Original Research Paper

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ESTIMATION OF NEUTRALIZING ANTIBODIES OF COVID 19 VIRUS AMONGST INFECTED, EXPOSED BUT NON INFECTED, FULLY VACCINATED, AND NON-EXPOSED NON VACCINATED INDIVIDUALS OF GWALIOR CHAMBAL REGION.

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ABSTRACT **Introduction:** Virus neutralization test are the gold standard for the detection and quantification of NAbs, but require BSL3 facilities. In contrast, surrogate enzyme linked immunosorbent assays (sELISA) offer the possibility of high-throughput testing under common laboratory safety condition. We used GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit for estimation of Neutralizing Antibodies of Covid 19 virus amongst infected, exposed but non infected, fully vaccinated, and non-exposed non vaccinated subjects. Method: 200 participants above 18 years of age were included using purposive sampling method. Four different groups C1 to C4 were formed of 50 subject each as infected, exposed but non infected, fully vaccinated, and non-exposed non vaccinated individuals respectively. The test was done as per the recommendation of the manufacturer and correlated accordingly. The result of the samples can be found out by finding the inhibition rate = (1- OD value of sample / OD value of negative control) * 100%. Cut off was taken as more than 30% of inhibition means neutralizing antibodies was present. Result: In our study out of total 200 subjects of all the groups 86.5% showed neutralizing antibodies whereas in 13.5% of subjects NAbs were absent. It also showed presence of NAbs in all the four-group ranging from 74% in C4 group to 94% in C1 group. Common vaccines used for Covid 19 showed nearly similar results. Conclusion: In all the four groups of our study subject we found more that 70% NAbs. We also found a high percentage of NAbs in fully vaccinated subjects. Though it was a small study but it could conclude that a good immune response was present in Indian population. Also, to note that few of the subjects were neither exposed nor vaccinated indicating herd immunity was prevalent in the community.

KEYWORDS : Neutralizing Antibodies (NAbs), Protective Antibodies, Covid 19 Vaccination, ELISA

1. Introduction:

The COVID-19 pandemic, caused by SARS-CoV-2, has lasted for more than two year with no off and on frequency. The pandemic has resulted in more than 517 million cases and close to 6.0 million deaths as of May, 2022. Several key unanswered scientific questions remain concerning the pandemic. One of these questions is the nature and longevity of protective immunity, which is highly important in the context of risk assessment for reinfection and vaccine development. [1][2][3][4]

In any viral infection, it is expected that both antibody and T-cell responses will play roles in protective immunity and there are published studies to suggest that this might also be true for SARS-CoV-2 infection. [1][5][6]

Government introduced several interventions to minimize the impact of pandemic which affected the daily lives of almost everybody. About nine months after the beginning of this pandemic, the scientists and policy makers have shifted their focus from the diagnosis to sero-study and its impact on the daily life. [7]

Enzyme-linked immunosorbent assays (ELISAs), the test to detect antibodies are mainly used to estimate the prevalence of SARS-CoV-2 binding antibodies. For neutralizing antibody Plaque Reduction Neutralization test (PRNT) is the gold standard and as such ELISA is not able to detect neutralizing antibodies. In viral infections, neutralizing antibodies are of prime importance due to viral neutralizing antibodies playing a crucial role in long term immunity, herd immunity, assessment of vaccine efficacy etc., hence the detection of virus neutralizing antibodies is of importance. [8]

Traditionally the neutralization assays in vitro could be performed in an appropriate biosafety level laboratory (BSL-3). It not only requires a high containment facility due to use of cell lines and live virus but is time consuming and unfit for mass screening. Recently there is an increasing demand for "surrogate virus neutralization tests" (sVNTs) to supplement viral neutralization test and for estimation of neutralizing antibodies. [9][10]

In patients who have recovered from COVID-19, some individuals have very low levels or absence of neutralizing antibodies, indicating that T-cell immunity could be the dominant mechanism, at least in some individuals. [11] However, high levels of neutralizing antibodies appear to correlate with protection against reinfection. [6]

The present study aims to detect the neutralizing antibodies among the recovered SARS-CoV2 infected people, their household contacts and its correlation with vaccinated people for Covid-19 and non-infected/ non exposed non-vaccinated adult population of Gwalior city by using Surrogate Viral neutralization test.

2. Aims and Objectives:

2.1. To estimate the neutralizing antibodies among all the study subjects.

2.2. To find out the effect of Covid 19 vaccination on the titer of neutralizing antibodies.

2.3. To device strategy and/or give recommendations on the basis of findings of the study.

3. Material and Method:

A cross sectional study was done for the duration of 3 month in urban area of Gwalior city. By using purposive sampling method, we included a total of 200 participants above 18 years of age, 50 participants each from the four different group's i.e.

Items	Cutoff	Result	Interpretation
SARS-CoV-2 neutralizing antibody test	≥30%	Positive	SARS-CoV-2 neutralizing antibody detected

	< 30%	Negative	No detectable SARS-CoV-2 neutralizing antibody

Real Time Polymerase Chain Reaction (RTPCR) and/or Rapid Antigen Test (RAT) confirmed/ positive SARS-CoV2 individual found positive between 30-90 days before the date of sample collection. Also not vaccinated for Covid-19 (denoted as C1),

(2) Household uninfected and unvaccinated contacts of first group i.e. C1 (denoted as C2),

(3) Individuals received Covid-19 vaccination (received all the recommended doses) who had neither declared positive by RTPCR and/or RAT nor any household contact of Covid positive patient (denoted as C3) and

(4) Individuals who are not vaccinated for any Covid vaccine and who had neither declared positive by RTPCR and/or RAT nor any household contact of Covid positive patient. (Denoted as C4)
The study was reviewed and approved by our Institutional Ethics Committee and conducted as per the guidelines.

All SARS-CoV2 positive individuals found positive less than 30 days before and more than 90 days after the day of sample collection, individuals partially vaccinated for Covid vaccine and not willing were excluded.

List of RTPCR/ RAT confirmed individuals was obtained from the Department of Microbiology of our institute and list of fully vaccinated persons was obtained from Office of the Chief Medical and Health Officer. Participants were contacted telephonically and purpose of study was explained to them and time of home visit was fixed simultaneously with those who were willing. During home visit written informed consent was taken. After the administration of questionnaire to elicit social and demographic details, a trained team member obtained 3 ml blood in a plain vial using standard sample collection procedure. Each vial was given a unique ID. The collected samples were taken to the laboratory using standard sample transport procedure securely packed in temperature controlled insulated boxes for maintaining the integrity of the sample. The serum was separated and stored in the lab at 2-4°C to process it within 24 to 48 hours of collection.

In this study we used a commercially available surrogate neutralization test kit (GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit USA). Which is a Surrogate virus neutralization test (sVNT). sVNT Kit can detect circulating neutralizing antibodies against SARS- Cov-2 that block the interaction between the receptor binding domain of the viral spike glycoprotein (RBD) with the ACE2 cell surface receptor (Hoffmann et al., 2020). The assay detects any antibodies in serum and plasma that neutralize the RBD-ACE2 interaction. The test is both species and isotype independent.

The tests were done as per the recommendation of the manufacturer and correlated accordingly. The result of the samples can be found out by finding the inhibition rate which is calculated as below:

Inhibition = (1- OD value of sample / OD value of negative control) * 100%

The cutoff interpretation was done as per the following:ItemsCutoffResultInterpretationSARS-CoV-2 neutralizing antibody test≥30%PositiveSARS-CoV-2 neutralizing antibody detected < 30%NegativeNo detectable SARS-CoV-2 neutralizing antibody

4. Result:

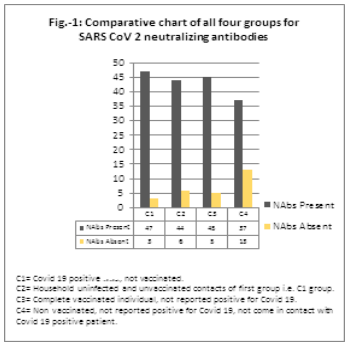
Out of 200 individuals of all the four groups (50 from each group) total females were 68 (34%) and 132 (66%) were males only 2 individuals reported presence of type 2 diabetes mellitus. Age wise distribution of individuals is as per the following table-1:

Table-1: Age wise distribution of individuals:

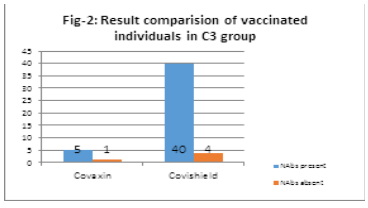
Age group in years	Neutralizing antibody present	Neutralizing antibody absent
<30	42	8
30-40	53	9
40-50	27	4
50-60	24	3
60-70	19	3
70-80	8	0

Our finding shows that neutralizing antibody production was usually same irrespective of the age, even geriatric age group shows production of neutralizing antibody in almost all individuals.

In our study (In Fig.-1) group C1 of non-vaccinated but Covid 19 positive individuals shows 94% positivity for neutralizing antibody and only 6% individuals do not have neutralizing antibody despite of infection. Similarly in group C2 which is of close contact but uninfected individuals 88% shows presence of neutralizing antibody which implies presence of herd immunity. Vaccinated persons which were taken as group C3 also showed 90% presence of neutralizing antibody but 10% of them fail to show neutralizing antibody despite of two doses of vaccination. In group C4 which comprised of naive individuals for Covid 19 a good percentage (74%) shows presence of neutralizing antibody which means a good herd immunity amongst the community only 26% of naïve individuals were at risk due to absence of neutralizing antibodies.



In our study (In Fig.-2) out of 50 vaccinated individuals 88% got two shots of Covishield and 12% got Covaxin. Though it is a small number to compare but in our study 83% individuals with Covaxin showed neutralizing antibody whereas 91% individuals with Covishield showed neutralizing antibody.



5. Discussion:

In our study irrespective of age neutralizing antibody production was profound meaning that even the elderly population was able to mount a good immune response against infection/vaccination. Though only two individuals (1%) reported presence of type 2 diabetes mellitus but both of them showed good immune response more elaborative study is needed to find out Covid 19 neutralizing antibody production in person suffering from type 2 diabetes mellitus. Our study also showed that 81% of individuals who were neither infected nor vaccinated showed a good neutralizing antibody production which supports the general thought of having good herd immunity in the community resulting in slowing down of infection leading to less morbidity and mortality.

6. Conclusion:

Though our study was done with limited resource and enrolled subject but it highlighted the prevalence of herd immunity as well as showed that a good number (90%) of individuals were able to mount neutralizing antibody response after 2 doses of vaccination and still 10% of individuals were not able to mount response after complete vaccination showing the need for booster vaccination.

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Conflict of Interest:

The author declared that they have no known competing personal relation or financial interests that could have appeared to influence the word reported in this paper.

Declaration:
The study was reviewed and approved by our Institutional Ethics Committee.
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Contribution of Author:
Vaibhav Misra: Conceptualization, Investigation, Supervision
Jyoti Sharma: Investigation, Writing
Rishika Khetan: Conceptualization, Investigation

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