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Quantitative SARS-CoV-2 Spike Receptor-Binding Domain on Vaccinated Individuals Compared to Natural Infection

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Abstract

BACKGROUND: Coronavirus disease 2019 (COVID-19) pandemic has been going on for more than 2 years, with various treatments and diagnostic methods available. One of the most prized structures, the receptor-binding domain (RBD) of the spike protein in severe acute respiratory syndrome coronavirus 2 has long been thoroughly researched for its function and becoming the target for various diagnostic methods and treatments, including a vaccine. The spike-RBD (sRBD) antibody count might be the parameter for antibody response in vaccinated and infected individuals. However, no direct comparison is made.

AIM: The study aims to compare the sRBD antibody count in the naturally infected individuals to the vaccinated ones.

METHODS: We conducted a cross-sectional study with 49 participants of the infected patients, and vaccinated individuals were included in this study from Prof. Dr. R. D. Kandou Hospital, Manado. The participants underwent a COVID-19 antibody test, using enhanced "Chemiluminescence" Immuno assay to analyze the anti-sRBD IgG quantitatively. Results were then analyzed and compared using IBM Statistical Package for Social Sciences ver 25.0 with Mann−Whitney non-parametric test.

RESULTS: The study shows a higher median antibody count in the naturally infected group compared to the vaccinated group (132.70 vs. 11.95 U/mL; $p < 0.001$). Further studies on the topic should be conducted to determine the comparison on a larger scale.

CONCLUSION: The s-RBD antibody titer is significantly higher in naturally infected patients than in vaccinated individuals.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has been going on for more than 2 years, with more than 635 million confirmed cases and more than 6.6 million deaths by November 22, 2022. However, the case growth has been successfully flattened with various efforts, particularly COVID-19 vaccine employment [1]. As of November 15, 2022, around 12.9 billion vaccine doses have been administered, with 68.4% of the world population receiving at least one vaccine dose, with daily vaccine administration reaching 2.5 million doses [2]. The continuous research and trial for the diagnosis and treatment of COVID-19 is based on the novel research of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine structure.

The SARS-CoV-2 virus or simply called coronaviruses belongs to the *coronaviridae* family. The SARS-Cov-2 is part of the RNA viruses with single-stranded positive-sense RNA (+ssRNA) with a genome size of 27–32 kb. The SARS-CoV-2 is constructed with four structural proteins (S, E, M, and N) and sixteen non-structural proteins (nsp1–16). The non-structural proteins are responsible for the reproduction and virulence of the virus itself. The spike protein (S) of SARS-CoV-2 is accountable for entering host cells with spike glycoproteins forming homotrimers that protrude from the viral surface. The S protein is also composed of two subunits, the S1, and S2. The S1 subunits consist of the N-terminal domain and receptor-binding domain (RBD) to bind the SARS-Cov-2 into the known receptor on host cells, namely Angiotensin-converting enzyme 2 receptor (ACE2) [3]. The RBD includes two structural domains, namely the core and external subdomains, composed of five β antiparallel strands and loops, stabilized with the di-sulphide bond [3], [5].

The composition of RBD in the spike protein, making the glycan coat paired with the flexibility of SARS-CoV-2 spikes, enables them to identify the host cell surface and bind with ACE2 receptors [6]. The RBD protein also functions through two different states, the closed "down" and open "up" structure, to enable human ACE2 receptor identification while shielding the receptor-binding regions from neutralizing antibodies [7]. The nature of RBD regions in the S

protein of SARS-CoV-2 also has been evaluated, with more sensitive S protein to ACE2 receptor than other coronaviruses. This might explain the 24% difference in structure domains in the RBD and S protein of SARS-CoV-2 compared to different SARS-CoV lineage [8]. The structure is furtherly evolving throughout the pandemic as new variants and mutations of the RBD protein, particularly in the arrangement of the amino acids, with increasing virulence and binding affinity along with antibody escape, particularly on the B.1.617.2 (Delta variant) and B.1.1.529 (Omicron variant) [9], [10].

The S protein, particularly RBD, has become the target for various diagnostic modalities. The SARS-CoV-2 RBD IgG test has been implemented as an antibody test for COVID-19 through the Enzyme-Linked Immunosorbent Assay (ELISA) method for detecting SARS-CoV-2 IgG in human serum. The RBD IgG test is developed for identifying individuals with an adaptive immune response to SARS-CoV-2 [11]. The ELISA to detect IgM, IgA, and IgG antibodies against RBD of SARS-CoV-2 has revealed sensitivity 47%, 80%, and 88%, respectively, with the specificity of 98–100% [12]. The diagnosis using the RBD domain with chemiluminescent reduction-neutralizing test also displays the use of diagnostic serology method for B.1.1.7 and B.1.351 variants using anti-RBD antibody qualitative assessment, with 99.1% and 100% sensitivity and specificity, respectively. The presence of anti-RBD antibodies also correlates with the emergence of neutralizing antibodies to guide the clinical or public health decisions during the pandemic [13], [15].

The RBD is also observed to be the potential target for treatment modalities and vaccine development using S protein has led to antibody-blocking therapy and small molecule inhibitors [8]. The RBD can also be used as an antigen, leading to many neutralizing antibodies isolated to be used in COVID-19 treatment. The presence of RBD-targeting antibodies, which have displayed neutralizing characteristics towards SARS-CoV-2, has been observed to achieve a cross-neutralizing effect which might lead to the development of antibodies targeting the SARS-CoV-2 specifically [16]. The RBD is also used as a target for vaccine development [17]. Several recombinant subunit vaccines containing the RBD of SARS-CoV-2 and Fc fragment of human IgG, such as Adimr SC-2f, S-RBD protein vaccine of China, ZF2001 using a dimeric fragment of RBD, VIR-7831, AZD7442, or LY-CoV555 [17]. The more well-known commercial vaccine such as Moderna mRNA-1273 or BioNTech-Pfizer BNT162b1 also uses RBD as the vaccine target by generating the protein through mRNA [18].

The use of quantitative spike-RBD (s-RBD) antibody quantitative assessment has been implemented, mainly to find antibody titers of infection patients or vaccinated subjects [19]. However, a direct comparison between the naturally infected and vaccinated subjects might not be made. Therefore, this study aims to compare the s-RBD quantitative antibody titers between naturally infected and vaccinated individuals.

Materials and Methods

Ethical consideration

All the participants were provided with an adequate explanation of the reasons for retaking the study and its procedures. The participants signed informed consent forms, and their demographic data and medical history were recorded. The study protocol was approved by the local ethics committee at each institution and conducted by the Declaration of Helsinki principles.

Study design and participants

This type of research is analytic observational with a cross-sectional study design. This research was conducted at the outpatient department and inpatient department of the Gastroenterology division of the Prof. Dr. RD Kandou Manado and colonoscopy was carried out at the Gastrointestinal Endoscopy Center, Prof. Dr. RD Kandou Hospital Manado. Examination of fecal SCFA and fecal calprotectin levels was carried out at the Manado Prodia Laboratory. The study was conducted from December 2021 to June 2022.

Population and sampling

The study was conducted on Prof. Dr. R. D. Kandou Hospital Manado COVID-19 patients. Patients with positive reverse transcription polymerase chain reaction results will be considered COVID-19 patients. COVID-19 patients of adult age (≥18 years old) within 2 weeks after diagnosis, without any autoimmune or immunodepression diseases or states, is eligible for the study. The participants must not use any steroid or immunosuppressant medications before the study. The team also searched for participants who have been vaccinated, with any vaccine, through consecutive sampling methods. Throughout the sampling process, a total of 49 participants, consisting of 21 infected participants and 28 vaccinated participants, were retrieved for the antibody analysis.

The participants were then assessed for their quantitative antibody count, using s-RBD as the protein target. The assessment used the Enhanced “Chemiluminescence” Immunoassay (ECLIA), using the Elecsys® Anti-SARS-CoV-2 kit [20]. The ECLIA method uses the binding of specific antibodies on serum plasma to a specific antigen in the reaction well. First, the samples will be incubated with recombinant antigens, namely the ruthenium complex, resulting in antibody/antigen complexes forming. The complex

was then added with streptavidin-coated microparticles to bind the complex into a solid phase. Next, the reaction mixture is aspirated to the measuring cell by magnetizing the microparticles to the electrode surface. The application of voltage to the electrode will induce chemiluminescent emission, which is then measured by the photomultiplier. The resulting signal will determine the level of specific antibodies in the patient's samples. As per the method above, 20 μL of the patients' samples or patients' serum will be incubated with a mix of biotinylated and ruthenylated nucleocapsid (n) antigens forming a double-antigen sandwich complex for 9 min. The streptavidin-coated microparticles were then added to create a solid phase of biotin and streptavidin for another 9 min for incubation. The reagent mixture was then measured on the measuring cell. A cut-off of <0.8 U/mL is considered non-reactive, with an antibody count of ≥0.8 U/mL considered reactive.

Data analysis

The data analysis is conducted using the IBM® Statistical Package for Social Sciences (SPSS®) ver. 25.0. First, patients' characteristics on age and gender are extracted. The outcome of the antibody count was then classified into a naturally infected group and a vaccinated group. Data were then assessed for their normality using the Saphiro−Wilk analysis. Finally, comparing both groups on the antibody count used the Mann-Whitney non-parametric test. A $p < 0.05$ is used to address the significance of the test result.

Results

A total of 49 participants (21 in the natural infection group and 28 in the vaccinated group) were included in the study (Table 1). The age of participants, based on the median value, is older in the vaccinated group compared to naturally-infection (38.5 vs. 34 years old). In addition, the gender distribution for females is higher in the natural infection group, with the same number of males and females in the vaccinated group. All data are not normally distributed; thus, information is displayed in median (min-max).

Table 1: Patients' characteristics and antibody count outcome on natural infection and vaccinated group

Variable (s)	Natural infection (n = 21)	Vaccinated (n = 28)	p-value (If available)	Total (n = 49)
Age (years old)	34 (25–67)	38.5 (25–78)		38 (25–78)
Gender				
Male	6	14		20
Female	15	14		29
Antibody count (U/mL)	132.70 (0.00–250.00)	11.95 (0.88–250.00)	<0.001[1)]	24.26 (0.00–250.00)

[1)]Mann–Whitney non-parametric test.

The antibody count based on the ECLIA antibody test for IgG on SARS-CoV-2 has displayed a significantly higher count in the naturally infected group than in the vaccinated group (132.70 vs. 11.95 U/mL) Figure 1. Also shows the considerably lower antibody count in most vaccinated participants than in the naturally infected group.

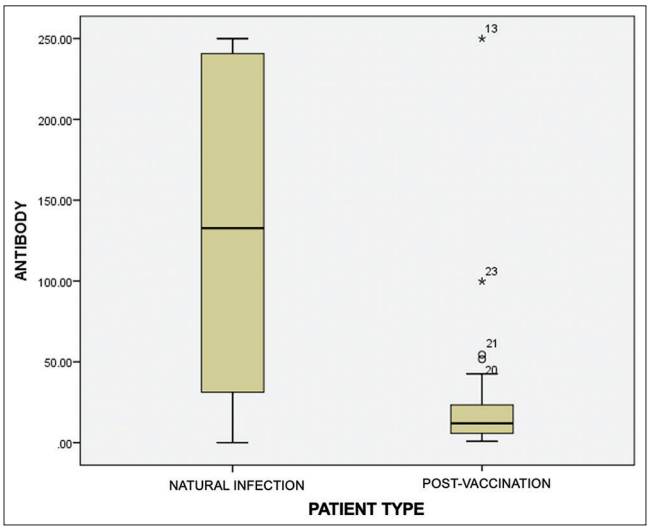

Figure 1: Antibody count using enhanced "chemiluminescence" immunoassay analysis on both the natural infection group and the vaccinated group. A higher value of antibody count in the natural infection group is observed

Discussion

Our study has compared the antibody count of the naturally infected individuals with the vaccinated individuals, with a significantly higher antibody count in the infected group than in the vaccinated group. Up to the writing of this manuscript, the authors believe this study is the first to compare the IgG antibodies toward s-RBD level between the naturally infected and vaccinated individuals. In addition, the increase in antibody levels and seroconversion rate after the use of the COVID-19 vaccine have been proven by various trials on COVID-19 vaccine efficacy.

However, other studies on a similar topic might not support our study. The COVID-19 vaccine achieves almost 98% seroconversion rate as compared to the antibody seroconversion rate of non-vaccinated individuals with prior infection [21]. The results are also in line with a study by Alatab *et al*., showing lower anti-s-RBD IgG levels in the previously infected subjects compared to the vaccinated subjects (2,110 vs. 1,341 BAU/mL), with higher IgG levels found in subjects with the third dose of vaccine (booster) [19], [22]. Roltgen *et al*. also stated the higher IgG levels observed in patients vaccinated with the BioNTech/Pfizer mRNA vaccine BNT162b2, as IgG is more dominant in the vaccinated patients as compared to increased IgM or IgA in the infected patients. The report is still suitable even with the new variants such as B.1.1.7 or B.1.351 [23]. Most

studies evaluated the comparison of antibody count from the vaccinated subjects with prior infection compared to vaccinated subjects without previous infection. Ali *et al*., report higher IgG, IgA, and neutralizing antibodies in the vaccinated with prior infection [24]. Similar results were also reported by Demonbreun *et al*. and Pantelidou *et al*., with higher values of median IgG and neutralization rate as compared to the non-infected patients [25], [26].

The result of this study, however, is quite in line with one of the results in a study by Jaworska *et al*., Despite the initial results of higher IgG value in the vaccinated group, the IgG decay curve was found steeper in the vaccinated group, particularly the BNT162b2 Thus, after 6 months of post-vaccination, a lower IgG level is observed in patients as compared to hospitalized COVID-19 patients 6 months earlier [27]. This also might explain the phenomenon found in this study, considering the non-defined vaccination period of the included participants.

Our study also factored in the age and gender of the participants. Sasso *et al*. reported higher s-RBD IgG levels in the female group compared to the male, despite a similar decay rate of the IgG [22]. Ikezaki *et al*. also reported that the mean titer of anti-spike IgG was lower in the older age group after adjustment for sex, body mass index smoking habits, and alcohol drinking habits. Other variables are found not significant on the IgG antibody count [28]. However, age is considered a significant positive negative correlation toward (s-RBD), with lower antibodies found between 30 and 39 years old [29]. Lower length of stay in the hospital and lower oxygen need are also associated with the increased s-RBD antibody count [30].

This study is not without limitations. Without defining the time frame, the diverse definition of COVID-19 cases and vaccination status might lead to non-conclusive results due to IgG decay status.

Conclusion

The s-RBD antibody titer is significantly higher in naturally infected patients than in vaccinated individuals. Further analysis and study on the topic, particularly in defining a suitable time frame, is needed to properly understand the effect of the COVID-19 vaccine compared to infection in terms of antibodies with a larger sample size.

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