ORIGINAL ARTICLE

Comparison of long-term anti-RBD SARS-CoV-2 antibody response following different vaccination schemes in Tunisia

Comparaison de la réponse à long terme des anticorps anti-RBD SARS-CoV-2 à différents schémas de vaccination en Tunisie

Awatef Ben Jemaa[1,2,3], Rihab Bouabsa[1], Meriam Ben Othmen[1], Ridha Oueslati[2], Hamdi Dhaouadi[1]

1. Biodhaouadi Laboratory, Center for Medical Analysis and Reproduction Biology, Bizerte, Tunisia.
2. Unit IMEC-Immunology Microbiology Environmental and Carcinogenesis, Faculty of Science of Bizerte, Bizerte, Tunisia.
3. Department of Biology, Faculty of science of Gafsa, ,University of Gafsa, Gafsa, Tunisia.

Abstract

Aim: The study aimed to compare long-term vaccine-induced humoral immunity following different vaccines regimens.
Methods: Anti-S-RBD total antibody levels were measured in blood samples of 167 participants nearly 6 months post-vaccination. Participants had received one; two or four doses of Pfizer vaccine or who received a third dose of mRNA vaccine (Pfizer) and primed with mRNA (Pfizer/Moderna), adenoviral (AstraZeneca/Jonson & Jonson) or inactivated (CoronaVac/Sinopharm) vaccine.
Results: Among all vaccination regimens, fourth dose of Pfizer achieved the highest S-RBD antibody titers. Nevertheless, the third dose of mRNA vaccine primed with adenoviral vaccine achieved the lowest titers of S-RBD antibody. Notably, the group that received a third dose of mRNA primed with two doses of mRNA vaccine exhibited higher S-RBD antibody compared to groups inoculated with a third dose of mRNA and primed with inactivated or adenovirus vaccine.
Conclusion: Our data showed the superiority of three mRNA vaccinations compared to third heterologous vaccine (inactivated of adenoviral) including mRNA as booster in terms of humoral immunogenicity. Our findings supporting the use of additional booster shot from a more potent vaccine type such as mRNA vaccines. Nevertheless, due to the limited number of subjects, it is difficult to extrapolate the results of our study to the whole of Tunisian population. Future studies should investigate a larger cohort and other potential correlates of protection, such as cellular immunity and how it is affected by different vaccination schemes after long-term post-vaccination.

Key words: COVID-19, mRNA, adenoviral, inactivated, humoral immunity.

Résumé

Objectif: L'étude visait à comparer l'immunité humorale induite par le vaccin à long terme après différents schémas vaccinaux.
Méthodes: Les anti-S-RBD ont été mesurés dans le sérum de 167 participants environ 6 mois post-vaccination. Les participants avaient reçu un, deux ou quatre doses du vaccin Pfizer ou une troisième dose de vaccin à ARNm (Pfizer) et amorcé avec un vaccin à ARNm, adénoviral ou inactivé.
Résultats: La quatrième dose de Pfizer a atteint les titres d'anticorps S-RBD les plus élevés. Néanmoins, la troisième dose de vaccin à ARNm amorcée avec le vaccin adénoviral a atteint les titres les plus bas. Le groupe qui a reçu une troisième dose d'ARNm amorcée avec deux doses de vaccin à ARNm a présenté des anticorps S-RBD plus élevés que les groupes inoculés avec une troisième dose d'ARNm et amorcés avec un vaccin inactivé ou adénovirus.
Conclusion: Nos données ont montré la supériorité de trois vaccinations à ARNm par rapport au troisième vaccin hétérologue. Nos résultats constituent une étude de validation de principe soutenant l'utilisation d'une injection de rappel du vaccin à ARNm qui pourrait être nécessaire. Néanmoins, en raison du nombre limité de sujets, il est difficile d'extrapoler les résultats de notre étude à l'ensemble de la population tunisienne. Les études futures devraient étudier une cohorte plus large et d'autres corrélats potentiels de protection, tels que l'immunité cellulaire et la manière dont elle est affectée par différents schémas de vaccination après une vaccination à long terme.

Mots clés: COVID-19, ARNm, adénovirus, inactivé, immunité humorale.

Correspondance
Awatef Ben Jemaa
Unit IMEC-Immunology Microbiology Environmental and Carcinogenesis, Faculty of Science of Bizerte, Bizerte, Tunisia.
Email: benjemaa_awatef@yahoo.fr

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Introduction

Unexpectedly, the first pandemic of the COVID-19 was caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus since 2019, continue to rise and spread around the world [1]. Evidence suggested that SARS-CoV-2 virus was evolving rapidly; however, widespread immunization of the world population leading to overcome and manage the COVID-19 pandemic [2]. Thus, vaccine availability remained a substantial global public health concern to fight against SARS-CoV-2 strains. Among several vaccination platforms, inactivated or attenuated viruses and recombinant proteins and vectors were developed and authorized for emergency use [3]. In Tunisia, seven vaccines were approved to be used during the mass of vaccination campaign which started on March 13, 2021. These vaccines were recommended as fellow: Pfizer-BioNTech, Moderna, Astrazeneca, Sinopharm, CoronaVac, Sputnik V and Jonson & Jonson were initially approved for a 21-days interval, 2 dose regimens except for Jonson & Jonson which was initially used for one dose [4]. However, the immunity conferred by SARS-CoV-2 vaccines showed to be declined in efficacy in parallel with the emergence of several variants of concern (VOCs) [5]. In Tunisia, the Alpha, Delta, and lately Omicron variants were responsible for the outbreaks during early 2021, mid 2021, and early 2022, respectively [4,6]. During the Delta wave in Tunisia in June 2021, there were evidences of breakthrough disease among fully vaccinated subjects [6]. Additionally, antibody levels after complete primary vaccination wane over time [7]. Thus, application of a booster dose (third dose of vaccination) was already started in Tunisia, especially in individuals at high risk. In Tunisia, the Pfizer-BioNTech mRNA vaccine has been the unique booster authorized as a third vaccine dose [8]. Despite the fact that the COVID-19 pandemic is in its third year, the discovery of highly transmissible variants raises worries about the repercussions of vaccine escape mutations [9].

The Omicron form is a SARS-CoV-2 variation of concern. This strain of SARS-CoV-2 emerged with its great ability to evade and resist humoral immune responses caused by infection and vaccination antibodies [10]. Due to lack of appropriate immunity against Omicron strains even in vaccinated individuals, the necessity of further booster vaccination (the fourth even fifth doses) was highly recommended to strengthen and broaden immunity against newly circulating SARS-CoV-2 strains [11]. Incomplete information on COVID-19 vaccination durability in Tunisian vaccinated with different type of vaccine remains. Therefore, we sought to compare levels of humoral immune response long-term following different vaccine schedules in the Tunisian population during period of SARS-CoV-2 Omicron variants.

Methods

Study design and participants

This study was conducted at Biodhaouadi Laboratory, Center for Medical Analysis and Reproduction Biology, Bizerte, Tunisia from June 21, 2022 to August 30, 2022. A total of 192 adult (age > 18 years old) Tunisian individuals vaccinated against SARS-CoV-2 virus interested and participated voluntarily in this study. Among them, 167 individuals meeting the inclusion criteria and signed the informed consent form were included in the study.

All consenting adults aged over 18 years old, excluding any immune deficiency disease or disorder, any disability (mainly mental disabilities), and without any history of symptoms suggestive of COVID-19 or a positive COVID-19 test were considered eligible for the study. The presence of contraindication to any of the vaccines used, pregnancy, or intent to conceive were considered as non-inclusion criteria. Moreover, the occurrence of a serious adverse event (death, anaphylactic shock, etc.), wishing to withdraw from the study, and the occurrence of a SARS-CoV-2 symptomatic infection during the follow-up period were also considered as exclusion criteria.

After obtaining the informed consent, all included subjects were interviewed face-to-face by trained interviewers in order to complete a paper questionnaire. The questionnaire included demographics (age, gender, comorbidities, body mass index (BMI)), type of vaccination, date of vaccination, pre- and post-vaccination medications, and need for medical attention.

The participants were categorized into six groups:

- Group 1: vaccinated with one dose of mRNA vaccine (Pfizer).
- Group 2: vaccinated with two doses of mRNA vaccine (Pfizer).
- Group 3: vaccinated with three doses of mRNA vaccine (Moderna/Pfizer).
- Group 4: vaccinated with four doses of mRNA vaccine (Pfizer).
- Group 5: vaccinated with adenoviral vaccine/Pfizer (3 doses) vaccine.
- Group 6: vaccinated with inactivated vaccine/Pfizer (3 doses) vaccine.

For all participants receiving three or four doses of SARS-CoV-2 vaccine, the booster dose was BNT162b2 (produced by Pfizer-BioNTech). Adenoviral vaccine was either AstraZeneca or Jonson & Jonson. Inactivated vaccine was CoronaVac or Sinopharm. Local guidelines recommended 3 weeks between the first and the second dose of vaccination. In addition, a period of at least 4 months between the administration of the second vaccine dose and the third dose booster was indicated. For the fourth dose booster, a period of at least 4 months between the third and the fourth dose booster was indicated.

The study was approved by the institutional ethics committee of the Center for Medical Analysis and Reproduction Biology, Bizerte, Tunisia. Informed consent was obtained from all subjects enrolled in the study. All research was conducted according to the declaration of Helsinki principles.

Sampling for the serology assay

The peripheral blood samples were strictly collected

nearly 6 months from the last dose of vaccination for each participant after the administration of the first dose of vaccine for the first group, after the second dose of vaccine for the second group, after the third vaccine dose for the third, fifth and sixth group and after the fourth dose of vaccine for the fourth group. For the serology, five milliliters of whole blood were taken in a tube without anticoagulant.

Immunogenicity assessment

Total anti-RBD-specific (receptor binding domain) antibodies in serum were assessed using a commercial kit test Elecsys® Anti-SARS-CoV-2 S (Cat. No. 09.289.267.190, Roche® Diagnostic, Switzerland). The Cobas® e411 analyzer was used for these tests. This test measures total specific antibodies directed against RBD proteins (mostly IgG, but also IgM and IgA). The UK National Institute of Biological Standards and Control calibrated the test to WHO First International Standard 20/136. Elecsys® Anti-SARS-CoV-2 S received FDA EUA on November 25, 2020. According to the manufacturer, the test had a sensitivity of 98.8% (95% CI 98.1-99.3%) and a specificity of 100%. The data are mentioned in IU/ml, which corresponds to 0.972 bound antibody units per ml. A sensitivity criterion of 0.8 U/mL, suggesting beyond viral exposure and a neutralizing antibody cutoff of 15 U/mL have been proposed. Consistent with the producer, serum levels greater than 15 U/ml indicate the existence of neutralizing antibodies and have a 100% positive predictive value. To start with, all sera have been tested undiluted. If the amount exceeds 25,000 IU/mL, dilute the serum to 1/100 and calculate the actual amount after multiplying by the dilution factor. However, if the diluted serum continues to be assessed as superior to 25, 000 IU/mL, the final results will be equal to the value of 25, 0000 IU/mL. We rigorously followed the manufacturer's commands whilst comparing anti-RBD antibodies in the serum of vaccinated subjects.

We set the anti-S-RBD antibody threshold at 6967 BAU/mL (equal to 7163 IU/mL), a number proved to be associated with 100% protection against Omicron strains by Dimeglio et al. 2022 [12].

Statistical analysis

All statistical analyses were performed by GraphPad Prism software 5 (GraphPad PRISMA 5.0 computer program). Shapiro-Wilk normality test was conducted to estimate the distribution of the data. Categorical variables were expressed as number or percentages, and significance was detected by Fisher's exact test. Continuous variables were expressed as medians and interquartile range (IQR) values. The Kruskal-Wallis test was used for continuous variables. The Spearman rank correlation coefficient was used for linear correlation analysis between groups. For all statistical analysis, $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics of participants

Between June 21, 2022 and August 30, 2022, 192 individuals were invited to participate in this study and 167 meeting the inclusion criteria and signed the informed consent form. Participants were all above the age of 18, with a median age of 51 years (IQR 33-63 years). Most subjects were female (58.6%). In this cohort, 19 (11.3%), 66 (39.5%) and 10 (5.9%) received one, two or four doses of Pfizer-BioNTech vaccine, respectively. Forty-nine (29.8%), 13 (7.7%) and 10 (5.9%) received three mRNA vaccine, the adenoviral vaccine/Pfizer (3 doses) and the inactivated vaccine/Pfizer (3 doses), respectively. Obesity (n = 33; 19.7%), diabetes (n = 20; 17.9%), and arterial hypertension (n = 31; 18.5%) were the most common co-morbidities (Table 1). Pain, redness at the site of vaccine injects, fever, fatigue, and headache, have all been described as side effects of SARS-CoV-2 vaccinations (data not shown). All individuals had no history of medication or food allergies or anaphylaxis.

Table 1. Main characteristics of the study population

	N	Median Age (years) (IQR)	Sex M/F	Diabetes N (%)	Hypertension N (%)	Obesity N (%)	Cardiovascular Disease N (%)
Pfizer-BioNTech (1 dose)	19	33 (25-69)	3/16	1 (5.2)	2 (10.5)	3 (15.7)	0 (0)
Pfizer-BioNTech (2 doses)	66	38 (27-56)	26/40	11 (16.6)	8 (12.1)	13 (19.6)	0 (0)
mRNA (3 doses)	49	59 (47-69)	23/26	14 (28.5)	14 (28.5)	5 (10.2)	1 (2)
Pfizer-BioNTech (4 doses)	10	64 (62-70)	7/3	3 (30)	3 (30)	6 (60)	2 (20)
Adenoviral vaccine/Pfizer (3 doses)	13	52 (45-61)	5/8	0 (0)	2 (15.3)	5 (38.4)	1 (7.6)
Inactivated vaccine/Pfizer (3 doses)	10	53 (28-77)	5/5	1 (10)	2 (20)	1 (10)	1 (10)
All Vaccines	167	51 (33-63)	69/98	30 (17.9)	31 (18.5)	33 (19.7)	5 (2.9)

Comparison of anti-RBD levels after different vaccination schemes

Given the importance of the SARS-CoV-2 S-RBD protein in the invasion and evasion of the immune response mechanisms, we assessed the levels of anti-S-RBD antibodies, as outlined in the materials and methods section. Although all vaccinated individuals developed significant anti-RBD antibodies (>15 IU/mL), these levels varied significantly between vaccinated groups (Table 2, Figure 1). All participants inoculated with three or four doses of SARS-CoV-2 vaccine received an mRNA-Pfizer vaccine (BNT162b2) as booster. Notably, the fourth dose of Pfizer vaccine gave the highest levels of anti-RBD antibodies (median (IQR) level of 14540 IU/mL (10983-25000)) followed by three doses of mRNA vaccine (median

(IQR) level of 8033 IU/mL (2882-11628)). However, we found no statistically significant difference between the two groups (p>0.05). Participants inoculated with two doses of Pfizer vaccine exhibited lower levels of anti-RBD antibodies than those vaccinated with one dose of Pfizer vaccine ((median (IQR) level of 4371 IU/mL (1939-8198) and 5606 IU/mL (1931-9325), respectively). Similarly, no statistical difference was observed between the serum samples from the group inoculated with one dose of Pfizer vaccine and those receiving two doses of Pfizer vaccine (p>0.05). The anti-RBD levels were increased between participants inoculated with two doses of Pfizer vaccine and those inoculated with three doses of mRNA vaccine (p=0.0142). Our data analysis revealed that, among all vaccination schemes, participants inoculated with adenoviral vaccine/Pfizer (3 doses) or with inactivated vaccine/Pfizer (3 doses) exhibited the lowest concentration of antibodies against S-RBD ((median (IQR) level of 3873 IU/mL (1170-6035) and 4027 IU/mL (2658-20645), respectively) (Table 2, Figure 1). The median anti-RBD titers of participants vaccinated with adenoviral vaccine/Pfizer (3 doses) were significantly lower than those inoculated with four doses of Pfizer vaccine (p=0.0042) (Table 2, Figure 1). Notably, a slight increase of anti-RBD levels was found after three doses of mRNA vaccine compared to three doses of the adenoviral vaccine/Pfizer and three doses of the inactivated vaccine/Pfizer. Nevertheless, comparing the vaccinated groups, we found no statistically significant difference between participants inoculated with three doses of mRNA vaccine, those receiving the adenoviral vaccine/Pfizer (3 doses) and those receiving the inactivated vaccine/Pfizer (3 doses) (p>0.05) (Table 2, Figure 1).

Table 2. Median levels and percentage of positivity of the humoral immune response obtained in the different groups of vaccines

	N	Anti-RBD Antibodies Median Level (IQR)	Anti-RBD Antibodies % of Positivity (>15 IU/mL)
Pfizer-BioNTech (1 dose)	19	5606 (1931-9325)	100
Pfizer-BioNTech (2 doses)	66	4371 (1939-8198)	100
mRNA (3 doses)	49	8033 (2822-11628)	100
Pfizer-BioNTech (4 doses)	10	14540 (10983-25000)	100
Adenoviral vaccine /Pfizer (3 doses)	13	3873 (1170-6035)	100
Inactivated vaccine /Pfizer (3 doses)	10	4027 (2658-20645)	100

We discovered significant variations between vaccination groups. The percentage of subjects with anti-S-RBD antibodies > 6967 BAU/mL were 42.%, 28.78%, 55.1%, 90%, 15.38%, and 40% for Pfizer (1 dose), Pfizer (2 doses), mRNA (3 doses), Pfizer (4 doses), adenoviral vaccine/Pfizer (3 doses) and inactivated vaccine/Pfizer (3 doses) vaccines, respectively (Figure 2). Thus, among vaccinated groups, the percentage of subjects with anti-S-RBD antibodies > 6967 BAU/mL was the lowest in the group receiving the adenoviral vaccine/Pfizer (3 doses). However, most subjects inoculated with four doses of Pfizer exhibited persistent levels of anti-S-RBD antibodies > 6967 BAU/mL after several months of vaccination (Figure 2).

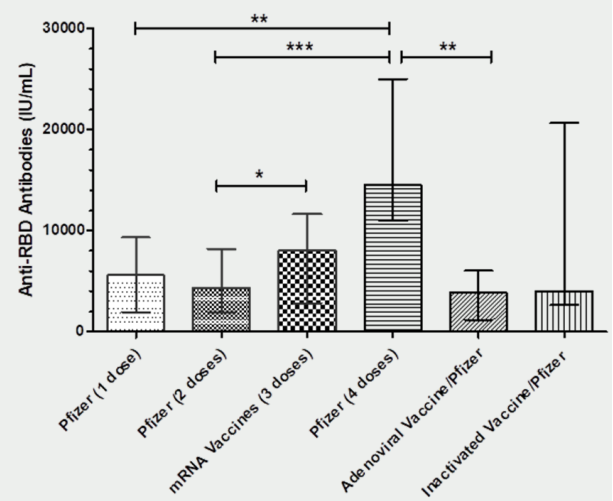

Figure 1. Humoral anti-RBD immunity in individuals vaccinated with different SARS-CoV-2 vaccination regimens.
*: p<0.05; **: p<0.01; ***: p<0.001

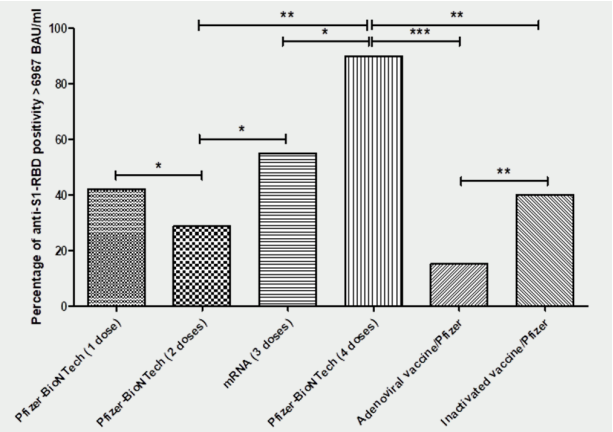

Figure 2. Percentage of positivity over the threshold of 6967 BAU/mL for anti-RBD antibodies in different groups of vaccine.
* = p<0.05; **: p<0.01

Correlation between anti-RBD levels and age after different vaccination schemes

As shown in Figure 3, in response to the first dose of Pfizer vaccine, Spearman's correlation indicated a positive correlation between age and serum anti-RBD concentrations (r=0.6974, p=0.0009). However, age was not significantly correlated with anti-RBD antibodies for other vaccine regimens.

Discussion

Several vaccination schemes were deployed worldwide against SARS-CoV-2 in order to control COVID-19 pandemic [3]. Among different vaccination platforms, mRNA ones in addition to inactivated and adenovirus vaccines were developed and authorized for use in Tunisia [4]. The Pfizer-BioNTech mRNA (BNT162b2) vaccine has been the unique booster authorized as a

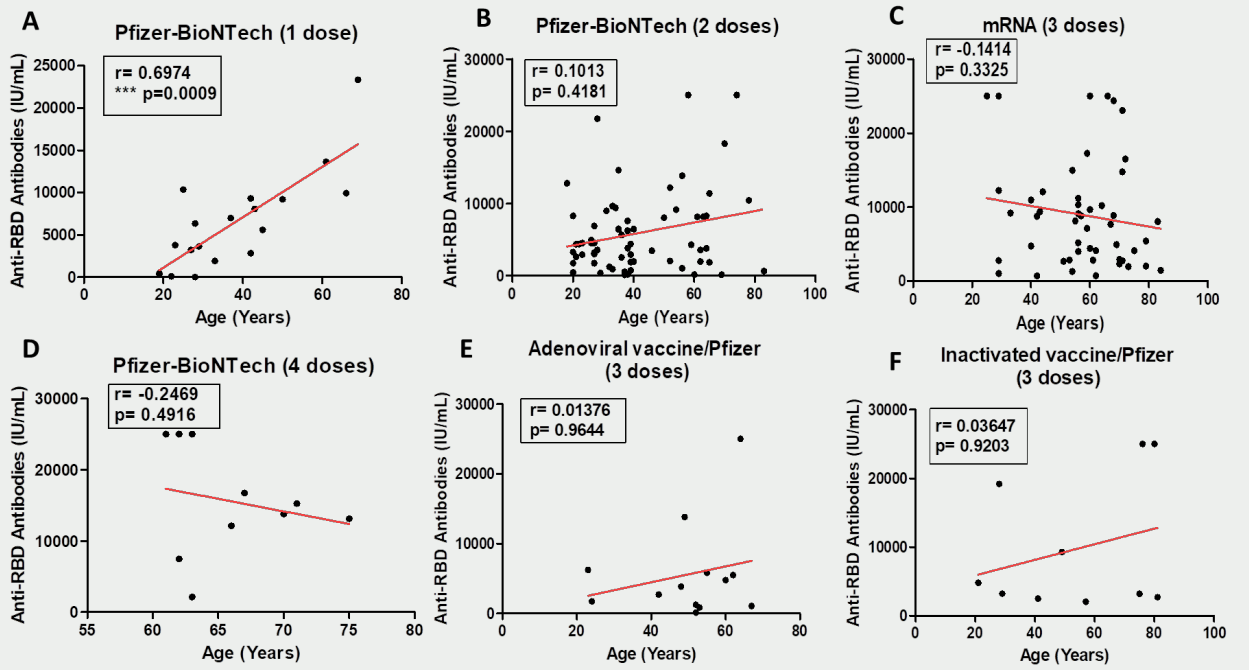

Figure 3. Correlation between humoral anti-RBD immunity and age of subjects vaccinated with different groups of SARS-CoV-2 vaccination regimens

third and fourth vaccine dose in Tunisia [8]. At present, immunization with homologous mRNA vaccine in vaccinated subjects has been widely investigated [13-15]. However, little data are available about the long-term third mRNA booster post-vaccination in individuals primed with mRNA, inactivated or adenovirus vaccines. Hence, the purpose of this study was to estimate the comparative long-term humoral immune response in vaccinated subjects with different vaccination regimens: Pfizer vaccine (1 dose), Pfizer vaccine (2 doses), Pfizer vaccine (4 doses), and third mRNA booster vaccination in individuals primed with mRNA, inactivated or adenovirus vaccines. Current COVID-19 vaccines primarily target the viral spike protein "S" or its receptor binding domain, aiming to induce a robust neutralizing antibody response [3,16,17]. In this study we assessed the anti-S-RBD binding antibody levels which demonstrated excellent concordance (r = 0.86) with the neutralizing antibody and a potential solution for COVID-19 antibody testing [18]. Increasing evidence suggests a strong association between neutralizing antibody titers and protection against SARS-CoV-2 variants of concern, mediated by a reduction in symptomatic infection and risk of severe disease [19]. It is important to note that after several months post-vaccination, individuals who received the second dose of Pfizer vaccine (BNT162b2) achieved lower S-RBD antibody titers than those inoculated with one dose of Pfizer vaccine (BNT162b2). Additionally, after long-term of vaccination, the group that received a third-dose of mRNA vaccine primed with two doses of mRNA vaccine exhibited higher titers of S-RBD antibody than the group inoculated with two doses of Pfizer vaccine. Our findings align with previous studies which reported that vaccination against various SARS-CoV-2 outbreaks has been poor after one and two doses of Pfizer vaccine [20,21]. Moreover, it was revealed that the third dose of Pfizer restored high immunity against SARS-CoV-2 and COVID-19 hospitalizations in persons 18 years of age and older [22,23]. In accordance with a previous study [4], we did not observe correlation between age and serum anti-S-RBD levels following vaccination regimens except for the group inoculated with one dose of Pfizer vaccine. Indeed, a positive correlation was found between age and titers of S-RBD antibody in the group that received one dose of Pfizer vaccine. Nevertheless, other studies have reported an age-related decline in immune responses and cellular responses, including those elicited by mRNA, viral vectors, or inactivated vaccines [24,25]. Notably, several studies have shown that the third mRNA antibody is effective in increasing Omicron-specific antibody titers [19,26]. However, protection against SARS-CoV-2 Omicron decreased rapidly after the third mRNA vaccine [9,27]. This phenomenon seems to be particularly important among older adults aged ≥80 years compared to younger patients. These results reinforce the importance of booster vaccination policies, particularly with respect to high-risk populations [27]. It should be noted that the fourth dose of COVID-19 vaccine is given due to declined efficacy of the first, second and three dose of vaccine due to decreased antibodies specific to SARS-CoV-2 [28]. Interestingly, among all vaccination regimens, the fourth dose of Pfizer vaccine (BNT162b2) achieved the highest S-RBD antibody titers after several months post-vaccination. Our results are consistent with a previous study since the authors showed significantly higher immunogenicity after the fourth BNT162b2 booster compared to the first, second and third BNT162b2 vaccine [15,28]. Several studies have shown that the fourth mRNA antibody is effective in increasing Omicron-specific antibody titers [15,28]. However, protection against SARS-CoV-2 Omicron decreased more rapidly after the fourth than the third dose of mRNA vaccine [9,27]. This rapid decline may be due to the reduction of the immune response of the Pfizer

vaccine against the Omicron variant [9,27]. We should note that the study was conducted during the Omicron outbreak. In discordance with previous studies, the antibodies S-RBD levels were maintained at high levels after several months of vaccination in the group receiving four doses of Pfizer vaccine. Despite vaccination, the potential primary cause of infection is the emergence of new SARS-CoV-2 variants and the waning of the immunity after several months post-vaccination [5]. Regardless of the vaccine type, a previous study found that three-dose-receivers were protected from infection to SARS-CoV-2 approximately 3.67 times more, four-dose-receivers 8 times more, and five-dose-receivers 27.77 times more from being infected with COVID-19 than any two doses of any COVID-19 vaccine type [29]. Authors concluded that higher antibody levels and administration of four and/or five doses of vaccines are more protective from COVID-19 than 2 or 3 dose [29].

Recent studies reported superiority of mRNA vaccines to elicit a stronger immune response against SARS-CoV-2 compared to adenovirus and inactivated vaccines [4,30,31]. In line with this view, after long-term post-vaccination, we found a slightly higher S-RBD antibody titers among the group that received a third dose of mRNA vaccine primed with two doses of mRNA vaccine compared to the group inoculated with a third dose of mRNA vaccine and primed with inactivated or adenovirus vaccine. However, no statistically huge variations have been established among vaccine groups. Several studies concluded that both homologous and heterologous vaccination regimens achieved high humoral immune responses against the Omicron variant, particularly when a booster dose of mRNA vaccine is included [19,26]. However, previous studies reported that vaccination regimens based on inactivated virus (CoronaVac) and the mRNA vaccine BTN162b2 as a booster have yielded longitudinal test facts indicating that infected people generated moderate neutralizing antibody(nAb) and anti-N IgG titers that declined after 9 months [32,33]. Despite the stated superiority of RNA vaccines for the induction of a selected immune response compared to inactivated or adenovirus vaccines [4,30,31], the use of inactivated and adenovirus vaccines were justified especially in developing country like Tunisia by their availability at times when other alternatives were not accessible yet. However, vaccine effectiveness wanes over time, with some accounts reporting efficacy falling below 50% after the first year following vaccination [34]. These data and our results reinforce the importance of booster vaccination policies, particularly with respect to high-risk populations.

One of the most relevant findings of our research was that SARS-CoV-2 antibodies attained high or low protective levels depending on the immunization protocol. For Omicron strains, the threshold of protective antibody levels against S-protein was greater than 6967 BAU/mL [12]. By setting up the protective threshold of 6967 BAU/mL, protection was deemed complete against SARS-CoV-2 Omicron strains since no individual at this level experienced illness [12]. Based in this threshold, we showed that the positivity rates were the highest for the fourth dose of Pfizer vaccine, followed by the group that received three doses of mRNA vaccine. However, the percentage of subjects with persistent anti-S-RBD antibodies > 6967 BAU/mL was the lowest in the group immunized with the adenoviral vaccine/Pfizer (3 doses). In terms of humoral immunogenicity, third homologous mRNA and third heterologous vaccine, including mRNA as booster, were previously tested. The results are in discordance with our data since the authors showed that significantly higher immunogenicity after the third-post-vaccination heterologous mRNA vaccination primed with inactivated or adenovirus vaccine compared to the three doses of mRNA vaccine [35]. An observational study showed that mRNA booster vaccines elicited strong humoral and cellular responses in cancer patients and comparable immune response between who were given two initial doses of either CoronaVac or BNT162b2 vaccine [36]. In line with published data, as compared to one and two doses of the Pfizer immunization, equivalent regimens of a three-dose Pfizer mRNA vaccine elicited greater titers of neutralizing antibodies against SARS-CoV-2 Delta and Omicron variants [37]. On the other hand, the fourth doses of the Pfizer vaccine delivered under a homologous stimulation regime offered a high level of protection against COVID-19 [15,27]. With regard to the effect of vaccination on SARS-CoV-2 cellular immunity, a recent study has documented that T cell response remained sustained after long-term post-vaccination, whereas the titer of anti-RBD antibodies as well as their neutralization function decreased significantly during that period [38]. Accordingly, T cell helper response primed by inactivated virus vaccines seems to be more persistent over time than this elicited by mRNA vaccines [39]. Moreover, it seems that CD4 cells elicited by the inactivated vaccine could be activated upon mRNA vaccination, thus facilitating the building of a stronger immune response and memory [40].

In addition to anti-RBD, a previous study showed that the titer of anti-N (nucleocapsid) antibody differs according to vaccination status. While anti-RBD is a good indicator of an effective response to vaccine, anti-N antibody play more important role when assessing the immune response to natural infection [41].

There are various limitations in our research. Although the study assessed the anti-RBD binding antibody levels which exhibited a strong correlation (r = 0.86) with the neutralizing antibody, there is a lack of neutralizing antibodies test, which are known to be strongly associated with protection. The second limitation is that each immunization regimen group has substantially different sizes. The third limitation is the scanty number of people in the Pfizer vaccination group who received the fourth dose. Another limitation is the lack of the cellular immunological response to each vaccination regimen.

Conclusion

Collectively, our data highlighted the superiority of the third dose of mRNA vaccine primed with two doses of mRNA vaccine than that primed with two doses of

adenoviral or inactivated vaccine in terms of humoral immunogenicity after long-term of vaccination. Moreover, the study demonstrated that the fourth dose of mRNA vaccine provided a sustained further humoral immunogenicity compared to three doses of mRNA vaccine. Our findings had implications for policy change and suggested that vaccinated subject with three doses of mRNA vaccine retain immunologic benefit from vaccination 6-months post-vaccination, though booster doses are needed to keep most reliable antibody levels over time. Nevertheless, due to the limited number of subject, it is difficult to extrapolate the results of our study to the whole of Tunisian population. Future studies should investigate a larger cohort and other potential correlates of protection, such as cellular immunity and how it is affected by different vaccination schemes after long-term post-vaccination.

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