COVID-19 Serological Study in Non-infected Vaccinated Subjects: Differences among Age, Sex, and Vaccine Brand

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Objective: To evaluate IgG antibody levels against SARS-CoV-2 in non-infected vaccinated subjects among vaccine brand, sex, and age.

Methods: Abbott's AdviseDx SARS-CoV-2 IgG II immunoassay was used to measure IgG levels within 6-9 months after the second dose vaccination; level >50 AU/mL was classified as a positive test.

Results: Data of 183 non-infected vaccinated subjects was analyzed according to the vaccine brand, time after second vaccination, sex, and age. Bivariate analysis showed that receiving the Moderna brand vaccine, being female, and younger were associated with higher antibody levels, p<.001. Conversely, no differences were observed between the IgG antibody levels against SARS-CoV-2 and time after second vaccination (6-7 months as compared to 8-9 months), p=.49.

Conclusion: After six to nine months post-vaccination, receiving the Moderna vaccine, being female, and being younger were significantly associated to higher IgG antibody levels to SARS-CoV-2 in non-infected vaccinated subjects. [*P R Health Sci J 2023;42(3):203-206*]

Key words: SARS-CoV-2 antibodies, COVID-19 antibodies, COVID-19 antibodies post-vaccination

The COVID-19 disease, which emerged in Wuhan, China in December 2019, rapidly became a global public health problem (1). In Puerto Rico, the first confirmed cases of COVID-19 were reported in March 2020 (2). The causative agent, SARS-CoV-2, is a single-stranded RNA virus of the human coronavirus family with structural surface glycoproteins that resemble spikes (S). These S proteins contain a receptor binding domain that binds with very high affinity to the angiotensin-converted enzyme receptor-2 (ACE-2) (3). It is precisely this binding between the S protein and the ACE-2 enzyme receptor that allows the virus to invade the human cells and initiate the infectious process. This S glycoprotein has also been the target antigen for the development of vaccines against SARS-CoV-2.

The natural humoral response typically initiates with the development of IgA and IgM antibodies within three (3) to six (6) days after a viral infection, and the development of IgG antibodies typically occurs by the second week of infection (4). Unfortunately, these naturally generated antibodies to SARS-CoV-2 have been shown to wane after three (3) months in some convalescent patients (1). The natural antibody response generated against the SARS-CoV-2 virus and the duration of this immunity was found to vary from person to person for reasons that are not fully understood (5, 6). Researchers have expressed the need for more research on the duration of natural immunity against COVID-19 (7) and even serological surveillance (8) to better understand and control COVID-19.

The vaccination process in Puerto Rico began in December 2020. By the time the study was conducted (August to December 2021), a substantial amount of the general population was fully vaccinated, mostly by Pfizer/BioNTech or Moderna vaccines. The objective of this study was to evaluate the concentration levels of IgG antibodies directed against SARS-CoV-2 in subjects fully vaccinated who never had COVID-19 disease.

Materials and Methods

A prospective study of adult subjects (age > 21 years) with at least two doses of Moderna or Pfizer/BioNTech and no history of COVID-19 who went to Colón Clinical Laboratories, Puerto Rico, between August and December 2021. After a brief explanation about the study and assessing their willingness to

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participate, the participant signed an informed consent, and answered a questionnaire to obtain information on their age, sex, municipality of residence, and vaccination status with dates. Lastly, we collected a 4 ml blood sample in a lavender-top tube (EDTA) to measure SARS-CoV-2 IgG antibodies. The timing of the blood draw was intended to approximate six to nine months after the second dose vaccination. Exclusion criteria included age less than 21 years, pregnant subjects, those infected nor vaccinated, and those vaccinated with the Janssen COVID-19 vaccine, mainly because it has a different mechanism of action.

The measurement of SARS-CoV-2 IgG antibodies was performed on the Architect i1000 equipment manufactured by Abbott with the AdviseDx SARS-CoV-2 IgG II assay. We measured the IgG antibody levels in arbitrary units (AU/mL), where a concentration equal to or greater than 50 AU/mL was considered a positive test result. The assay's upper limit of quantification (LoQ) is 25,000 AU/mL.

This study (#B0740121) was approved by the Institutional Review Board at the Medical Sciences Campus, University of Puerto Rico. All data were de-identified to protect the participant's identity before statistical analysis. The independent categorical variables chosen for analysis were the vaccine brand (Pfizer/BioNTech vs. Moderna), subject's sex (male vs. female), subject's age grouped into three categories: i.e., 21-40 years, 41-60 years, and over 60 years, and time after second SARS-CoV-2IgG (6-7 months vs. 8-9 months).

The continuous dependent variable was the concentration of IgG antibodies to SARS-CoV-2. Descriptive statistics were done using measurements of central tendency and dispersion for continuous variables, and with frequencies and percentages for categorical variables. Comparison between study variables were done using the Wilcoxon Rank Sum Test or the Kruskal-Wallis test with multiple comparisons, as appropriate. Statistical significance was set at $p<0.05$. The statistical significance of the multiple comparisons was adjusted according to the number of comparisons performed ($p<0.05/3=<0.02$). The statistical software STATA version 15 (STATA Corp.; College Station, TX, USA) was used to perform the analyses.

Results

The study consisted of 183 vaccinated participants; 64% (n=117) received at least two doses of the Pfizer/BioNTech vaccine, while 36% (n=66) received at least two doses of the Moderna vaccine. As shown in Figure 1, subjects vaccinated with the Moderna brand had significantly higher antibody levels (n=66, median=1723.80) than those with Pfizer (n=117, median=905.30); p<.001. Serology in females (n=122, median=1277.55) was significantly higher than in males (n=61, median=674.10); p=.007. Subjects' age categories of 21-40 years (n=54), 41-60 years (n=74), and over 60 years (n=55) showed median antibody levels, respectively, of 1705.75, 1197.35, and 676.90, p<.001. In pairwise comparison testing of age categories; marginal differences were observed between subjects between the age group of 21-40 years as compared to those between the ages of 41-60 years, and among those aged 41-60 as compared to participant's >60 years of age (p=.02). Significant differences were observed when comparing participant's aged 21 thru 40 years and those >60 years of age (p<.001). Finally, no significant differences were observed in the levels of IgG antibodies among people whose samples were collected between 6-7 months after vacination vs. those that were taken between 8-9 months (p=0.49, data not shown). (Table 1).

Discussion

The COVID-19 pandemic presented a global public health emergency (9), for which many efforts have been implemented for its control, including a widespread vaccination process. Serological tests of SARS-CoV-2, initially used as the first available diagnostic tests, have now been proposed to measure true infection rates, such as the seroprevalence surveys conducted by the Centers for Disease Control and Prevention (CDC), which include large-scale geographical, community, and special populations (8). For another study, on the other hand, the purpose of using serological tests was a measure suggesting immunological protection against COVID-19 (10).

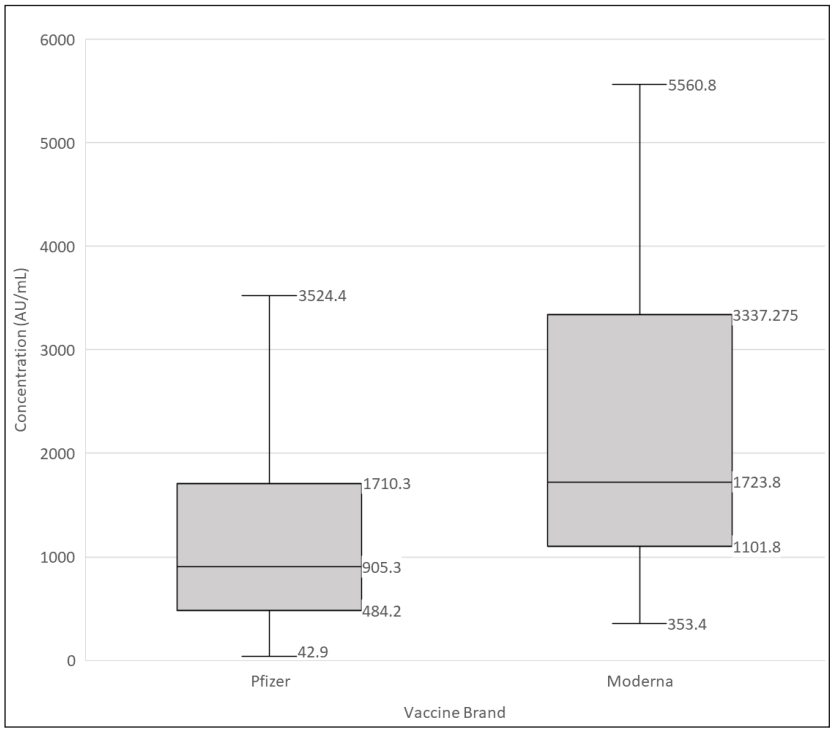

Figure 1. Comparison of antibody levels according to the vaccine brand (n=183)

Table 1. Subject characteristics, antibody levels, and statistical comparison of variables

Variables	n	Antibody levels (AU/mL) median value (IQR)	p-value
Vaccine brand			p < .001
Pfizer	117	905.9 (484.2-1710.3)	
Moderna	66	1723.8 (1101.8-3337.28)	
Sex			p = .007
Male	61	674.1 (379-1850.2)	
Female	122	1277.55 (793.1-2451.63)	
Age (categories), years			p < .001
21-40	54	1705.75 (970.23-2973.05)	
41-60	74	1197.35 (643.55-2051.40)	
>60 years	55	676.90 (385.70-1595.90)	
Months after the second vaccination			p = .486
6-7	152	1214.85 (619.60-2486.00)	
8-9	31	985.5 (622.50-1929.70)	

IQR: interquartile ranges are presented as percentiles (25th percentile – 75th percentile)

Some researchers have indicated that seropositivity alone does not guarantee high levels of antibody-mediated protection after vaccines (11). Thus, much research is required to definitely conclude what concentration of antibody levels confers the best immunity as well as what concentration of antibody levels indicates the need for further vaccination to boost immunity.

In this study, we measured the concentration levels of IgG antibodies directed against SARS-CoV-2 among those vaccinated without a history of the COVID-19 disease (n=183). We found that 100% revealed a positive serological test (>50 AU/mL) at the time of sampling (within 6-9 months, mean=215, range of 167-281 days after second vaccine dose). Statistical analysis of this data revealed that the IgG antibody levels were significantly higher in those vaccinated with the Moderna brand (vs. Pfizer/BioNTech), in females (vs. males), and in younger ages of 21 to 40 (vs. over 60 years). Our research findings are similar to a study in Belgium where people who received the Moderna vaccine had greater IgG antibody levels than those who received the Pfizer vaccine (14). Similar variables were analyzed in a study conducted in Ceuta, Spain, where they measured IgG antibody levels two months after the first vaccine (Moderna, Pfizer, or AstraZeneca) and then again six months after the second dose (12). The positivity rate was 99% (295/298) at the 6-month interval, initially higher with the Moderna brand at the 2-month interval (but not statistically different at the 6-month interval), with no differences in sex and initially higher levels among the elderly at the 2-month interval only. Although this study had more subjects, it was mainly a post-infection study, and they combined the vaccinated and unvaccinated together, which may account, in part, for the different findings.

Differences in SARS-CoV-2 antibody response according to the vaccine brand have been described in other studies (13, 14), where they suggested a possible association with the timing of the second vaccine dose; i.e., 28 days for mRNA-1273 (Moderna) vs. 21 days for BNT162b2 (Pfizer/BioNTech). Differences in serological response according to sex have been described in post-infection studies, where relatively high IgG levels were found in women three weeks after the onset of symptoms, while men took four weeks to attain similar levels (15). COVID-19 outcome studies also reveal antibody responses to be higher and longer lasting in women vs. men (15). Differences in serological response according to age have also shown an inverse relationship in a study that found the highest levels in those younger than 35 (14), but not so in other studies where older adults had higher levels than young adults (12,16). The fact that the later studies were conducted among infected subjects (vaccinated and unvaccinated) may explain the differences in findings.

A major limitation of the study was that it was conducted within certain time constraints, which affected the recruitment of subjects for two of our initially proposed groups (infected only or infected and vaccinated).

Conclusion

In conclusion, non-infected vaccinated people exhibited significantly higher IgG antibody levels against SARS-CoV-2 if they received the Moderna vaccine, were female, and were of a younger age when measured within 6 to 9 months after their second vaccine dose.

Resumen

Objetivo: Evaluar los niveles de anticuerpos a SARS-CoV-2 en individuos no-infectados vacunados en relación a vacuna administrada, sexo y edad. Métodos: Se utilizó el inmunoensayo AdviseDx SARS-CoV-2 IgG II de Abbott para medir los niveles de IgG dentro de 6-9 meses después de la segunda dosis de vacunación; nivel >50 AU/mL es una prueba positiva. Resultados: Sujetos adultos reclutados fueron 183. Dicho grupo fue subdividido de acuerdo a la vacuna administrada, sexo y edad. Un análisis bivariado demostró que los sujetos vacunados con Moderna, del sexo femenino y más jóvenes tuvieron niveles de anticuerpos significativamente más altos que aquellos con Pfizer, p<.001. No se observaron diferencias significativas en los niveles de anticuerpos IgG contra SARS-CoV-2 de acuerdo al tiempo transcurrido luego de la segunda dosis (6-7 meses vs 8-9 meses), p=.49. Conclusión: Sujetos no-infectados vacunados exhibieron niveles de anticuerpos IgG a SARS-CoV-2 significativamente más altos con la vacuna Moderna, si son mujeres y si son más jóvenes cuando fueron medidos dentro de 6-9 meses post-vacunación.

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References

1. Zhu N, Zhang D, Wang W, et al. China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China. The New England Journal of Medicine, 2019; 382(8): 727–733. https://doi.org/10.1056/NEJMoa2001017
2. Puerto Rico Department of Health. Vaccination https://covid19datos.salud.gov.pr Accessed April 25, 2022.
3. Fung TS & Liu DX. Human Coronavirus: Host-Pathogen Interaction. Annual Review of Microbiology, 2019; 73: 529–557. https://doi.org/10.1146/annurev-micro-020518-115759
4. Guo L, Ren L, Yang S, et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clinical Infectious Diseases, 2020; 71(15), 778-785. https://doi.org/10.1093/cid/ciaa310
5. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid Decay of Anti-SARS-CoV-2 Antibodies in Persons with Mild Covid-19. The New England Journal of Medicine, 2020; 383(11), 1085–1087. https://doi.org/10.1056/NEJMc2025179
6. Liu X, Wang J, Xu X, Liao G, Chen Y, & Hu CH. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerging Microbes & Infections 2020; 9(1),1269–1274. doi: 10.1080/22221751.2020.1773324
7. Lagunas-Rangel FA & Chávez-Valencia V. What do we know about the antibody responses to SARS-CoV-2? Immunobiology, 2021; 226(2), 152054. Advance online publication. https://doi.org/10.1016/j.imbio.2021.152054
8. Centers for Disease Control and Prevention. Uncovering SARS-C0V-2 Infections through Serology (or Antibody) Testing. U.S. Department of Health and Human Services. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/about-serology-surveillance.html Accessed September 20, 2022
9. World Health Administration. Statement on the eleventh meeting of the international Health Regulations (2005) Emergency Committee regarding the coronavirus disease (COVID-19) pandemic. https://who.int. Accessed April 13, 2022
10. Khoury DS, Cromer D, Reynaldi A,, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nature Medicine, 2021; 27(7): 1205–1211. https://doi.org/10.1038/s41591-021-01377-8
11. Demonbreun AR, Sancilio A, Velez MP, et al. Comparison of IgG and neutralizing antibody responses after one or two doses of COVID-19 mRNA vaccine in previously infected and uninfected individuals. EClinicalMedicine, 2021; 38, 101018. https://doi.org/10.1016/j.eclinm.2021.101018
12. Dominguez-Padilla, M. (2021). Comparative analysis of the natural and artificial humoral immunity of SARS-CoV-2 in health care workers of the National Institute of Health Management of Ceuta. Escuela Nacional de Sanidad. 2021; repisalud.isciii.es
13. Voysey M, Costa Clemens SA, Madhi SA, et al. Oxford COVID Vaccine Trial Group. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomized trials. Lancet. 2021; 397(10277):881-891. doi:10.1016/S0140-6736(21)00432-3
14. Steensels D, Pierlet N, Penders J, Mesotten D & Heylen L. Comparison of SARS-CoV-2 Antibody Response Following Vaccination With BNT162b2 and mRNA-1273. JAMA, 2021; 326(15):1533–1535. https://doi.org/10.1001/jama.2021.15125
15. Huang B, Cai Y, Li N, et al. Sex-based clinical and immunological differences in COVID-19. BMC infectious diseases, 2021;21(1), 647. https://doi.org/10.1186/s12879-021-06313-2
16. Varona JF, Madurga R, Peñalver F, et al. Seroprevalence of SARS-CoV-2 antibodies in over 6000 healthcare workers in Spain. International Journal of Epidemiology, 2021; 50(2):400–409. https://doi.org/10.1093/ije/dyaa277
17. Kang SJ & Jung SI. Age-Related Morbidity and Mortality among Patients with COVID-19. Infection & Chemotherapy. 2020;52(2):154–164. https://doi.org/10.3947/ic.2020.52.2.154
18. Krammer F, Srivastava K, Alshammary H, Amoako AA, Awawda MH, Beach KF, et al.. Antibody Responses in Seropositive Persons after a Single Dose of SARS-CoV-2 mRNA Vaccine. The New England Journal of Medicine, 2021;384(14):1372–1374.