ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

An epidemiological study to determine demographic factors influencing COVID-19 IgG antibody production among the adult population of urban area in Malegaon, Maharashtra - A cross sectional study

Nirmalkumar Adhar Rawandale[1], Vikrant Sayaji Pagar[2], Madhuri Magan Suryawanshi[3], Sushant Shamrao Chavan[4], Jinendra Mohan Jain[5], Pallavi Supriya Prabhakar Saple[6], Madhuri Rajeev Kanitkar[7]

[1]Deputy Dean, [5]Assistant Professor, Department of Medicine, [2,4]Assistant Professor, Department of Community Medicine, [3]Assistant Professor, Department of Microbiology, [6]Dean, Department of Paediatrics, Shri Bhausaheb Hire Government Medical College, Dhule, Maharashtra, India, [7]Vice Chancellor, Department of Paediatric Nephrology, Maharashtra University of Health Sciences, Nashik, Maharashtra, India

Submission: 14-07-2022 Revision: 02-09-2022 Publication: 01-10-2022

ABSTRACT

Background: Seroprevalence of COVID-19 antibody production in a person can be dependent on many physiological and demographic aspects such as previous infection, age, sex, body mass index, and also status of vaccination. It is of immense value to know about demographic aspect of COVID-19 antibody production so as to know about vulnerable population and suggest preventive measures. **Aims and Objectives:** The present study was aimed to determine effect of demographic variables COVID-19 antibody production in population of urban area. **Materials and Methods:** In this study, a total of 2454 subjects were screened for COVID-19 neutralizing antibody by ELISA technique. Subjects more than 18-year-old were selected for the study. We used cluster sampling method for data collection. A pre-structured questionnaire was administered after informed consent and 5 mL venous blood was collected in plain bulb for testing. **Results:** The prevalence of neutralizing antibody was found to be 93.9%. Female had 95% positive antibodies against males (92.34%). Maximum positive antibody status was seen in age group of 20–40 (55.6%). About 77.9% subjects following mixed diet were having positive COVID-19 antibody test as compared to subjects following pure vegetarian (10.2%). About 83.2% subjects who received vaccine showed positive antibody test. The lowest positivity is seen in underweight subjects (8%) followed by obese subjects (12.7%). Maximum inhibition % was seen in subjects using Vitamin C Zinc tablets (92.1%). The lowest inhibition was seen in subjects using Unani Kadha. A one-way ANOVA revealed that there was not a statistically significant difference in prophylactic measures for prevention of COVID-19 infection other than vaccination and COVID-19 neutralizing antibody inhibition %. ([F-1.363], P = 0.244). **Conclusion:** COVID-19 neutralizing antibody prevalence was found to be much higher in the population (96%), which was mostly associated with younger age, gender, diet, and vaccination status of the population. Extensive studies are required to establish any association between prophylactic methods other than vaccination and COVID-19 antibody response.

Key words: COVID-19; IgG antibody; Vaccine; Seroprevalence

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v13i10.46722

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

The COVID-19 outbreak began in December 2019 in Wuhan, Hubei Province, China, and was caused by the novel infectious disease agent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or 2019-nCoV.[1] Scientists have discovered that the range of disease manifestations and immune responses that occur after

Address for Correspondence:
Dr. Sushant Shamrao Chavan, 48, Swami Vivekananda Colony, Nakana Road Deopur, Dhule - 424 002, Maharashtra, India.
Mobile: +91-8805481514. **E-mail:** drsushantchavan@yahoo.in

SARS-CoV-2 infection varies significantly.[2] We now know that many people have asymptomatic or mild disease[3] and that the number of laboratory confirmed cases is an inaccurate measure of true disease prevalence because many of these cases have not yet been identified by conventional diagnostic testing methods. Antibody or serologic testing has been critical in our understanding of disease prevalence to date, and it may continue to be so in addressing these concerns. Antibodies are one of our primary defenses against viruses, created to identify particular proteins on the surface of a virus and initiate processes that gradually neutralize and eventually remove them. The serological tests to detect the presence of IgG antibodies may provide a more reliable estimation of the prevalence of SARS-CoV-2 past infection in the population, as is likely to persist for a more extended period after cleaning up the viral infection.[4] The IgG represents the most robust and long-duration antibody against the SARS-CoV-2 virus and can be detected after a median of 14 days (interquartile range [IQR] 10–18 days) from the onset of symptoms during infection.[5]

Seroprevalence is a critical measure used by researchers and public health organizations around the world to better understand the spread of COVID-19 disease. Seroprevalence is the proportion of people in a population who are infected with a disease at a given time, based on positive serum antibody testing.[6] It is difficult, if not impossible, to test every individual in a population due to resource and time constraints. A serosurvey can be carried out by sampling a small number of participants from a population that represents the general population. Serosurveys provide critical information about true disease prevalence when using a serologic test with high sensitivity and specificity.

Malegaon corporation area which is the area under this study was a hotspot of infection in the first wave witnessing 178 deaths and 4560 positive patients with a positivity rate of 18.5%. In second wave, positive cases were 6222 and 120 reported deaths. The spread of COVID-19 infection was low among the people of Malegaon town in second wave even when there was high vaccine hesitancy in the population. The magic Malegaon project was taken up by Maharashtra University of Health Sciences to understand the reasons why COVID-19 cases continued to be low in Malegaon city unlike the trend elsewhere in Nashik district which had registered a spike in in COVID cases in third wave.[7]

Various methods were used by Indian population for prevention of COVID-19 infection during pandemic. That included Vitamin C tablets, Ayurveda medicine, Homeopathic medicine, Unani medicine, and also many types of liquid preparation.[8] The efficacy of many preventive therapy is not yet established. In this study, one of the objective is to address effectiveness of most commonly used home based and over the counter used substances and their effect on COVID-19 antibody status.

Seroprevalence of COVID-19 antibody production in a person can be dependent on many physiological and demographic aspects such as previous infection, age, sex, body mass index (BMI), and also status of vaccination. It is of immense value to know about demographic aspect of COVID-19 antibody production so as to know about vulnerable population and suggest preventive measures. We propose a study to determine effect of demographic variables COVID-19 antibody production in population of urban area.

Aim and objectives

The present study was aimed to determine effect of demographic variables COVID-19 antibody production in population of urban area.

MATERIALS AND METHODS

This study was done in the urban area of Malegaon, district Nashik, Maharashtra. This district was one of the hotspot for COVID infections during first wave, but reported less number of positive cases during second wave as compared to other regions in Nashik district. The reason remains unknown, so this study was proposed under the "Magic Malegaon" project by the Maharashtra University of Health Sciences (MUHS) to investigate why the region, which was once a hotspot in the first wave, continues to report low COVID-19 cases unlike other regions. It was a cross-sectional study. Sample size was calculated using epiinfo software with 80% power and design effect of 1. A total of 2700 subjects were screened for COVID-19 neutralizing antibody by ELISA technique, but only 2454 samples were tested as other samples got hemolyzed during transport. Subjects more than 18 years old were selected for the study. We used cluster sampling method for data collection. The urban area was divided into four zones and from these zones 16 wards were selected randomly. From each ward, 154 subjects were selected randomly for interview and COVID-19 antibody testing. A pre structured questionnaire was administered after informed consent and 5 ml venous blood was collected in plain bulb for testing.

Ethical approval

Ethical clearance was taken from ethical committee. Patient's informed consent was taken before enrolling them in the present study.

Statistical analysis

Testing instruments were calibrated to standard units. ELISA technique was used to detect COVID-19 neutralizing antibodies and subjects who showed >30% inhibition on testing were labeled as antibody positive.[9] Data were analyzed using SPSS version 22 with proportion, Chi-square, and one-way ANOVA statistical methods.

RESULTS

Table 1 demonstrates the demographic variables of 2454 subjects. One thousand two hundred and forty (50.4%) of the subjects belonged to age group of 20–40 years followed by 60–80 years (32.2%). Male constituted 1258 (51.3%).

Table 1: Distribution of demographic variables of subjects

Demographic variables	N	%
Age group (year)		
20–40	1240	50.5
40–60	125	5.1
60–80	791	32.2
80–100	298	12.1
Gender		
Male	1258	51.3
Female	1196	48.7
Religion		
Buddhist	8	0.30
Christian	1	0.10
Hindu	594	24.2
Muslim	1849	75.3
Sindhi	2	0.1
Education		
Postgraduate	77	3.1
Graduate	345	14.1
Intermediate or diploma	67	2.7
High school certificate	474	19.3
Middle school certificate	755	30.8
Primary school certificate	348	14.2
Illiterate	388	15.8
Occupation		
Professional	194	7.9
Semi-professional	73	3.0
Clerical, shop owner, and farm owner	214	8.7
Skilled worker	270	11.0
Semiskilled worker	330	13.4
Unskilled worker	263	10.7
Unemployed	1110	45.2
Diet		
Mixed	2044	83.3
Non-vegetarian	153	6.2
Vegetarian	257	10.5
BMI		
Underweight	219	8.9
Healthy weight	1168	47.6
Overweight	732	29.8
Obese	335	13.7
Vaccination history		
No	372	15.2
Yes	2082	84.8

One thousand eight hundred and forty-nine (75.3%) subjects were of Muslim religion while Hindu consisted of 594 (24.2%). Most of the subjects had education up till Middle school (30.8%) followed by high school (19.3%) and 15.85 subjects were illiterate. About 45.2% subjects were unemployed. About 13.4% subjects were semiskilled worker and 115 were skilled worker. About 83.3% subjects were following mixed diet while non-vegetarian were 6.2% and pure vegetarian consisted of 10.5%.

About 47.6% subjects belonged to healthy category of BMI, 8.95 were underweight, 29.8% were overweight, and 13.75 subjects were obese. About 84.8% subjects had at least taken one vaccine dose for COVID-19 prevention.

Figure 1 shows various prophylactic measures used by subjects other than vaccination for the prevention of COVID-19 infection. One thousand seven hundred and twenty-seven (70.37%) subjects had not taken any kind of home based prophylactic measures, while 17.52% subjects consumed Unani kadha. About 5.34% subjects consumed Vitamin C and zinc tablets while 5.26% subjects used Arsenic album.

Figure 2 shows COVID-19 neutralizing antibody status of subjects. About 93.9% of the subjects were positive for 19 neutralizing antibody while only 6.1% individuals were negative.

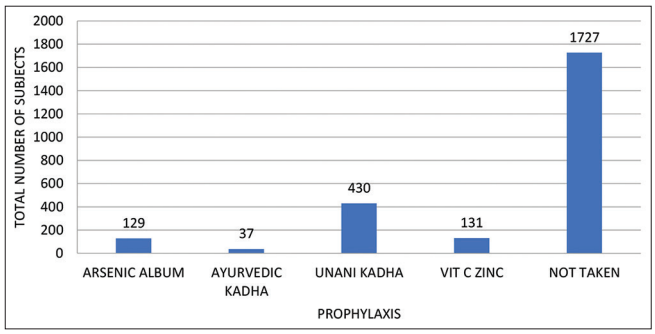

Figure 1: Prophylactic measures status by subjects other than vaccination

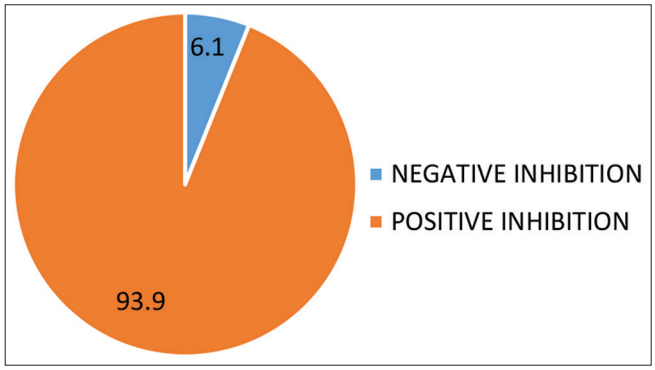

Figure 2: COVID-19 neutralizing antibody status of subjects

Table 2 demonstrates relation between various demographic variables and COVID-19 neutralizing antibody status among the subjects. Out of the total subjects, 47.45% subjects were male and 46.6% were females that showed positive antibody test. About 95% females had positive antibody test as compared to males (92.34%). A significant relation was seen between gender and COVID-19 antibody status (Chi-square = 11.007[a], df=1, P=0.001).

Maximum positive antibody status was seen in age group of 20–40 (55.6%) followed by 40–60 year (30.9%). Lowest positivity was seen in age group of 80–100 year (3%). A significant association was seen in age group and COVID-19 antibody status of the subjects (Chi-square=9.215[a], df=4, P=0.05).

About 77.9% subjects following mixed diet were having positive COVID-19 antibody test as compared to subjects following pure vegetarian (10.2%) and non-vegetarian (5.8%) diet. A significant relation was seen between type of diet and COVID-19 antibody status (Chi-square=7.030[a], df=2, P=0.030).

Out of the total subjects, 10.7% subjects who did not received COVID vaccination showed positive antibody test, while 83.2% subjects who received vaccine showed positive antibody test. A significant relation was seen between COVID-19 vaccination history and COVID-19 antibody status (Chi-square=414.86[a], df=1, P=0.000).

About 45% subjects in healthy category of BMI showed positive antibody test. The lowest positivity is seen in underweight subjects (8%) followed by obese subjects (12.7%). A significant relation was seen between BMI category and COVID-19 antibody status (Chi-square=7.66[a], df=3, P=0.05).

A one-way ANOVA was performed to compare the effect of various prophylactic measures for prevention of COVID-19 infection on COVID-19 neutralizing antibody inhibition %.

Maximum inhibition % was seen in subjects using Vitamin C zinc tablets (92.1%). The lowest inhibition was seen in subjects using Unani kadha (Table 3).

A one-way ANOVA revealed that there was not a statistically significant difference in prophylactic measures for prevention of COVID-19 infection other than vaccination and COVID-19 neutralizing antibody inhibition % ([F-1.363], P=0.244).

DISCUSSION

Antibody evolution occurs in germinal centers, where antigens are stored for long periods of time as immune complexes on the surface of follicular dendritic cells through somatic mutation and selection. Antibodies recognize the SARS-CoV-2 S-protein and specifically target and bind to the RBD of S protein within the S1 sub-domain through adaptive immunity.[10] This causes the antibody-dependent cell cytotoxicity and complement cascade to be activated, resulting in the elimination of infected cells.[11] This binding gives antibodies the ability to block viral entry into cells, which is critical in the protective immune response to SARS-CoV-2 infection.[12,13] SARS-CoV-2-specific B and T cell responses are also easily triggered by infections.[14] COVID-19 patients' SARS-CoV-2-specific B cell responses result in the development of specialized antibody-secreting cells (ASCs). The pathogen-specific antibodies are then secreted in large quantities by these ASCs.[1]

Antibodies against SARS-CoV-2 are critical for outwitting the virus, as a proper neutralizing response would significantly reduce the number of virions capable of infecting Angiotensin converting enzyme-2 receptor-expressing cells. As a result, research on antibody responses to SARS-CoV-2 should be a top priority for the scientific community responding to the pandemic, both in terms of prevention and treatment.

In our study, we found that 94% of the population were having positive neutralizing antibody status which was way more than reported by different studies.[15,16] ICMR NIRT Chennai has conducted a nationwide seroprevalence survey which indicated of the 28,598 serum samples from the general population, 4585 (16%) had IgG antibodies against the N protein, 6647 (23.2%) had IgG antibodies against the S1-RBD protein, and 7436 (26%) had IgG antibodies against either the N protein or the S1-RBD protein. Weighted and assay-characteristic-adjusted seroprevalence against either of the antibodies was 24.1% (95% confidence interval [CI]: 23.0–25.3%).[12] Our study indicates seroprevalence much higher than this study indicating more exposure to the viral antigen. Earlier nationwide COVID-19 serosurveys conducted in India indicated an increase in seroprevalence from 0.73% (95% CI: 0.34–1.13%) in May–June 2020 to 6.6% (95% CI: 5.8–7.4%) in September–October 2020 and 24.1% (95% CI: 23.0–25.3%) in December 2020–January 2021.[17] A study done in same population by Saple et al., in year 2020 found overall seroprevalence of anti-SARS-CoV-2 of IgG antibody as 40% (95% CI 35–45%).[18] Our study shows significant increase in seroprevalence after second wave of COVID-19. The substantial seroprevalence of anti-SARS-CoV-2 antibodies in the Indian population should provide some measure of protection against future waves of COVID-19 in the country. Furthermore, the study reported that 83.2% individuals were received at least one dose of COVID-19 vaccine. Vaccination status was significantly associated with the antibody status. Sarah E.

Table 2: Relation between demographic variables and COVID-19 antibody status

Demographic variables	COVID antibody		Total	Chi-square	DF	P value
	Negative inhibition	Positive inhibition				
Gender						
Male						
Count	96	1162	1258	11.007[a]	1	0.001
% of total	3.9%	47.4%	51.3%			
Female						
Count	53	1143	1196			
% of total	2.2%	46.6%	48.7%			
Total						
Count	149	2305	2454			
% of total	6.1%	93.9%	100.0%			
Age group						
20–40						
Count	99	1266	1365	9.215[a]	4	0.05
% of total	4.0%	51.6%	55.6%			
40–60						
Count	33	758	791			
% of total	1.3%	30.9%	32.2%			
60–80						
Count	16	274	290			
% of total	0.7%	11.2%	11.8%			
80–100						
Count	1	7	8			
% of total	0.0%	0.3%	0.3%			
Total						
Count	149	2305	2454			
% of total	6.1%	93.9%	100.0%			
Diet						
Mixed						
Count	133	1911	2044	7.030[a]	2	0.030
% of total	5.4%	77.9%	83.3%			
Non-vegetarian						
Count	10	143	153			
% of total	0.4%	5.8%	6.2%			
Vegetarian						
Count	6	251	257			
% of total	0.2%	10.2%	10.5%			
Total						
Count	149	2305	2454			
% of total	6.1%	93.9%	100.0%			
Vaccine Taken						
No						
Count	109	263	372	414.861[a]	1	0.000
% of total	4.4%	10.7%	15.2%			
Yes						
Count	40	2042	2082			
% of total	1.6%	83.2%	84.8%			
Total						
Count	149	2305	2454			
% of total	6.1%	93.9%	100.0%			
BMI category						
Underweight						
Count	22	197	219	7.660[a]	3	0.05
% of total	0.9%	8.0%	8.9%			
Healthy						
Count	63	1105	1168			
% of total	2.6%	45.0%	47.6%			
Overweight						
Count	41	691	732			
% of total	1.7%	28.2%	29.8%			
Obese						
Count	23	312	335			
% of total	0.9%	12.7%	13.7%			
Total						
Count	149	2305	2454			
% of total	6.1%	93.9%	100.0%			

Table 3: Relation between various prophylactic measures taken by subjects and COVID-19 antibody Inhibition %

Prophylaxis taken by subjects other than vaccine	N	Mean inhibition %	Standard deviation	F	Sig.
Arsenic album	129	88.6%	18.7%	1.363	0.244
Ayurvedic Kadha	37	90.7%	15.3%		
Unani Kadha	430	87.9%	19.7%		
Vitamin C Zinc	131	92.1%	16.1%		
Not taken	1727	88.2%	20.5%		
Total	2454	88.4%	20.0%		

Wheeler et al., in their study revealed a peak of antibody induction after the vaccine boosting dose with a gradual decline of antibody levels at later time. Anti-nucleocapsid antibody was not induced by spike protein-encoding vaccines and this may continue to serve as a marker of the previous SARS-CoV-2 infection. No differences between COVAXIN and COVISHIELD vaccines in terms of antibody response were revealed.[19]

The proportion of COVID-19 neutralizing antibody was found to be distinct in different age groups. Young age group showed more positivity in antibody testing than the older groups. That can be attributed to physiological difference between the younger and the older people. A significant association was seen in age groups and COVID-19 antibody positivity. He S Yang et al., in a cross-sectional study used 31 426 SARS-CoV-2 antibody test results from pediatric and adult patients. The SARS-CoV-2 IgG level correlated negatively with age in the pediatric population ($r=-0.45$, $P=0.001$) and moderately but positively with age in adults ($r=0.24$, $P=0.001$). The findings of this study suggest that SARS-CoV-2 viral specific antibody response profiles differ between age groups. Age-specific disease screening and management strategies, as well as vaccine development, may be necessary.

Females had showed more antibody response than males. The past studies have shown that sex has a considerable effect on the outcome of infections and has been associated with underlying differences in immune responses to infection.[20,21] Women mount a more robust immune response to vaccines.[22] Takahashi et al., demonstrated that the T cell response was significantly and negatively correlated with patients' age in male, but not female, patients. These data indicate key differences in the baseline immune capabilities in male and female patients during the early phase of SARS-CoV-2 infection, and suggest a potential immunological underpinning of the distinct mechanisms of disease progression between sexes. These analyses also provide a potential basis for taking sex-dependent approaches to prognosis, prevention, care, and therapy for patient with COVID-19.[23]

Several studies have hypothesized that dietary habits may play an important role in COVID-19 infection, severity of symptoms, and duration of illness. In our study, we found that COVID-19 antibody positivity was more in people having mixed diet as compared to the pure vegetarian and non-vegetarian diet. That could simply because mixed diet can fulfill the nutrient and protein demand more effectively than the other diets. Kim et al., have suggested that plant-based diets or pescatarian diets were associated with the lower odds of moderate-to-severe COVID-19. These dietary patterns may be considered for protection against severe COVID-19.[24] The future studies with detailed macro and micronutrient data are warranted to study associations between dietary intake and COVID-19 antibody titer.

Significant difference in mean IgG antibody inhibition was seen in subjects in healthy category of BMI. Least inhibition was seen in subjects in underweight category and overweight category. BMI significantly affected the IgG antibody inhibition. Contradictory results were shown in a study by Ozgocer et al., where SARS-CoV-2 antibodies are higher in individuals with older age, whereas BMI, concomitant chronic disease, and medications had no effect on antibody titers.[25] in another study by Yamamoto et al., higher BMI was associated with the lower titers of SARS-CoV-2 spike antibodies in men, but not in women, suggesting the need for careful monitoring in men with obesity, who are at high risk of severe COVID-19 outcomes.[26]

The Indian Traditional System of Medicine is one of the world's oldest medical systems and has played an important role in providing health-care services to human civilization since its inception. Although India has its own recognized traditional medicine; Ayurveda, Yoga, Unani, Siddha, and Homoeopathy (AYUSH),[27] we found no significant association between various home-based methods used for COVID-19 prevention and antibody positivity.

It should be noted that all these methods were unsupervised and mostly taken on voluntary basis with no scientific dosing and pattern. A series of studies are needed to prove the efficacy of these methods to establish their

Table 4: Contributions to the current project

S. No.	Name of Team Member	Designation
Maharashtra University of Medical Sciences, Nashik	Lt. Gen. Madhuri Kanitkar	Vice-Chancellor, MUHS, Nashik
	Dr. Kalidas D. Chavan	Registrar, MUHS, Nashik
	Prashant P. Shivgunde	Assistant Professor, University Research Department, MUHS, Nashik
	Pravin Ghatekar	Statistical Officer, Statistical Cell, MUHS, Nashik
Motiwala (National) Homoeopathic Medical College & Hospital, Nashik	Name of Team Member	Designation
	Dr. Farooq Motiwala	Professor
	Dr. Swanand Shukla	Professor
	Dr. Kamlesh Bagmar	Lecturer
	Dr. Sachin Bhalerao	Reader
	Dr. Mita Gharte	Reader
	Dr. Vishal Nimbhore	Reader
	Dr.Sharmila Roy	Lecturer
	Dr. Shweta Patil	Lecturer
	Dr .Tejashree Thakare	Lecturer
	Dr.Smitha Nair	Lecturer
	Dr.Dhanashree Chaudhari	Lecturer
	Dr. Ruta Patharkar	Lecturer
	Dr .G .B. Prasad	Professor
	Dr.Manisha Ahiwale	Lecturer
	Dr. Dipalee Paraskar	Reader
	Dr.Gitanjalee Pawar	Reader
	Dr. Swati Patil	Lecturer
	Dr. Khuzaim Lokhandwala	Professor
	Dr.Gayatri Nimbhore	Reader
	Dr.Kirti Kadam	Assistant Lecturer
Mohammadia Tibbia College and Assayer Hospital, Malegaon	Dr. Mohammad Zubair Mohammad Shabbir Khan	Principal & Superintendent
	Dr. Abdul Majid Abdul Salam	In charge MTC
	Dr. Abul Irfan Abdul Ahad	Professor
	Dr. Sayyed Minhaj Mushtaque Ahmad	Convenor
	Dr. Sharique Zohaib Ashfaque Ahmed	Associate Professor
	Dr. Taufiq Ahmad Safiullah Khan	Associate Professor
	Dr. Mohd. Hameed Ahmed Md. Abdul Samad	Associate Professor
	Dr. Mohammad Shakir Neyaz Ahmad	Associate Professor
	Dr. Momin Shahzad Aamir Mohammad Sadique	Associate Professor
	Dr. Rahemani Shaheda Parveen	Associate Professor
	Dr. Mohd. Sajid	Associate Professor
	Dr. Naseem Ahmad	Assistant Professor
	Dr. Musarrat Ali Anwar Husain	Assistant Professor
	Dr. Zaid Ahmed Mohammed Iqbal	Assistant Professor
	Dr. Ifra Abdul Qaiyyum	Assistant Professor
Malegaon Corporation, Malegaon	DR Sapana Thakare	Health Officer
	Dr Sunita Rokade	Medical Officer
General Hospital, Malegaon	Dr. Hitesh Mahale	Medical Superintendent
KC Ajmera Ayurved College, Dhule	Dr. Rajesh Shah	Principal
	Dr. Sanjivan V. Kulkarni	Reader
	Dr. Manojkumar B. More	Professor
	Dr. Avinash D. Jagtap	Professor
	Dr. Anil S. Patil	Professor
	Dr. Shrikant S. Bagul	Lecturer
	Dr. Kaustubh Y. Salunke	Lecturer
	Dr. Subodh C. Patil	Reader
	Dr. Kushal N. jain	Lecturer
Alameen Unani Medical College, Malegaon	Dr. Salim Shah	Principal
	Dr. Shamshad Aalam	Professor
	Dr. Obaidurrahman	Assistant Professor
	Dr. Sahiba Firoz Ahmed	Assistant Professor
ACPM Medical College and Hospital, Dhule	Dr Vijay Patil	Principal

effectiveness under the expert guidance. Shankar Gautam et al., in his study explains immune-modulatory, antiviral, anti-oxidant, anti-inflammatory, anti-platelet, anti-atherosclerotic, hepatoprotective, renoprotective properties in immunoregulation for controlling viral infections like COVID-19. Further pre-clinical and clinical trials need to be done for the evaluation of safety and efficacy of this polyherbal formulation.[28]

Limitations of the study

As this was a cross-sectional study, the temporal link between the IgG antibody inhibition and the demographic variable under study cannot be determined because both are examined at the same time.

Generalizability

We used cluster sampling method as a sampling technique and sample size was fairly large so the results of this study are fairly generalizable to the population under study.

CONCLUSION

COVID-19 neutralizing antibody prevalence was found to be much higher in the population (96%), which was mostly associated with younger age, gender, diet, and vaccination status of the population. Extensive studies are required to establish any association between prophylactic methods other than vaccination and COVID-19 antibody response.

ACKNOWLEDGMENT

We acknowledge the guidance we got from Maharashtra University of Health sciences Nashik. We are immensely grateful or Municipal Corporation of Malegaon, Nashik, Motiwala (National) Homoeopathic Medical College & Hospital, Nashik, Mohammadia Tibbia College and Assayer Hospital, Malegaon, KC Ajmera Ayurved College, Dhule, Alameen Unani Medical College, Malegaon, ACPM Medical College and Hospital, Dhule for their help in data collection and designing this study [Table 4].

List of project team members participated in Phase One Data Collection on Malegaon Research

We acknowledge the team member as follows from different institution for their kind support and guidance.

Declaration

Manuscript has been read and approved by all the authors and requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

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Authors Contribution:
NAR- Acquisition, analysis, or interpretation of data for the work. **VSP-** Accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **MRK-** Drafting the work or revising it critically for important intellectual content. **SSC-** Analysis of data. Final approval of the version to be published.

Work attributed to:
The project work is attributed to the Shri Bhausaheb Hire Government Medical College, Dhule, Maharashtra and Maharashtra University of Health sciences Nashik, Maharashtra, And the research work was conducted in the urban area of Malegaon, district Nashik, Maharashtra.

Orcid ID:
Dr. Nirmalkumar Adhar Rawandale - https://orcid.org/0000-0002-3340-4533
Dr. Vikrant Sayaji Pagar - https://orcid.org/0000-0001-5870-7613
Dr. Madhuri Magan Suryawanshi - https://orcid.org/0000-0001-9012-4346
Dr. Sushant Shamrao Chavan - https://orcid.org/0000-0001-5883-433X
Dr. Jinendra Mohan Jain - https://orcid.org/0000-0003-1465-9204
Dr. Pallavi Supriya Prabhakar Saple - https://orcid.org/0000-0002-1732-5122
Dr. Madhuri Rajeev Kanitkar - https://orcid.org/0000-0002-1644-3565

Source of Support: None, **Conflicts of Interest:** None.