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Brief Communication

Neutralizing antibody responses to SARS-CoV-2: A population based seroepidemiological analysis

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ABSTRACT

This study (August–September 2021) estimated the seroprevalence of SARS-CoV-2 neutralizing antibodies in the general population of Delhi and correlated it with their anti-SARS-CoV-2 IgG levels. Samples were selected by simple random sampling method. The neutralizing capacity was estimated by performing a surrogate virus neutralization test (sVNT) (GenScript), Piscataway, NJ, USA.

A total of 2233 (87.1%, 95% C.I. 85.7, 88.3) of the 2564 SARS-CoV-2 IgG seropositive samples had detectable SARS-CoV-2 neutralizing antibodies. In samples with S/CO $\geq$ 4.00, the neutralizing antibodies ranged from 94.5% to 100%. The SARS-CoV-2 neutralizing antibody seroprevalence strongly correlated with the S/CO range of IgG SARS-CoV-2 ($r = 0.62$, $p = 0.002$).

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic with enormous morbidity, mortality, and economic losses. India has till date recorded 34,633,255 cases and 473,326 deaths [1].

Protection from SARS-CoV-2 infection is accorded from previous infection or vaccination that substantially reduces the risk of symptomatic and severe disease and that of reinfection [2,3]. Neutralizing SARS-CoV-2 antibodies once produced are expected to provide durable and effective protection lasting at-least several months afterwards [4].

Several population-based SARS-CoV-2 seroepidemiological studies have been conducted in India but these have usually screened for antibodies recognizing SARS-CoV-2 spike antigens and/or nucleoprotein [5,6]. It is therefore uncertain as to the extent of the population having the protective SARS-CoV-2 neutralization antibodies. A surrogate virus neutralization test (sVNT) is capable of measuring majority of the neutralizing antibodies against SARS-CoV-2 viral spike (S) protein Receptor Binding Domain (RBD) without requirement of the live virus cells [7].

This study was conducted with the objective of estimating the seroprevalence of neutralizing antibodies in the general population and to further correlate it with the anti-SARS-CoV-2 IgG levels.

2. Methods

This present cross-sectional analysis was conducted as a sequel of a state level community-based seroepidemiological study. The anti-SARS-CoV-2 IgG antibodies were screened in the general population of Delhi aged$\geq$ 5 years that were selected through a two-stage random sampling method from 11 districts spanning 274 wards in Delhi, India. A total of 25,622 (89.5%) of the 27,811 samples had detectable anti-SARS-CoV-2 IgG antibodies that were detected using the VITROS® assay on VITROS® 3600 (Ortho Clinical Diagnostics, Raritan, NJ, USA). This chemiluminescent technology-based assay tests against the recombinant SARS-CoV-2 spike antigen and has 90% sensitivity and 100% specificity [8]. The detailed methodology and results of the study have been previously reported elsewhere [9].

Nearly 10% of the anti-SARS-CoV-2 IgG seropositive samples ($n = 2564$) representing the entire range of signal to cut off (S/CO) were

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screened for the SARS-CoV-2 neutralization antibodies. The samples were selected through computer-based simple random sampling method and the sample size was adequate at 95% confidence levels with 1.2% absolute precision. Neutralizing capacity was estimated by performing a surrogate virus neutralization test (sVNT) (GenScript, Piscataway, NJ, USA) having a specificity of 93.8% and sensitivity of 99.4% as per manufacturer instructions. This sVNT assay uses purified RBD from the SARS-CoV-2 viral spike (S) protein and the host cell receptor ACE2 that purposively mimics the virus-host interaction [7,10].

Neutralizing antibodies to SARS-CoV-2 was operationally considered as detected when the signal inhibition was ≥30%. Data were analysed with IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. To estimate the seroprevalence of the anti SARS-CoV-2 neutralizing antibodies in the general population of Delhi, the formula used was: Estimated number of cases with anti SARS-CoV-2 neutralizing antibodies * 100/(Total number of cases in the serosurvey). Since, 10% of seropositive samples were tested for the presence of neutralizing antibodies, this seroprevalence fraction was extrapolated to the complete IgG seropositive cohort of the serosurvey sample (n = 25,622). The denominator included total number of cases in the serosurvey sample inclusive of both IgG SARS-CoV-2 seropositive (n = 25,622) and IgG SARS-CoV-2 seronegative participants (n = 2189). The chi-square test was used to find the association between the independent variables (age, sex, Diabetes, Hypertension) and the dependent variable (anti-SARS-CoV-2 neutralizing antibody). A p-value < 0.05 was considered statistically significant.

The study was approved by the Institutional Ethics Committee F.1/IEC/MAMC/85/03/2021/No428 dated 21.08.2021. Adult participants provided informed electronic consent, while minor participants provided parental consent and informed electronic assent.

3. Results and discussion

A total of 2233 (87.1%, 95% C.I. 85.7, 88.3) of the 2564 SARS-CoV-2 seropositive samples had detectable anti-SARS-CoV-2 neutralizing antibodies. The anti-SARS-CoV-2 neutralizing antibody seroprevalence in the serosurvey sample on extrapolating the findings from this study was estimated as 80.2%.

On bivariate analysis but not on adjusted analysis, Covid-19 vaccination showed a statistically significant association with the presence of these neutralizing antibodies (p < 0.001). However, age, sex, diabetes or HTN comorbidity were not independently associated with the presence of neutralizing antibodies (Table 1).

The signal/cut off ratio (S/CO) of anti-SARS-CoV-2 IgG ranged from 1.00 to 22.8 (median 11.40). In cases with S/C ratio between 1 and 1.99 (n = 432), anti-SARS-CoV-2 neutralization antibodies were observed in 232 (52%) while in those cases having S/C ratio between 2 and 2.99 (n = 309), the anti-SARS-CoV-2 neutralization antibodies were observed in 237 (76.7%) cases. Furthermore, in the cases with S/C ratio between 3 and 3.99 (n = 111), anti-SARS-CoV-2 neutralization antibodies were observed in 88 (79.2%). Among the samples with S/CO ≥ 4.00 (n = 1698), the prevalence of neutralizing antibodies ranged from 94.5 to 100%, while in samples with S/CO < 4.00 (n = 535), it ranged from 52.0 to 79.2%. The anti-SARS-CoV-2 neutralizing antibody seroprevalence strongly correlated with the S/CO range of anti-SARS-CoV-2 IgG (r = 0.62, p = 0.002). Previous studies have suggested that SARS-CoV-2 neutralizing antibodies are able to block the viral infection and provide durable immune response despite the likelihood of waning of overall antibody levels. A large-scale study at Mount Sinai hospital, USA among health care workers also found strong correlation of IgG antibody responses against the viral spike protein generated in those with natural SARS-CoV-2 infection [11]. Our study results indicate that in populations with high SARS-CoV-2 seroprevalence with either infection or vaccine induced immune response, neutralizing antibodies are generated in nearly 9 of 10 seropositive individuals, with no statistically significant variation in the observed risk based on the individuals' age, sex, and comorbidity status.

Table 1
Factors associated with positive neutralization antibodies (N = 2564).

Variable	Total[a]	Neutralizing Ab present	Adjusted odds	p-value
Age				
<18	487	403 (82.8)	1.1 (0.77, 1.57)	
≥18	2077	1830 (81.1)	1	0.59
Sex				
Male	1093	944 (86.4)	1	
Female	1467	1286 (87.7)	1 (0.78, 1.3)	0.84
History Covid-19				
Present	559	495 (88.6)	1.1 (0.83, 1.5)	
Absent	1300	1117 (85.9)	1	0.40
HTN				
Yes	173	148 (85.5)	1	
No	1686	1464 (86.8)	1.2 (0.77, 2.1)	0.35
DM				
Yes	119	101 (84.9)	1	
No	1740	1511 (86.8)	1.2 (0.66, 2.1)	0.58
Vaccine doses				
0 dose	929	769 (82.8)	1	
1 dose	430	400 (93.0)	2.1 (0.7, 6.0)	
2 doses	500	443 (88.6)	1.7 (0.5, 3.2)	0.09
Vaccine type				
BBV152	185	164 (88.6)	1	
CHADOX1 NCOV-19	742	676 (91.1)	1.2 (0.7, 2.0)	0.49

[a] Not equal to 2564 due to missing questionnaire data.

This study also suggests that a higher signal to cut-off ratio may be considered as an indirect marker for the presence of neutralizing antibody response. This finding may have a clinical implication towards recommending booster doses in vulnerable populations like the immunocompromised and healthcare workers.

Certain variants of concern especially the Delta have demonstrated the ability to bypass existing immune response and elicit symptomatic disease even in vaccinated and more rarely in recovered individuals [12]. However, in this study, vaccination with at-least one dose of vaccine, either CHADOX1 NCOV-19 (Covishield) or BBV152 (Covaxin) was likely to induce robust neutralizing antibody response. Evidence from mostly in-vitro studies also indicate that neutralization titres highly correlate with protection against the SARS-CoV-2 variants of concern with waning of antibodies predictive of rapid loss of protection, which can be restored through booster vaccination [13].

Study limitations were that the entire IgG seropositive cohort from the serosurvey were not examined for anti-SARS-CoV-2 neutralizing antibodies due to resource limitations. Nevertheless, the representative and adequately powered sample size enable generalization of the study findings to the general population of Delhi.

Author contribution statement

Pragya Sharma and Ekta Gupta: Conceptualization.
Pragya Sharma, Ekta Gupta, Suruchi Mishra: Data curation.
Ekta Gupta and Saurav Basu: Formal analysis.
Pragya Sharma, Suruchi Mishra, Ekta Gupta, Reshu Agarwal: Investigation.
All authors: Methodology.
Pragya Sharma, MM Singh, Ekta Gupta, Nutan Mundeja, Gautam K Singh: Resources.
Ekta Gupta and Suruchi Mishra: Supervision;
Pragya Sharma: Ekta Gupta, Reshu Agarwal, Pratibha Kale: Validation.
All authors provided final approval of the version to be submitted.
Saurav Basu: Writing - original draft;
All authors contributed to writing - review & editing.

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Conflicts of interest

None.

Declaration of competing interest

All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

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