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ANALYTICAL STUDY OF THE CORRELATION BETWEEN THE LEVEL OF IGG ANTIBODIES AND PREVIOUS EXPOSURE TO SARS-COV-2 ANTIGEN IN A PRIVATE HEALTH INSTITUTION IN THE MONTH OF JULY 2021

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ABSTRACT

The COVID-19 is a disease caused by the infection of coronavirus, SARS-CoV-2. Serological tests, performed using conventional Enzyme Linked Immunosorbent Assay (ELISA) by detection on chemiluminescence (CLIA) or electrochemiluminescence (EIA) platforms, are intended to identify antibodies to SARS-CoV-2 and contribute to the identification of people who have been exposed to the virus, through active immunization or by previous coronavirus infection. To correlate the amount of IgG antibodies in the serological samples analyzed by chemiluminescence, and previous exposure to the SARS-CoV-2 antigen, in a private health institution, specialized in clinical pathology/laboratory medicine, in the period of July 2021. An analytical, observational cross-sectional study was carried out. The level of IgG antibodies obtained in the serological samples and the type of vaccine against SARS-CoV-2 received by each employee with a result detected were evaluated in July 2021. There was a significant association between IgG level and previous Covid-19 (p=0.008): of 220 individuals with a very high IgG level, 54.1% had previous Covid-19; at the low IgG level, 91 (63.6%) did not have Covid-19. It is concluded that among employees who were vaccinated, those who had already acquired COVID-19 prior (about 54%) produced high and very high levels of IgG.

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INTRODUCTION

Regarding the immunopathogenesis of the COVID-19, it is known that, in a physiological way, the human body has, as one of the mechanisms of protection against the presence of pathogens, the development of the immune response that grows after exposure to the virus, resulting from the simultaneous actions of B lymphocytes, responsible for the production of antibodies characteristic of humoral immunity, and T lymphocytes, responsible for cellular immunity and for helping B cells to promote humoral immunity (Sewell *et al.*, 2020, Taefehshokr*et al.*, 2020, Dan *et al.*, 2021). It is known that the immune response is paramount for the control and destruction of the coronavirus infection, however, an unbalanced response can result in more serious cellular damage. Thus, the human organism has innate immunity, responsible for preventing viral replication, promoting viral clearance, inducing tissue repair and promoting a probable prolonged immune response against the virus. In view of this pandemic scenario, screening tests have become extremely important in the medical routine, with serological tests performed using conventional enzyme immunoassay techniques as representatives, such as Enzyme Linked Immuno sorbent Assay (ELISA) by detection on chemiluminescence (CLIA) or electrochemiluminescence (EIA) platforms. These are intended to identify antibodies to SARS-CoV-2 and contribute to the identification of people who have been exposed to the virus, through active immunization (with attenuated or inactive virus) or by previous infection with COVID-19 who have been cured (Manalac *et al*., 2020, Bryan *et al*., 2020).

The accuracy of these tests may vary according to the method and type of antigen used in the reaction, in addition to the collection period at the onset of symptoms (ideally after 7 to 10 days for IgM and IgA and after 15 days for IgG) (Dias *et al*., 2020). Such tests are performed only in clinical laboratories and seek to identify IgG, IgM, IgA and total antibodies in a qualitative or semi-quantitative manner. They also allow the patient to be monitored for reassessment of possible serological changes in IgG class antibodies or increase in their titers, when the results are very close to the cut-off point, in the first collection.The tests are performed by collecting the patient's blood, and identify the presence of IgM and IgG antibodies, markers of recent and late infection, respectively. The presence of IgM antibodies tends to indicate a recent immune response, while the detection of IgG antibodies indicates a later stage called memory, both of which are used for surveillance and screening. Vaccination against COVID-19 has generated a significant demand from those vaccinated for serological tests, to know if the individual has antibodies against the SARS-COV-2 virus. However, the mechanism of post-vaccination immunity or even after the natural disease is complex, as it involves an immune response that does not depend only on neutralizing antibodies, but also on innate immunity, cellular response and humoral response (CDC, 2020). Like SARS-CoV-1, the SARS-CoV-2 S protein binds to the cell surface through angiotensin-2 (ACE2) converting receptors. Neutralizing antibodies seem to be predominantly directed to the S protein (spike), while the nucleoprotein (NC) protein plays a crucial role in viral replication and induces earlier antibody production (Hoffman *et al*., 2020). Antibody recognition after the onset of infection is analyzed in the first few weeks, with seroconversion to IgM and IgG generally occurring 1 to 3 weeks after the onset of symptoms (Kowitdamrong*et al*., 2020, Krajewski *et al*., 2020). In this way, patients can be classified as anti-IgG reagent, anti-IgG non-reactive or indeterminate, and it is also possible to quantitatively analyze the IgG titer of each patient based on serology results such as low, moderate, high and very high. Thus, this method stands out as the method of choice for the direction of the present research, which aims to highlight the influence of previous exposure to SARS-Cov2 on the amount of IgG antibodies in employees who worked in the fight against COVID-19, submitted to testing at a reference diagnostic center in Belém-PA, in the period of July 2021.

MATERIALS AND METHODS

Analytical, observational, cross-sectional correlation study between the level of IgG antibodies and previous exposure to the SARS-COV-2 antigen in a private health institution, obtained from serological samples and the type of vaccine against SARS-CoV-2 received by each employee with a result detected in the analysis of the blood serum, in the period of July 2021. The sample consisted of 621 tested employees. The research and data collection were carried out between February and May 2022. Were analyzed the amount of IgG antibodies of health workers, vaccinated or not, that worked on the front line against the coronavirus, who chose to carry out the serological test for detection of IgG antibodies during the month of July 2021 and who filled out a form with information about vaccination and previous diagnosis of COVID-19, that is currently under domain of a referral laboratory service in Belém-PA, Brazil, which analyzed theirs blood serum. All employees whose results were non-reactive, indeterminate and those whose notification form was poorly filled out, incomplete or illegible were excluded from the study. Variables studied: sociodemographic characteristics; Amount of IgG in the serology; Received vaccine; Previous diagnosis of COVID-19; Close contact with a patient diagnosed with COVID-19; Time elapsed since each of these events.Vaccines evaluated in the work were Pfizer, CoronaVac and AstraZeneca. Due to the still scarce number of studies on the disease and the lack of a consensus in the literature about the quantitative classification of anti-IgG against the coronavirus, in this work were categorized, for organizational and didactic purposes, the numerical values of IgG in AU/ml in: Low for IgG values <100 AU/ml; Moderate for IgG values between 100-250 AU/ml; High for IgG values >250 AU/ml; Very high for IgG ≥ 400UA/ml values.

Data were organized in Microsoft Excel 2010 program. Tables were built using tools available in Microsoft Word, Excel and Bioestat 5.5 programs. All tests were performed using the Bioestat 5.5 software. Quantitative variables were described by minimum, maximum, mean, median and standard deviation and qualitative variables by frequency and percentage. Confidence intervals of 95% were calculated for the proportion to infer how the prevalence behave in relation to the population from which they were obtained. The independence or association between two categorical variables was tested by the chi-square test and the significant associations were detailed by the analysis of standardized residues, to identify the categories that most contributed to the result. Results with $p \leq 0.05$ (bilateral) were considered statistically significant. Documents that ensured ethical aspects of the research were used. All research subjects were studied according to the precepts of the Declaration of Helsinki, of the Nuremberg Code and respected the Norms of Research Involving Human Beings (Res. CNS 466/12) of the National Health Council. The research was carried out after approval of the project by the Research Ethics Committee with human beings of the University Center of the State of Pará. Data were collected after the researchers signed the Term of Commitment to Use Data and the authorization term signed by the directors of the laboratory of interest.

RESULTS

A total of 621 patients were included in the study. Most (466 or 75%) were female, and the predominant age group was 20 to 39 years old.Almost all subjects (609 or 98.1%) had a reagent test result. Of the total number of employees, 10 (1.6%) had a non-reactive result and 02 (0.3%) had an undetermined result. To assess the generalizability of proportions, 95% confidence intervals were calculated for prevalence (95%CI). We found 609 reactive tests (98.1%), 10 non-reactive (1.6%) and 2 indeterminate (0.3%), with a 95% CI, respectively, of 96.6 – 98.9, 0.8 – 3.0 and 0.1 – 1.3. Numerical IgG values in AU/ml were categorized as low (<100), moderate (100-250), high (>250) or very high (≥ 400). 220 (35.4%) subjects had a very high level of IgG, and 161 subjects (25.9%) had a moderate level. About half (319 or 51.4%) of participants with analyzed samples had no previous diagnosis of COVID-19. Another 30.4% of subjects had 13 to 18 months since their last COVID-19 infection (range 0.4-18 months, mean 11.3 ± 4.9 months). About 86.3% had had close contact with a positive case. Table 1 shows that most patients (569 or 91.6%) were vaccinated, the majority being vaccinated with the vaccine from the manufacturer AstraZeneca (337 or 54.3%), while the CoronaVac and Pfizer vaccines corresponded, respectively, to 34.6% and 2.1% of the vaccinated. Most employees had received the 1st dose (572 or 92.1%) and the 2nd dose (523 or 84.2%) of the Pfizer, CoronaVac and AstraZeneca vaccines. The values of months since the first dose ranged from 0.0 to 8.1 months, with a mean of 4.1 ± 1.6 months. The months since the second dose ranged from 0.0 to 6.0, with a mean of 2.3 ± 1.9 months.

Table 1. Vaccination carried out on employees who worked in the fight against Covid-19, evaluated in July 2021, Belém-Pará

Variable	Frequency	Percentage
Vaccine		
Yes	569	91.6
No	50	8.1
Vaccine		
AstraZeneca	337	54.3
CoronaVac	215	34.6
Pfizer	13	2.1

The percentages are relative to the total number of patients (n=621).
Source: Prepared by the authors.

In the very high IgG level, 84.9% were female, this proportion being higher than expected by the statistical test (†); in the low IgG level, 31.5% were male, and this proportion was also higher than expected. That is, females were associated with a very high level of IgG and males were associated with a low level.

Table 2. Relationship between the levels of IGG, age and sex of employees working in the fight against Covid-19, evaluated in July 2021, Belém-Pará

Variable	Low (n=143)	Moderate (n=161)	High (n=97)	Very High (n=219)	p-value
Sex					<0.001
Feminine	*98 (68, 5)**	116 (72.0)	66 (68.0)	*186 (84, 9)†*	
Male	*45 (31, 5)†*	45 (28.0)	31 (32.0)	*33 (15, 1)**	
Age					0.001
0 to 19 years	6 (4.2)	4 (2.5)	2 (2.1)	9 (4.1)	
20 to 39 years	101 (70.6)	112 (69.6)	*74 (76, 3)†*	*115 (52, 3)**	
40 to 59 years	32 (22.4)	40 (24.8)	*18 (18, 6)**	*89 (40, 5)†*	
60 to 85 years	4 (2.8)	5 (3.1)	3 (3.1)	7 (3.2)	

Source: Prepared by the authors.

Table 3. Relationship between previous IgG and Covid-19 levels of employees who worked in the fight against Covid-19, evaluated in July 2021, Belém-Pará

Variable	Low (n=143)	Moderate (n=161)	High (n=97)	Very High (n=220)	p-value
Covid-19					0.008
Yes	*52 (36, 4)**	82 (50.9)	49 (50.5)	*119 (54, 1)†*	
No	*91 (63, 6)†*	79 (49.1)	48 (49.5)	*101 (45, 9)**	

Source: Prepared by the authors.

Table 4. Relationship between IgG and time since the last infection of employees who worked in the fight against Covid-19, evaluated in July 2021, Belém-Pará

Variable	Low (n=51)	Moderate (n=82)	High (n=49)	Very High (n=116)	p-value
Months Since Last Infection					0.022
0 to 6 months	*22 (43, 1)†*	18 (22.0)	13 (26.5)	*22 (19, 0)**	
7 to 12 months	8 (15.7)	9 (11.0)	5 (10.2)	12 (10.3)	
13 to 18 months	*21 (41, 2)**	55 (67.1)	31 (63.3)	*82 (70, 7)†*	

Source: Prepared by the authors.

In relation to IgG level and age group, there was a significant association (p=0.001): of the 97 individuals with a high IgG level, 76.3% were aged between 20 and 39 years, a proportion greater than expected by the statistical test; of the 220 individuals with a very high IgG level, 89 (40.5%) were aged between 40 and 59 years, a proportion greater than expected (†). In other words, age 40-59 years was associated with a high level of IgG (Table 2). There was a significant association between IgG level and previous Covid-19: of the 220 individuals with a very high IgG level, 54.1% had Covid-19, a proportion greater than expected by chance; at the low IgG level, 91 (63.6%) did not have Covid-19, in a higher proportion than expected (Table 3). Of the 51 individuals with a low IgG level, 43.1% had been between 0 and 6 months since the last infection, this proportion being higher than expected by the statistical test; in the very high IgG level, 82 (70.7%) had 13 to 18 months since the last infection, a proportion higher than expected by chance. That is, a very high IgG level was associated with a longer time since the last infection. (Table 4) The level of IgG and vaccine were significantly associated (p<0.001): of the 95 individuals with a high IgG level, 75 (78.9%) had AstraZeneca vaccine, which was higher than expected by the statistical test; of the 215 individuals with a very high IgG level, 160 (74.4%) had AstraZeneca vaccine, which was higher than expected by the statistical test (†); of the 109 individuals with a low IgG level, 78% had the CoronaVac vaccine, and this proportion was higher than expected. That is, AstraZeneca was more associated with high and very high levels of IgG. Between IgG level and months since the first vaccine dose, there was a significant association (p<0.001): in the very high IgG level, 4.8% had up to 1 month since the first dose, this proportion being higher than expected; of the individuals with a high IgG level, 47 (52.2%) had 2 to 3 months since the first dose, this proportion being higher than expected by the statistical test (†); of the subjects with a very high IgG level, 145 (69%) had 4 to 6 months since the first dose, this proportion being higher than expected; of the individuals with low IgG levels, 38.3% had 6 to 8 months since the first dose, with this proportion being higher (†) than expected. Between IgG level and months since the second dose, there was a significant association (p<0.001): in the high IgG level, 56 (63.6%) had up to 1 month since the second dose, a proportion higher than expected by chance; of the 183 subjects with a very high IgG level, 70 (38.3%) had 2 to 3 months since the second dose, this proportion being higher than expected by the statistical test (†); of the low IgG subjects, 68 (75.6%) had 4 to 6 months since the second dose, which is a higher proportion than expected.

DISCUSSION

Since the beginning of the pandemic, especially during the vaccination campaigns against COVID-19, a great concern has arisen within the lay and scientific communities about the possible quantitative serological outcomes in patients undergoing different types of vaccine (Oliveira Silva *et al*, 2021). The present study analyzed health workers in the fight against COVID-19 in sociodemographic and laboratory aspects with the objective of reaching a population whose social isolation was lower and whose chances of infection during the pandemic were higher, since they continued to carry out their work activities. During all or most of the isolation period (Teixeira *et al*., 2020). In addition, since the distribution of workplaces varies between hospital, outpatient and laboratory environments, with different degrees of exposure to the virus, a less biased assessment of the variables studied was allowed. The method of choice for the quantitative serological analysis of specific IgG anti-S1 and IgG anti-S2 antibodies against SARS-CoV-2 in the present study is the CLIA, which has sensitivity values of 97.4% and specificity of 98.5%, both results evaluated and reinforced by Escribano *et al* (2020). Chemiluminescence is measured in relative light units (relative light unit, RLU), and by comparing the sample RLU with the calibrator's RLU, the signal/ cutoff index (S/C) is obtained. Thus, there is a direct relationship between the measured RLU production and the number of antibodies present in the sample (Manalac *et al*., 2020, Bryan *et al*., 2020). A total of 621 patients were analyzed in the study. Being about 75% female and 24.8% male. Furthermore, 402 (65%) participants were aged between 20 and 39 years and 179 (28.8%) were aged between 40 and 59 years. Such findings are different from those found in the survey "Working Conditions of Health Professionals in the Context of Covid-19", published by Leonel, F, which shows most health professionals on the front line in the fight against Coronavirus in the range aged 36 to 50 years (Leonel *et al*., 2021). About 536 individuals (86.3%) had close contact with a positive case; of these, only 302 (56% of the 536

contacts) developed infection with the virus. This shows that, despite the high contact of these professionals, only about half of them contracted the disease; the study by Luo L *et al* reveals that the chance of contagion after close contact in health institutions is 10%, different from what we can observe in the present study (Luo *et al*., 2020). Such discrepancy may be due to the different safety protocols observed in the different hospitals of the different studies; however, data such as climate, public investment in population orientation and level of proximity to the infected patient cannot be ruled out as influencing this variation. Most (569 or 91.6%) had been vaccinated, with only 50 (8.1%) employees not vaccinated. More than half (337 or 54.3%) had received the vaccine from the manufacturer AstraZeneca, which was due to its greater availability at the time of interest in the present study. For Lima EJ da F, the probable cause of this number of unvaccinated people stems from the still existing fear of receiving a newly manufactured product or even the lack of trust in the health authorities (Lima *et al*., 2021, Oliveira *et al.*, 2021). Since, as previously described in this work, there are multiple types of vaccines and mechanisms of action of human immunity, it is expected that different patients will have different reactions to the same vaccine and that the same patient will have different reactions to vaccination, depending on the prior contact or not to the disease antigen (Fernandes, 2021).Factors such as sex, age, previous diagnosis, contact with diagnosed patients and the time elapsed since diagnosis and/or the first and second doses of the vaccine were identified as influencing agents in the production of anti-IgG by the patients studied, data also observed in studies such as the one by Fernandes (2021).

Contrary to what is shown in the study by Pollán, M *et al.* (2020), which highlighted the indifference in the production of anti-IgG by both sexes, in our series we observed a higher production of antibodies by female patients, most of whom were (252 or 54.1%) at high and very high levels, in a greater proportion than expected by the statistical test, while most of those males (90 or 58.4%) were associated with low and moderate levels. This finding is consistent with what Santos *et al* (2021) analyzed in their 2021 article. Regarding age, it was noted that the age group of 20-39 years was found to have a high IgG level in a greater proportion than expected by the statistical test, representing 76.3% of the 97 patients at this level; however, despite this proportion, the majority of these patients concentrated on low and moderate IgG levels (213 or 52.9%), while the 40-59 age group was associated with a high and very high IgG level (107 or 59.7%). We can, therefore, state that there was an increase in the production of anti-IgG with the gradual increase in age, a finding that is different from those commonly described by various authors, such as Vilas Boas *et al.* (2021) and Oswaldo Cruz Foundation (2021), who reports that there is a drop in the levels of IgG produced with the gradual increase in the age of patients. In addition, there was a significant association between IgG level and previous Covid-19 (p=0.008): of the 220 individuals with a very high IgG level, 54.1% had Covid-19, a proportion greater than expected by chance. As stated by Peixoto *et al.* (2022), it can be observed that, of the employees with high (50.5%) and very high (54.1%) levels of anti-IgG, the majority correspond to those with a previous diagnosis of the disease, (50.5 and 54.1% respectively), a higher number than expected by chance, which, according to the authors, would be related to the immunological action of memory B cells. At the low IgG level, 91 (63.6%) did not have Covid-19, also in a higher proportion than expected. According to the study by Krammer *et al.* (2021), the lack of previous contact with the coronavirus antigen triggered relatively smaller and variable responses compared to those who were previously seropositive, which coincides with the findings of the present study.

Regarding the association between IgG level and months since the last infection, of the 51 individuals with a low IgG level, 43.1% had 0 to 6 months since the last infection, a proportion greater than expected by the statistical test; in the very high IgG level, 82 (70.7%) had 13 to 18 months since the last infection, a proportion also higher than expected by chance. That is, a very high IgG level was associated with a longer time since the last infection. In this sense, patients previously infected with COVID-19 tend to have a more pronounced increase in their levels of antibodies against the disease and to maintain these levels after long periods of diagnosis and vaccination. Such analysis corroborates studies such as those by Krammer *et al* (2021), Zollner*et al* (2021)and Alegre *et al*. (2021), who reported a faster and more uniform increase in IgG titers in these patients compared to those who were previously seronegative. A significant association was found between IgG level and vaccine: of the 95 individuals with a high IgG level, 75 (78.9%) had AstraZeneca vaccine; of the 215 subjects with a very high IgG level, 160 (74.4%) had AstraZeneca vaccine. Of the 109 individuals with a low IgG level, 78% had the CoronaVac vaccine. That is, AstraZeneca was more associated with high and very high levels of IgG, while CoronaVac with low levels. This is certainly due to the different composition and form of action of each vaccine in the body. However, according to several studies, such as the one published by Teixeira LCS in 2021, comparing the effectiveness of vaccines and defining the best option is unfeasible, since they are not similar in terms of composition and preparation technique (Teixeira, 2022). Thus, the most sensible thing is for each vaccine to be studied and compared with other similar ones, to obtain more accurate and reliable results. Between IgG level and months since the first vaccine dose: in the very high IgG level, 4.8% had up to 1 month since the first dose, this proportion being higher than expected; of subjects with a high IgG level, 47 (52.2%) had 2 to 3 months since the first dose; of subjects with a very high IgG level, 145 (69%) had 4 to 6 months since the first dose; of the individuals with a low IgG level, 38.3% had 6 to 8 months since the first dose, and this proportion was higher than expected. Were observed, then, that the peak of the amount of IgG antibodies produced by the analyzed employees was in the period of 4 to 6 months after the first dose, a number that decreases over the following months. This finding reveals the need to administer the second dose of the vaccine to ensure the seropersistence of antibodies against the virus in question. Finally, there was a significant association between IgG level and months since the second vaccine dose: in the high IgG level, 56 (63.6%) had up to 1 month since the second dose; of the 183 subjects with a very high IgG level, 70 (38.3%) had 2 to 3 months since the second dose, this proportion being higher than expected by chance; of those with a low level, 68 (75.6%) had 4 to 6 months since the second dose. It is concluded, then, that the level of anti-IgG produced by those vaccinated with the 2nd dose declines over the months, which is also evidenced by Levin *et al* (2021)and Vilas Boas *et al.* (2021) in their articles published at the end of 2021.

CONCLUSION

It was observed that the proportions of individuals of different sexes and different age groups varied significantly. There was a higher production of antibodies in female patients. It was found that the age at which higher amounts of IgG were produced was in the age group between 40-59 years, representing about 60% of the analyzed sample. In addition, about 91% of employees were vaccinated. Among the immunized individuals, it was observed that about 54% received the AstraZeneca vaccine, of which 70% produced very high and high IgG levels. Of the 215 employees who received the CoronaVac vaccine, only 30% produced very high and high IgG amounts. Finally, only 2% received the Pfizer vaccine and among these, 61% of the individuals produced very high and high IgG amounts. It can also be concluded that there was an increase in the production of IgG for individuals who were immunized. About 78% of the immunized employees had very high and high IgG levels. It has been shown that among employees who were vaccinated, those who had previously acquired COVID-19 produced high and very high levels of IgG. The coronavirus pandemic caused millions of deaths worldwide, in addition to countless sequels after acquiring the disease. However, there was a low amount of reliable literature on the subject. Therefore, there is a need for more concrete studies to expand the understanding of COVID-19 and thus reduce technical, scientific, social and moral damages.

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