B cells may be the cause of vaccinees' virus-specific immunoglobulin M responses [11].

Avidity of Anit-spike S1 RBD IgG specific to SARS-CoV -2 levels was assessed after vaccination with Pfizer-BioN Tech approved mRNA vaccines or SARS-CoV-2 natural infection [13]. IgG is initially produced during seven days

Group		N	Mean	Std. Deviation	
Antispike IgM	Vaccinated non infected	30	1.3940	1.90448	<0.0001
	Infected non-vaccinated	30	21.4640	10.07719	
Antispike IgM	Vaccinated noninfected	30	1.3940	1.90448	<0.0001
	Infected vaccinated	30	14.9520	6.28225	
Antispike IgM	Vaccinated noninfected	30	1.3940	1.90448	0.04
	Control	30	.6440	.12489	
Antispike IgM	Infected non-vaccinated	30	21.4640	10.07719	0.004
	Infected vaccinated	30	14.9520	6.28225	
Antispike IgM	Infected non-vaccinated	30	21.4640	10.07719	<0.0001
	Control	30	.6440	.12489	
Antispike IgM	Infected vaccinated	30	14.9520	6.28225	<0.0001
	Control	30	.6440	.12489	

Table 1: Association of Anti- spike S1 RBD IgM serum level in all groups of study

Group		N	Mean	Std. Deviation	P value
Antispike IgG	Vaccinated non infected	30	618.6713	82.63897	<0.0001
	Infected non-vaccinated	30	821.7997	263.02618	
Antispike IgG	Vaccinated non infected	30	618.6713	82.63897	<0.0001
	Infected vaccinated	30	913.9780	172.09034	
Antispike IgG	Vaccinated non infected	30	618.6713	82.63897	<0.0001
	Control	30	51.3340	24.16198	
Antispike IgG	Infected non-vaccinated	30	821.7997	263.02618	0.11
	Infected vaccinated	30	913.9780	172.09034	
Antispike IgG	Infected non-vaccinated	30	821.7997	263.02618	<0.0001
	Control	30	51.3340	24.16198	
Antispike IgG	Infected vaccinated	30	913.9780	172.09034	<0.0001
	Control	30	51.3340	24.16198	

Table 2: Association of Anti- spike S1 RBD IgG serum level in all groups of study

Group		N	Mean	Std. Deviation	P value
Antispike IgA	Vaccinated noninfected	30	357.28373	44.068095	<0.0001
	Infected non-vaccinated	30	531.72100	149.560504	
Antispike IgA	Vaccinated noninfected	30	357.28373	44.068095	<0.0001
	Infected vaccinated	30	574.31000	104.749120	
Antispike IgA	Vaccinated noninfected	30	357.28373	44.068095	<0.0001
	Control	30	25.80333	9.386283	
Antispike IgA	Infected non-vaccinated	30	531.72100	149.560504	0.2
	Infected vaccinated	30	574.31000	104.749120	
Antispike IgA	Infected non-vaccinated	30	531.72100	149.560504	<0.0001
	Control	30	25.80333	9.386283	
Antispike IgA	Infected vaccinated	30	574.31000	104.749120	<0.0001
	Control	30	25.80333	9.386283	

Table 3: Association of Anti-Sike S1 RBD IgA serum level in all groups of study

after vaccination or natural infection. According to previous studies, individuals with a high IgG level were less expected to develop symptoms, had a shorter improvement time, and had higher titers of IgM [14].

Severe COVID-19 instances were invariably linked to greater antibody production and neutralization titers, even in the sample population's heterogeneity [15]. According to a complete cohort study conducted on recipients of the mRNA-based vaccine, the Anti S1 RBD IgG response elicited by COVID-19 vaccination peaked up to 15 days after the second dose. It gradually decreased until six months or even more after vaccination [9].

Viral neutralization is mediated by SARS-CoV-2 S IgA, which is generated by spontaneous infection and is a crucial part of natural immunity. It has been studied how the COVID-19 vaccine affects IgA responses, particularly in comparison to mRNA-based vaccinations. The COVID-19 mRNA vaccine elicits S1-specific immunoglobulin A with comparable kinetics to S1-specific immunoglobulin G, according to Chan et al. [16]. However, the serum of vaccines decreases more quickly after both the first and second doses. It has been found that the mRNA-based medication induces serum SARS-CoV-2 S1 RBD -specific immunoglobulin A. However, the medication may also generate S1-specific immunoglobulin A in the mucosa of the nasal cavity. Moreover, anti-S1 IgA was secreted in women's milk [17] and vaccine recipients' saliva [10] in response to mRNA-based vaccinations. Lipid nanoparticles, particularly ones carrying mRNA-based vaccines, have been discovered in distant organs, such as the lung [18]. In contrast, the intramuscular vaccination technique does not produce mucosal immunity [19].

This study revealed that Anti-spike S1-RBD IgA levels in (the CoV group, V-CoV group, and V group) are higher compared to the (C) group (non-vaccinated, non-infected individuals), indicating that the response of Anti spike S1 RBD IgA is more prominent due to natural infection [16],

[20].

V-CoV individuals generated higher responses of IgG and IgA against S antigens than their V or CoV participants. It was observed that this variation in reactions persisted for five months following vaccination. Regardless of whether the disease develops before or after a vaccine, Bates et al. have shown an increase in humoral immune responses that include binding and neutralizing antibodies [21].

5. Conclusions

Specific SARS-CoV-2 anti-S1 RBD IgM, IgA, and IgG were significantly increased in CoV, V-CoV, and V groups individuals after one month of Pfizer-BioNTech complete vaccination, COVID-19 infection after Pfizer-BioNTech full vaccination, COVID-19 infection and.

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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