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Original Article

Internal Medicine Section

Hybrid Immunity in SARS-CoV-2: Are Antibody Responses Similar in all Infected and AZD1222 Vaccinated Persons?

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ABSTRACT

Introduction: There is a diversity in population regarding the number and doses of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) vaccines and past infection status and the antibody titres may be different across various groups. The antibody titres determined in the same time frame after the immune evoking event may give clues regarding the prioritisation for boosters and factors causing variability in titres.

Aim: To compare and assess the Immunoglobulin G (IgG) anti-spike (S) antibody titres among the Healthcare Workers (HCWs) with history of Adenovirus vector based vaccine AZD1222 (Covishield) and infections, in different orders.

Materials and Methods: An observational cross-sectional cohort study was conducted in Government Medical College, Kerala, India, during November to December 2021. The antibody titres of a healthy cohort of HCWs (n=178) who were either double vaccinated with no history of SARS-CoV-2 infection or vaccinated but along with a history of SARS-CoV-2 infection were determined six weeks after the last event (infection/vaccination). They were grouped based on the order of vaccination (V) and infection (I).

Results: The major groups were group 1 (V+V), group 2 (I+V+V), group 3 (V+V+I) and group 4 (V+I+V). The highest titres of anti-S IgG antibody observed in vaccinated with breakthrough infection group 3-V+V+I (n=71) {20662 (10853-34744)}. The group with double vaccination but with no history of infection {group 1-V+V (N=49)} had the lowest titres-{2395 (844.4-7443)}. The hybrid immunity group (those who had infection which was followed by vaccination) group 2 (I+V+V) had titres 4241 (2220-7373) and group 4 (V+I+V) had titres 6542 (3772-11700) which were lower than those with breakthrough infection.

Conclusion: Anti-S antibody titres are highest among vaccinated with breakthrough infections and lowest in those with two doses of vaccines but no history of previous confirmed infections and booster doses may be prioritised for the second group. The timing of previous infection can also be a criterion for further booster doses.

Keywords: Anti-spike antibody, Anti-S titres, Breakthrough infections, Heathcare workers, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

Even after two years there are difficulties encountered in the fight against SARS-CoV-2 virus. There is non uniformity in vaccine distribution in low-income countries. The variants of concern like delta and omicron continue to cause deaths and there are still unmitigated concerns regarding emergence of new variants. Vaccination remains the greatest armour against SARS-CoV-2 pandemic. Even though, the Variant of Concern (VoC) like delta and omicron made their way, vaccination provided the defence against severe infections and played a great role in reducing mortality [1]. It has been shown that previously infected people have greater protection against reinfection [2]. On the other hand the immunity starts waning after few months hence the choice of population who need booster doses and timing of booster depends on the antibody titres of the population [3,4].

Since, vaccination was rolled out when the epidemic was raging in waves of varying intensity, there is a diversity in population regarding the number of doses of vaccine and past infection status and the order of these events. There is a knowledge gap regarding the protective antibody titres in this diverse population. Several studies have shown that neutralising antibody titre is a good indicator of vaccine efficacy against SARS-CoV-2 reinfection and can dictate need for further booster doses [5-7]. Neutralising antibody titres are readily not available and a more practical immune correlate of protection like anti-S-IgG titre can also be used as a surrogate for protection against reinfection [2,8,9]. There is evidence that a single dose of messenger Ribonucleic Acid (mRNA) vaccine (Pfizer or Moderna) after a documented SARS-CoV-2 infection produces a robust antibody response that is superior to the antibody response generated by natural infection alone [5,10,11]. There have not been studies comparing the antibody response in AZD12222 (Covishield vaccination) in double vaccinated with no history of natural infection and vaccinated individuals who had breakthrough infections (infections after vaccination) and individuals who had infections first and then got vaccinated (hybrid immunity) [11-13].

Hence, the present study was aimed to assess and compare the IgG anti S antibody titres among the HCWs with history of two doses of adenovirus vector based vaccination (AZD1222) and infections in different orders.

MATERIALS AND METHODS

This observational cross-sectional cohort study was conducted in Government Medical College, Kerala, India, during November to December 2021. Healthy HCWs who had received two doses of AZD1222 (Covishield) vaccine were included in the study. History of molecular confirmed Coronavirus Disease-2019 (COVID-19) (confirmed covid infection), the symptoms during the infection and co-morbidities were elicited using a structured proforma after getting consent. Institutional Ethical Committee (IEC) approval (IEC No: 115/2021, IRB, Govt Medical College, Kerala) and informed consent obtained from all participants.

Inclusion criteria: The HCWs with age >18 years having history of two doses of vaccination with Covishield with or without history of molecular confirmed SARS-CoV-2 infection. The last immune evoking incident (infection or vaccination) was six to eight weeks prior to the time of sera collection were included in this study.

Exclusion criteria: Vaccination with any other vaccine and history of moderate and severe COVID-19 infection as per World Health Organisation (WHO) criteria at the time of study [3].

Sample size calculation: Sample size was calculated for comparison of two means using the formula $N=(Z_{\alpha/2}+Z_{\beta})^2\times2\times\sigma^2/d^2$. Based on the study conducted by Shenoy P et al., it was found to be less than 10 in each group [13]. So, considering attrition a minimum of 20 individuals in each category was considered.

Study Procedure

The persons satisfying inclusion criteria were grouped based on the number and order of vaccination and infection. The four major groups identified were (1) double vaccinated with no history of previous confirmed infection (V+V), (2) individuals who had both doses of vaccination after confirmed infection-hybrid group (I+V+V), (3) double vaccinated individuals with breakthrough infections (infections after two stipulated doses of vaccine) (V+V+I). (4) Individuals who were infected between two doses of vaccine {V+I+V}.

The sera was collected four to six weeks after the last event (infection/vaccination). The demographic details, history of co-morbidities, symptoms during COVID-19 infection were noted.

Antibody assay: IgG-Receptor Binding Domain (RBD) antibodies against the S1 subunit of the spike protein of SARS-CoV-2 was assessed using SARS-CoV 2 IgG @ Quant (Abbott, Ireland) Chemiluminescent microparticle immunoassay (CMIA) [12,14]. SARS-CoV-2 IgG 2 Quant assay (CE marked) was performed in all groups on the Abbot Architect 12000 platform in accordance with the manufacturer's package insert. In this antibody CMIA test, the SARS-CoV-2 antigen coated paramagnetic microparticles bind to the IgG antibodies that attach to the virus spike protein in human serum and plasma sample. The result in chemiluminescence in Relative Light Units (RLU) following the addition of antihuman IgG acridinium labelled conjugate in comparison with IgG 2 calibrator indicates the strength of response which reflects the quantity of IgG present and is represented as Arbitrary Units (AU). Fifty Arbitrary units/millilitre (50 AU/mL) and above is considered positive. This quantitative measurement is helpful to evaluate an individuals humoural response.

STATISTICAL ANALYSIS

All intragroup comparisons of antibody titres were done by independent samples t-test after log transformations of antibody levels. Data are expressed as Mean and Standard Deviation (SD) or median and Interquartile Range (IQR) based on Shapiro-Wilk test for normality. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software (IBM SPSS Statistics for windows, version 25.0) with a p-value of <0.05 as statistically significant, all reported values were two sided.

RESULTS

A total of 178 persons were recruited in the study and anti-S antibody titres estimated. Total 11 persons who were included initially and found to have only single vaccination later on detailed enquiry (6), I+V (5) and were excluded from final analysis. A total of 167 belonged to the four groups, group 1 double vaccinated with no history of previous confirmed infection V+V (n=49), (2) individuals who had both doses of vaccination after confirmed infection- group 2 I+V+V (n=30), (3) double vaccinated individuals with breakthrough infections (infections after two stipulated doses of vaccine) V+V+I (n=71) and (4) individuals who were infected between two doses of vaccine group 4 V+I+V (n=17). They were included in the final analysis.

There was no association between antibody levels and age in the four groups. The mean age was comparable across various groups. The co-morbidities observed were hypertension in five persons, diabetes in four, asthma in two, coronary artery disease in one and hypothyroidism in one person [Table/Fig-1]. Fever was the commonest symptom during the infection in three groups (I+V+V- 40%; V+V+I - 39.44% and V+I+V- 58.82%) [Table/Fig-2].

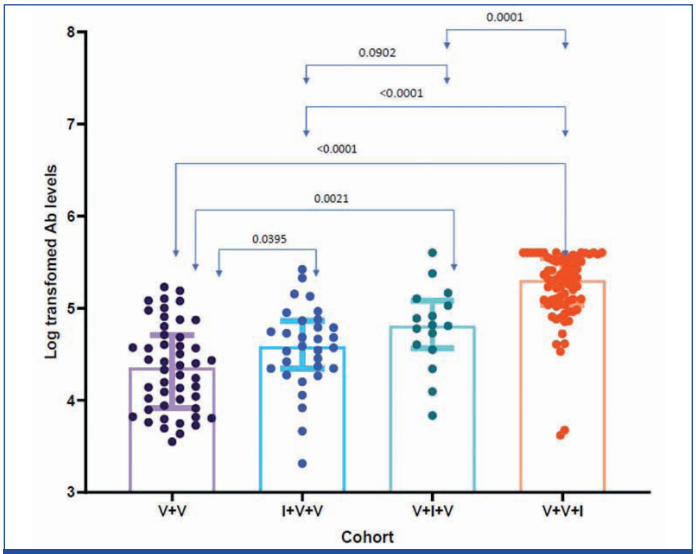
Antibody levels across various groups: The antibody levels were found to be highest among the double vaccinated group with breakthrough infections V+V+I (20662 (10853-34744)} compared to all other groups which was statistically significant [Table/Fig-3]. The levels were lowest among vaccination alone group (V+V) {2395 (844.4-7443)}. The hybrid groups with infection followed double vaccination had titres I+V+V {4241 (2220-7373) and V+I+V {6542 (3772-11700} which were lower than those with breakthrough infections.

Characteristics \ Groups	V+V (1) (n=49)	I+V+V (2) (n=30)	V+V+I (3) (n=71)	V+I+V (4) (n=17)
Age (years) (range)	21 (20-27)	29.50 (23.75-39)	30 (21-38)	29 (23.50-45)
Gender (Female), n (%)	20 (40.81)	2 (6.67)	51 (71.83)	1 (5.88)
Co-morbidities, n (%)	1 (2.04)	2 (6.67)	8 (11.26)	2 (11.76)
Asthma	1		1	
Hypertension		1	2	2
Diabetes mellitus			4	
Hypothyroidism		1		
Coronary artery disease			1	
Anti SARS-CoV-2S Ab (Au/mL)	2395 (844.4-7443)	4241 (2220-7373)	20662 (10853-34744)	6542 (3772-11700)

[Table/Fig-1]: Baseline characteristics.
AU/mL: Arbitrary units/Millilitre

Symptoms \ Groups	V+V (1) (n=49)	I+V+V (2) (n=30)	V+V+I (3) (n=71)	V+I+V (4) (n=17)
Fever	-	12 (40%)	28 (39%)	10 (59%)
Headache	-	5 (17%)	18 (25%)	1 (6%)
Fatigue	-	-	3 (4%)	3 (18%)
Joint pain	-	-	1 (1%)	-
Pain at site	-	-	-	1 (6%)
Myalgia	-	5 (17%)	24 (34%)	6 (35%)
Flu like	-	-	1 (1%)	1 (6%)
Cough	-	2 (7%)	13 (18%)	5 (29%)
Cold	-	4 (13%)	10 (14%)	1 (6%)
Loss of taste/Smell	-	10 (33%)	16 (23%)	5 (29%)
Loss of sleep	-	-	3 (4%)	-
Diarrhoea	-	-	2 (3%)	-
Dyspnoea	-	-	-	1 (6%)
Sore throat	-	2 (7%)	-	-

[Table/Fig-2]: Symptoms during infection.



[Table/Fig-3]: Anti-S antibody level comparison between four groups.

In subgroup analysis of the V+V+I group that had breakthrough infections, anti-spike antibody titres were maximum with the V+V+I group and significantly high compared to all other groups. Duration

between date of last vaccine dose and breakthrough infection showed no association.

DISCUSSION

The study compared the antibody titres across the various subgroups (depending on the doses of vaccines and order of vaccination and infection) measured at a uniform timeframe after the last immune activation (infection or immunisation). It shows that anti-S IgG titres were highest in the group who had breakthrough infections (V+V+I) after the stipulated two doses of vaccination compared to vaccination alone group (V+V) or the group with infection initially followed by vaccination (I+V+V). The vaccinated group with no history of infections (V+V) had the lowest titres compared to the groups with history of vaccination and infection in whichever order. Not withstanding the chance of them having a previously undetected/asymptomatic infection, this group with history of no previous confirmed infections had the lowest titres. With further evidence in waning of immunity prioritisation of booster/augmentation doses maybe done for persons without previous confirmed infections in low income countries [15,16].

The breakthrough infections (infections after both doses of vaccination) generate better antibody titres due to exposure to an array of antigens [11]. Hence infection, like vaccination is a potent immune evoking event. This was shown by neutralisation assays in previous studies also [9]. There was no association between the antibody titres and duration from last dose of vaccination to the infection in this group on subgroup analysis. There is ample evidence by previous studies that, a single dose of mRNA vaccines Pfizer/BioNtech or Moderna mount robust immune response in previously infected individuals [6-9,17,18]. A study in acute inflammatory rheumatoid disease patients in India with AZD12222 also showed that hybrid immunity is superior to vaccine alone induced immunity [10].

There are very few studies comparing the titres obtained after breakthrough infections (V+V+I) and titres obtained after vaccination following an earlier infection (I+V+V). A study by Bates TA et al., assessed the humoural response between vaccinated alone, breakthrough infections and hybrid immunity (vaccinations after infection) with antibody titres and neutralisation assays, showed that infections before or after vaccination significantly improves neutralisation responses [12]. In this study, there was no significant differences between the break through infection group and hybrid immunity group. This study by Bates TA et al., showed 8.5 and 15.7-fold improvements against the delta variant for the breakthrough and hybrid immune groups, respectively, compared with two vaccine doses alone group [12]. Earlier studies suggested that delta variant neutralising titers improved by 6-12 fold after booster vaccination eight months after a second dose [19]. Hence, in fully vaccinated individuals magnitude of improvement in natural infection is similar to that of booster doses.

In the present study, comparison done between the two groups I+V+V group 2 and V+V+I group 3 at a uniform time frame after the last immune response showed statistically significant high titres in V+V+I group. Compared to vaccine alone (V+V) group 1 they had very high titres. A neutralisation assay study done on AZD1222 recipients with history of COVID-19 infection and breakthrough infection also revealed similar results with high neutralising antibody levels in breakthrough infections [20,21].

Booster/augmentation doses are being rolled out in India now for those who are already vaccinated with two doses. They are given for individuals with history of two doses of vaccination and nine months after the date of last vaccination [22]. The infections are not considered as an immune provoking event and vaccinations can be done after three months of last documented infection in India. The vaccine roadmap by WHO-Strategic Advisory Group of Experts on Immunization (SAGE) is awaiting further studies in hybrid immunity to determine the importance of infection in deciding timing and number of booster doses [23]. Considering that a large population had infections and are having already robust titres, booster doses may be needed only at a later time after the last infection in the population with history of breakthrough infections. Prior infection can be considered as a criterion similar to the last vaccine dose for further vaccination. This would lead to effective utilisation of vaccines in low income countries where vaccine procurement maybe a problem.

Limitation(s)

A small sample size, lack of neutralisation assays were the limitations of the study. The study needs follow-up with further assays to assess rate of waning and effects of reinfections.

CONCLUSION(S)

Anti-S antibody titres after AZD1222 were highest among vaccinated with breakthrough infections and lowest in those with two doses of vaccines but no history of previous confirmed infections and booster doses may be prioritised for the second group. The timing of previous infection can also be a criterion for further booster doses. The strengths of the study are comparison across the groups with different orders of vaccines and infection at a uniform time frame and a fairly uniform study group (young, frontline HCWs).

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