Original Article

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Internal Medicine Section

Antibody Response following Exposure to SARS-CoV-2: Is It a Reliable Marker of Immunity?

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ABSTRACT

Introduction: Infection and vaccination with the viral vector vaccine Covishield are both expected to produce immunity in the body against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Production of neutralising antibodies as a result of the humoral immune response plays a key role in defending against this deadly infection. A lack of virus-specific antibodies in the serum does not always imply a lack of immunological memory. The immune response mediated by T cells is also important.

Aim: To check for the humoral immune response after exposure to the SARS-CoV-2 virus.

Materials and Methods: This cross-sectional observational study was carried out at Central Referral Hospital (CRH), a tertiary care hospital in Gangtok, Sikkim, India, from May to June 2021. A total of 90 participants were divided into three equal groups; unvaccinated with a history of infection with SARS-CoV-2 in the recent past, vaccinated but no infection and history of vaccination and infection both, respectively. The test was performed with COVISCREEN. It's a double antigen sandwich immunoassay that can detect total antibodies (IgM+IgG+IgA) simultaneously to the SARS-CoV-2 virus. The Statistical Package for the Social Sciences (SPSS), version 16.0 for Windows, was used to analyse the data.

Results: Overall, 30 (33.3%) participants showed positive antibody tests out of total 90. Participants with prior infection exhibited more antibody responses irrespective of the vaccination status as compared to vaccinated participants with no prior infection, this difference was statistically insignificant (p=0.165).

Conclusion: Both B cell, as well as T cell immune responses following infection and vaccination, need to be evaluated to predict long term immunological memory and protective immunity against future infections with SARS-CoV-2.

Keywords: Agglutination, B cell, Severe acute respiratory syndrome coronavirus-2, T cell, Vaccine

INTRODUCTION

Infection and vaccination with the non replicating viral vector vaccine Covishield is expected to achieve immunity in the body against infection with SARS-CoV-2 [1]. Production of neutralising antibodies plays a key role in defending against this deadly infection [2], although the protective efficacy and the duration of the antibody-mediated immune response following primary infections are not known [3]. After infection with this virus, protective immunity might extend anywhere from months to years [4]. Several studies on Coronavirus disease-2019 (COVID-19) antibodies, as well as other components of the immune system, are now underway. Presently, it is not known whether cell-mediated or humoral immunity, or a combination of both, can protect us.

Immunoglobulin (Ig) M antibodies, which are formed early in an infection, and IgG antibodies, which are more likely to appear later, are the two types of antibodies formed in response to any infection. Antibodies against the Receptor Binding Domain (RBD) may linger in the blood for months or years in individuals recovering from COVID-19 [5], and those directed against the RBD are exceptional indicators of both old and current infection. Antibody isotypes can assist in distinguishing between recent and previous illnesses. IgG antibodies linger for several months after the initial infection and are intimately associated with neutralising antibodies. Hence, IgG level measurements are more accurate in predicting the immune response in the body [6]. The antibody levels that may protect against reinfection are unknown. Moreover, differences in laboratory techniques make this even more complex to decide. According to some findings, antibodies to SARS-CoV-2 do not persist in the serum after recovery from illness. Nonetheless, the lack of particular antibodies in the serum does not always imply that immunological memory is absent [7]. T cell-mediated immunity may potentially have a role in the prevention of reinfection. The main challenge with this immunity is that virus-specific T cells are hard to measure in a laboratory test. It's costly and not widely available. Although, antibodies may not be the sole criteria to check for the body's immune response and may play only a supporting role in defending against viruses, they are easier to measure in blood tests. In this study, authors aimed to check the humoral immune responses following infection, vaccination, or both in the studied population.

MATERIALS AND METHODS

This cross-sectional observational study was carried out at CRH, which is a tertiary care hospital in Gangtok, Sikkim, India. Sikkim is a small north-eastern state of India. The study period was two months May to June 2021. Prior ethical approval was obtained from the Institutional Ethics Committee (IEC) of the Sikkim Manipal Institute of Medical Sciences (ethical approval no-SMIMS/IEC/2021-38). Before recruitment into the study, informed consent was obtained from each participant.

Inclusion criteria: All consecutive participants >18 years and <65 years of age who consented to participate in the study were included. Clinically stable participants who attended medical Outpatient Department (OPD) in the study period were included.

Exclusion criteria: Participants <18 years or >65 years of age, unwilling, acutely ill or admitted patients, participants with some known immunocompromising conditions like active cancer treatment, consumption of immunosuppressive drugs due to any cause, chronic liver disease, or chronic kidney disease, uncontrolled diabetes mellitus, Human Immunodeficiency Virus (HIV), Acquired Immunodeficiency Syndrome (AIDS), and postsplenectomy were excluded from the study, 13 participants who did not satisfy inclusion criteria were excluded from the study.

Sample size calculation: A sample size of 90 was chosen as the study period was small and, as per the standard statistical rule, a minimum of 30 participants in each category should be included. The prevalence of 50.3% was found in another north-eastern state, Assam, according to the third sero survey report of the Indian Council of Medical Research (ICMR) [8]. A random sampling of eligible patients was done.

Study Procedure

A 90 participants, after fulfilling the inclusion criteria, were divided into three groups. Group 1 included unvaccinated participants with a history of infection with SARS-CoV-2 in the past 1-2 months. Group 2 participants were those with a history of vaccination with two doses of non replicating viral vector COVID-19 vaccine, Covishield in the last 3-4 months. Group 3 participants had both a history of vaccination with two doses of Covishield in the last 3-4 months followed by infection in the past 1-2 months. Demographic characteristics like age, sex, marital status, profession, and co-morbidities were noted.

The test was performed with COVISCREEN, a rapid, qualitative test kit. It's a double antigen sandwich immunoassay that can detect total antibodies (IgM+IgG+IgA) simultaneously to the SARS-CoV-2 virus in serum, plasma and whole blood as per the instruction card of the manufacturer. These antibodies are binding antibodies. This test cannot measure neutralising antibodies. Only the PRNT50 assay can measure those antibodies. The test kit is manufactured by Zephyr Biomedicals in Goa, India. A sensitivity of 100% and a specificity of 99.07% were observed in an in-house study by the company. Antigens that are specific to SARS-CoV-2 are coated as capture in the region 'T', a test region, and biotinylated bovine serum albumin in the control region 'C', as an assay control. The coloured virus-specific recombinant antigen indicator colloidal particle complexes with the virus antibodies, if present in the specimen, as the test specimen flows through the membrane assembly within the test device. The SARS-CoV-2 recombinant antigens immobilise this complex as it travels through the membrane to the region 'T'. This is coated as capture on the nitrocellulose membrane, which leads to the formation of a coloured band in the region 'T'. This confirms a positive test result. A negative test is indicated by the absence of the coloured band in the region 'T'. The unreacted conjugate and the unbound complex, along with the streptavidin colloidal gold conjugate, move further along the membrane. These are then immobilised by the biotinylated bovine serum albumin, which is coated in the region 'C' and forms a coloured band. This control band is used to confirm the test results.

To perform the test, 2 mL of blood was drawn and of that, 20 µL were dispensed carefully into the specimen "A" port of the kit and immediately checked for the presence of antibodies. The negative result is indicated by the presence of only one pink, purple coloured band area 'C' whereas the presence of viral-specific total (IgM+IgG+IgA) antibodies and a positive test is indicated by the presence of two pink-purple coloured bands both in the region 'C' and 'T'.

STATISTICAL ANALYSIS

The SPSS, version 16.0 for Windows, was used to analyse the data (SPSS 16; Chicago, IL, USA). The Chi-square test was used to compare categorical variables. The $p<0.05$ was considered statistically significant.

RESULTS

Out of 90 participants, 33 were males and 57 were females. A 21 of the 90 participants were between the ages of 18-20 years, 34 were between the ages of 21-39 years, 32 were between the ages of 40-59 years and three were between the ages of 59-65 years. A total of 54 participants were healthcare professionals and 57 were married. A significant association was found between variables like age, sex, marital status, and profession and the study groups ($p<0.05$), whereas it was insignificant between comorbidities and the groups ($p=0.935$) [Table/Fig-1].

Overall, 30 (33.3%) participants showed positive antibody tests. The number of participants with positive antibody tests was similar in group 1 and 3 suggesting that those with prior infection exhibited more antibody response irrespective of the vaccination status as compared to participants with no prior infection and only vaccination. Although, the difference was found to be statistically insignificant at $p<0.05$ [Table/Fig-2].

Variables	Unvaccinated and infected (Group 1)	Vaccinated and uninfected (Group 2)	Vaccinated and infected (Group 3)	Total	p-value
Gender					
Male	12	06	15	33	0.049
Female	18	24	15	57	
Age group (in years)					
≤20	12	0	9	21	<0.00001
21-39	6	22	06	34	
40-59	12	8	12	32	
≥60	0	0	3	3	
Marital status					
Married	9	18	30	57	<0.00001
Unmarried	21	12	0	33	
Profession					
Healthcare	2	25	27	54	<0.00001
Others[#]	28	5	3	36	
Co-morbidities					
Present	6*	6*	7*	19	0.935
None	24	24	23	71	

[Table/Fig-1]: Demographic details of the participants.
[#]All participants other than healthcare professionals.
*Group1-Hypertension (HTN) in 4 patients and Diabetes Mellitus (DM) in 2 patients.
*Group2-HTN in 6 patients.
*Group 3-HTN with DM in 3 patients, HTN with dyslipidemia in 3 patients, and one patient had a history of operated Ca breast

Vaccination status	n	Positive antibody test	Negative antibody test
Unvaccinated and infected (Group 1)	30	12 (40%)	18 (60%)
Vaccinated and uninfected (Group 2)	30	6 (20%)	24 (80%)
Vaccinated and infected (Group 3)	30	12 (40%)	18 (60%)
Total	90 (100%)	30 (33.33%)	60 (66.67%)

[Table/Fig-2]: Antibody response in study groups.
p=0.165

DISCUSSION

Infection and vaccination with Covishield both induced antibody responses in the present study. Of the total, only 30 (33%) participants exhibited a positive antibody response following exposure to the virus. Those with prior infection had a higher antibody response as compared to the uninfected population, irrespective of their vaccination status. Antibodies provide immunity against COVID-19. According to a study done by Iyer AS et al., there is a long lasting immune response against SARS-CoV-2, especially after severe infection [6]. It provides optimism that the people who have been infected with the virus will acquire long term immunity. According to the research, the IgG levels in COVID-19 patients increased for almost four months. This finding was linked to the presence of protective neutralising antibodies. On measuring serum and/or plasma antibody responses to the RBD of the spike (S) protein of SARS-CoV-2, it was found that anti-S neutralising antibody titres were closely associated with IgG antibodies to the SARS-CoV-2 RBD, which remained nearly constant throughout 75 days after infection. As a result of this antibody response, the likely duration of protection was at least four months. In the present study, only 40% of the participants had positive antibody responses between 1-2 months following infection. This insufficient response was similar to a drop in neutralising antibody titers as well as antibodies against S protein or nucleocapsid (N) protein reported in other studies [9,10].

According to a report, antibodies against this virus may only be short-lived, raising doubts about the virus's long term immunity [11]. It is predicted to decline because IgG has a half-life of around 21 days [12]. In mild cases, a fast drop in antibodies have also been reported [13]. These investigations, however, were restricted by small sample numbers. Furthermore, the majority of the samples were taken within two months after the illness [10,14]. According to some studies, antibodies may not be detected after infection [15,16]. In a Japanese study, highly variable antibody responses were observed in various conditions [3]. According to this study, when compared to those who did not, patients who received mechanical ventilation or Extracorporeal Membrane Oxygenation (ECMO) had greater neutralising antibody titres. Increased antibody titre was shown to be linked to a higher Body Mass Index (BMI) and fever. Patients with advanced illness had a stronger immune response as well. On the contrary, most patients who did not require oxygen assistance (97%) also had neutralising antibodies, suggesting that even minor infections may elicit long term immunological responses.

In the present study also, low positivity following infection could be due to various reasons, like mild infections, duration of postinfection being less than two months, or other factors discussed in the above studies. The decrease in antibody titres observed in many studies during the relatively early stages of recovery simply reflects a reduction in the number of short-lived plasmablasts. This is a normal immunological reaction and should not be a reason for concern. As a result, long-lived plasma cells in the bone marrow play an active role in keeping neutralising antibodies in the circulation [2]. The titres of antibodies may vary according to the severity of the illness and the viral load. In this study, only six participants out of 30 elicited an antibody response between 3-4 months after the second dose of COVID-19 vaccination. It is yet unknown which antibody titers and tests best relate to vaccination effectiveness. On review of the literature, two studies related to a postvaccination antibody response were found. As per the first study, antibody responses induced by the mRNA-1273 vaccine persisted for upto six months after the second dose [17]. According to the second study, after a single vaccination, people with recent SARS-CoV-2 infection elicited higher levels of antibodies after three weeks as compared to those without a history of infection. They had antibodies against four SARS-CoV-2 antigens and even higher antibodies with neutralising characteristics [18]. In this study also, the antibody response was higher (40%) in participants with a history of vaccination and infection as compared to vaccination alone.

While antibody response has been used as a sign of protection, it is vital to recognise that in this situation, antibody response cannot be equated with immunity. Understanding cell-mediated immunity might aid in understanding the immune response to infection. Antiviral antibody loads that are too high can cause immunological dysregulation, which can be detrimental. So, there is a need to look above and beyond humoral immunity to better predict disease severity and patient prognosis [19].

B cells and T cells can survive even if blood antibody levels are undetectable. Humoral immune maturation is linked to T follicular helper cells. Long term protection against infection requires virus-specific memory T cells and B cells. The presence of follicular T cells indicates the formation of a pool of memory B cells that respond swiftly to reinfection. Certain T cells are drawn from a pool of T cells that have been preconfigured to recognise certain viral antigens. Specific CD4+ T cells trigger a robust B cell response, which leads to antibody affinity maturation. Levels of S protein-specific T lymphocytes are linked to serum IgG and IgA titers [20]. Tools for measuring T-cell response may provide a better picture of the immune response generated by infection or vaccination. A T cell response has been detected in individuals infected with this virus approximately one week after symptom onset, whereas an antibody response can be seen between 10-12 days of infection [21,22].

Limitation(s)

The small sample size was definitely a limiting factor in this study. Serological cross-reactivity across the other coronavirus groups may occur in certain patients with prior exposure to Human coronavirus HKU1 (HCoV-HKU1) or NL63 or OC43 or 229E or SARS-CoV-2 or MERS-CoV etc. False-positive or false-negative findings can occur owing to the presence of interfering chemicals in the specimen or for reasons beyond the control of the manufacturer, such as testing related technical or procedural problems. The immunocompetence of the patient and the viral dose on exposure play a role in the generation of the antibody response and eventually the test result. Long term follow-up of at least six months to a year postinfection and vaccination is required to look for the persistence of antibodies.

CONCLUSION(S)

The humoral immune response alone is not sufficient to predict long term immunological memory and protective immunity against future infections following exposure to SARS-CoV-2. Both B, as well as a T cell immune response following infection and vaccination, needs to be evaluated.

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