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Seropositivity Of COVID-19 Among Healthy Voluntary Blood Donors and Its Association with Blood Groups: A Reflection of Burden of Asymptomatic COVID-19 Cases in The District of South Gujarat

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ABSTRACT

Introduction: First case of COVID-19 in the district was reported on 19th March 2020, thereby increasing exponentially, presenting with various symptoms to having few or no symptoms, posing challenge to prevent disease transmission by being a significant source of infection. This study conducted to assess relationship between COVID-19 infection within blood groups and burden of reported cases in the district to seropositivity among donors.

Methodology: This is a prospective observational study from second largest blood bank of south Gujarat, catering to a population of seven million, 4916 donors from the month of August 2020 to May 2021 participated in the study.

Observations: Seropositivity was found to be 54.6%, with higher prevalence (68.63%) among female compared to male (54.45%), there was no statistical difference among age groups & RH groups, blood group AB was found to have highest seropositivity followed by A, B and O group. First time donors seropositivity was higher compared to frequent donors among the study participants. Seropositivity among donors was positively corelated with RTPCR Positivity rate reported in the district.

Conclusions: Looking at similar trend of active surveillance and its correlation to blood bank positivity, we urge policy makers to strengthen hospital-based surveillance for real time insights.

Key words: Seropositivity, Blood Bank, blood group and COVID-19, Natural history of COVID-19

INTRODUCTION

Towards the end of 2019, many cases were reported with severe pneumonia of unknown etiology in different parts of Wuhan, China. Soon after the first case was reported, within a flicker of time, the outbreak progressively spread across the country and the globe. The beta coronavirus – SARS-CoV-2 was found to be causative agent and it produced a severe acute respiratory syndrome (SARS) called COVID-19[1]. Many countries implemented unparalleled measures to control SARS-CoV-2 including international and domestic travel restrictions, nationwide lockdowns, physical and social distancing, quarantine were implemented for many countries these measures helped in slowing down of initial epidemic wave, however cases were still in rising trend.

COVID-19 often causes mild symptoms like cough, muscle pain and anosmia which may later progress into high fever, pneumonia, respiratory distress and in some cases death. Many a times there have been COVID-19 cases having few or no symptoms posing a challenge to prevent disease transmission by being a

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significant source of infection[2].

By published systematic reviews and reports asymptomatic persons could account from 5% to 80% [3] and, they do not require or seek medical attention, contributing to rapid spread of disease in the community[4].

Health authorities and policy makers cannot rely on confirmed cases by Reverse transcription polymerase chain reaction (qRT-PCR) as it could potentially miss large proportion of asymptomatic individuals and on the other hand studies have shown a high rate of false negative tests given the factors such as type of biological sample, insufficient collection, fluctuation of viral load, and the time between sample collection and symptom onset that can influence the test result[5]. World Health Organisation to overcome such issue had recommended population based Seroepidemiological studies that generate data to implement containment measures[6] nationwide serosurvey among 21 states conducted by Indian council of Medical Research (ICMR) reported population weighted seroprevalence of 0.73% between May and June 2020, while hospital-based survey from northern parts of India (Srinagar) reported 3.6%in July 2020[7]

It is critical to adopt a simple, sensitive, and specific test that provides instant and accurate findings for quickly identifying SARS-CoV-2-infected individuals in order to empower viral transmission control and timely public health intervention[8] such as Serological testing, which aid in the detection of acute-phase (IgM) or memory (IgG) antibodies even in asymptomatic individuals for considerable duration of time.

The index case in this district was reported on 19th march 2020, reaching 13331 cases at beginning of study (august 2020) to 140894 cases by end of study (may 2021)[9], we designed hospital based cross sectional study to determine seropositivity among healthy voluntary blood donors from 6 months of index case for a total duration of 10 months at second largest blood bank under public sector of south Gujarat attached to a tertiary care teaching hospital catering to nearly 7 million population of the city along with its urban health centres spread across the city, this blood bank using different strategies like maintaining blood group directory, conducting blood donation camps in collaboration with a number of social organisations it has been collecting on an average of 10,000 units of blood annually by standard protocol and screening methods for collection, storage, processing and distribution.

We hope findings of the study will help health authorities in disease containment and add valuable data in understanding natural history of disease among researchers.

METHODS AND MATERIALS

Study Setting: The study was conducted in blood bank attached to tertiary care teaching hospital, serving as referral hospital for 44 urban health centers of the city spread across 9 administrative zones along with another tertiary care teaching hospital inclusively catering about 7 million population, the city has average annual growth rate of 4.5% [10]. Health care in the city is delivered through Government health system and private practitioners, apart from health care the blood bank has strong community connections through various non-governmental organizations (NGO's) and Voluntary groups.

Study Design and Study Period: We designed hospital-based cross-sectional study at blood bank from August 2020 to May 2021among all voluntary blood donors with eligibility criteria for blood donations per drug & cosmetic act,1940 and its amendments, gazette of India notification[11,12]

Sample Size and Data Collection:

Data Source 1 (Primary data): All voluntary blood donors were screened for IgG type of COVID-19 antibodies using an enhanced chemiluminescent method as part of a routine protocol introduced during the COVID-19 pandemic period with a view to the patient's safety and to check for eligibility for convalescent plasma donors.

During the study period, 5046 blood units were collected, among which 130 units were discarded as part of the exclusion criteria for blood donation. A total of 4916 blood units were tested and included in this study and data has been analyzed accordingly.

All data were entered in Hospital management and information software of the tertiary care teaching hospital and stored locally with system generated unique ID for each donor.

The record of all these donors included informed written consent, demographic details, clinical history and screening test as part of blood banking protocol, additionally were asked for history of COVID-19 infection either laboratory confirmed or had flu like symptoms in past one month. All Voluntary blood donors declared themselves as not having any such symptoms.

Data Source 2 (Comparator / Secondary data): COVID-19 reported cases of city

As part of open Government data policy, gazette notification and national data sharing and accessibility policy 2012, data from various public sectors including health is made available for sharing and building knowledge base.[13]

During the pandemic, Government of India launched nationwide common platform for COVID-19 data under https://www.mygov.in/covid-19domain making it openly available.

From the early days of COVID-19, a team of 300 technical volunteers-built Application Programme interface (API) to make COVID-19 data available and easily accessible at website *https://www.covid19.org* and it was active till October 2021. Later, IIT Madras

took up the website and APIs which was openly available and built website *https://www.incovid19.org* and all data and data sources are available at this portal open and free to use.[14]

Data of daily number of cases district wise were accessed from website https://www.covid19.org. (Last access date 20th August 2021) and used as comparator for sero positivity from blood bank to positive cases reported from the city.

Sample processing and analysis: The serum was separated and used to test for presence of COVID-19 IgG antibodies using Anti SARS CoV2 IgG assay kits by Vitros 3600 instrument (Ortho clinical diagnostic, USA), it is an Enhanced chemiluminescent immunoassay method, by means of recombinant protein like the SARS-CoV-2 spike protein for the detection of high affinity IgG antibodies specifically against SARS CoV2. The assay has a signal to cut off ratio (S/C) calculated automatically from built in calibration standards, (S/C) of 1.00 or more as positive/ reactive, any value of (S/C) less than 1.00 is considered as negative/non-reactive.

Statistical analysis: We have used Panda's package of Python version 3.8.5 and MS Excel for statistical analysis. Positive IgG was considered for all results of antibody titer greater than or equal to 1.00. Positivity was calculated as a percentage value according to groups and subgroups by gender, age, blood group, and Rhesus (RH) group. According to the HMIS unique ID query function, the frequency of donation was classified as first-time donors and regular donors for repeat donors. Findings have been described as time-place and person distribution. Cases reported from the city were compared in time period reference with the same time period as the blood donation date. Statistical tests for association and goodness of fit have been applied. Attack rates during the different times of pandemics have been calculated. Risk differences across various groups have also been calculated.

Ethical Consideration: This study was approved by the human research and ethical committee of the institute before the onset of the study. All participants are voluntary blood donors, and the procedure and tests are free of charge to the donors. Snacks and juice were provided to all donors as part of standard blood donation collection practice. The results of the test were communicated to them.

RESULTS

In this study, we were able to enrol 4916 eligible blood donors during the study period. Almost all of them (98.96%) were male, and 92.75% were from the age group of 18 to 45 years. For the ABO profile, it was found that 34.68% were B blood group, 31.36% were O group, 23.71% were A group, and 10.23% were AB group. RH typing showed 94.89% to be RH positive. The majority (58.62%) were first-time blood donors (table 1).

All collected samples were tested for SARS-CoV-2 antibodies as described above.

The value for antibody titer for the 3rd centile among reactive individuals was 1.2 and for the 97th centile it was as high as 22.1. The median value was 5.7. The mean value of the titer was 7.6 with a SD of 6.05 In addition, the value for the coefficient of variation of titer was almost eighty percent (79.49%).

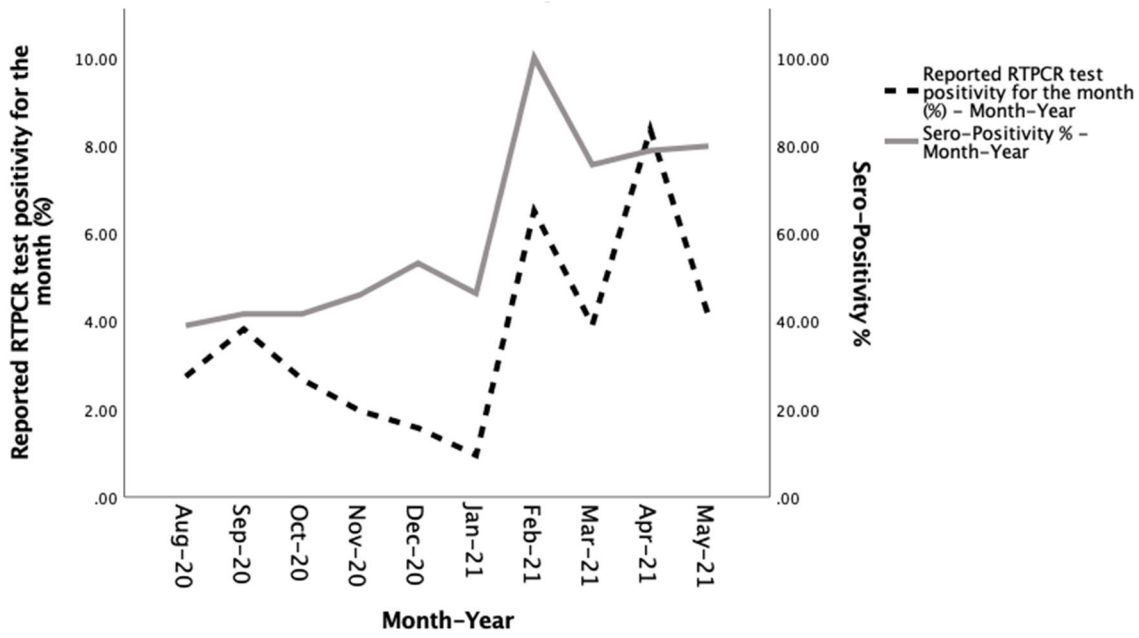
Table 1: Factors associated with Sero-Positivity among voluntary blood donors

Group	Positive IgG	Negative IgG	Total tested	Positivity (%)	$\chi 2$	p value
Gender						
Male	2649	2216	4865	54.45	4.0924	0.0431
Female	35	16	51	68.63		
Subtotal	**2684**	**2232**	**4916**	**54.6**		
Age						
18-30	1270	1102	2372	53.54	2.062	0.3567
31-45	1216	972	2188	55.58		
46-65	198	158	356	55.62		
Subtotal	2684	2232	4916	54.6		
Blood Group						
A	668	498	1166	57.29	20.92	<0.001
B	942	763	1705	55.25		
AB	300	203	503	59.64		
O	774	768	1542	50.19		
Subtotal	**2684**	**2232**	**4916**	**54.6**		
RH						
Positive	2542	2123	4665	54.49	0.4168	0.5185
Negative	142	109	251	56.57		
Subtotal	**2684**	**2232**	**4916**	**54.6**		
Frequency of Donation						
First time donors	1652	1230	2882	57.32	20.85	<0.001
Regular donors	1032	1002	2034	50.74		
Subtotal	**2684**	**2232**	**4916**	**54.6**		

Table 2: Association of Sero-Positivity to the cases reported in the city

Month Year	Reported COVID-19 cases in the Surat city			Sero-Positivity among Voluntary blood donors			
	Reported Cases	Daily Average cases ±SD	RTPCR test positivity (%)	Positive IgG	Negative IgG	Total tested	Sero-Positivity (%)
Aug-20	7626	246.0 ±15.6	2.74	87	136	223	39.01
Sep-20	8500	283.3 ±11.9	3.82	337	472	809	41.66
Oct-20	7660	247.1 ±21.5	2.68	287	402	689	41.65
Nov-20	6799	226.6 ±43.5	1.96	322	378	700	46
Dec-20	5628	181.5 ±35.1	1.57	512	450	962	53.22
Jan-21	2956	95.4 ±29.3	0.95	88	102	190	46.32
Feb-21	1382	49.4 ±17.2	6.51	3	0	3	100
Mar-21	11923	384.6 ±238.4	3.91	310	100	410	75.61
Apr-21	51431	1714.4 ±661.1	8.36	350	94	444	78.83
May-21	23910	771.3 ±488.1	4.12	388	98	486	79.84
TOTAL	**127815**	420.4 ±539.6		2684	2232	4916	54.6

Chart 1: Comparison of trends in reported RTPCR test positivity in the district to Sero-Positivity among healthy voluntary blood donors



Overall antibody test positivity observed was as high as 54.6%, with 54.45% in males and 68.63% in females. The difference was statistically significant (p = 0.04).

The trend of positivity among age groups was 53.54%, 55.58%, and 55.62% in an increasing order of age groups as 18–30, 31–45, and 46–65 years, among voluntary blood donors of this blood bank respectively.

The difference in positivity among blood groups was found to be in the range of 50.19% among the O group, with the highest (59.64%) among the AB group. The difference in positivity was significant as tested by a chi-square test p 0.0001, indicating AB was most susceptible, followed by A, B, and O groups. The RH type showed no such association.

First-time blood donors had a higher proportion of positivity (57.32%), which was statistically significant (p<0.000001).

The blood bank usually receives maximum blood donations during winter and spring seasons. During study period it reduced to less than 4% (3.9%), due to lockdown and pandemic situation, post august period Blood bank started receiving blood donations at usual pace, reaching little more than 45% (46.6%) and it continued in proportion of 50% (49.16%) of its routine collection December onwards (table 2).

The number of samples collected from all blood donors which was subjected to antibody testing have shown with COVID-19 cases reported from the city in table2. Majority of samples (72.68%) were tested from august 2020 to January 2021, where monthly reported positive cases in the city was in the range of 2956 to 8500 with daily averaging 94.4 to 283.3 cases. However monthly cases crossed 10,000 during the month of March 2021 to May 2021 (11923 to 51431) with daily averaging from 384.6 to 1714.4 cases each day during these months.

Strong positive correlation of r = 0.738 (p= 0.015) was observed between reported RTPCR test positivity in the district to Sero-Positivity among healthy

voluntary blood donors which was statistically significant.

DISCUSSION

The results of our study showed sero-positivity by presence of IgG antibodies against SARS-CoV-2 among healthy voluntary blood donors to be as high as 54.6%, indicating a large proportion of individuals were exposed to the agent without developing any symptoms nor being tested positive in routine screening activity of the health authorities. The finding is similar to sero prevalence conducted in the slum area of south India and systematic review and meta-analysis conducted for proportion of asymptomatic infections [153], however it is much higher when compared to studies in Pakistan and France which showed seropositivity ranging from 24.4% to 35.9% obtained at different points in time among blood donors [1617]. In Odisha India, seropositivity was reported to be at 35.9% [18]. The higher positivity in this study suggests wider circulation of the virus at the community level by the end of second wave of COVID-19 pandemic. This can be attributed to much denser population in Surat city which is 10052 persons / sq.km[19] as compared to density of Odisha state being 270 persons/ Sq. Km with range of 91-682 persons/ Sq. Km[20], while Pakistan and France have population density of 287 and 119 persons/ Sq. Km respectively[2122]

When risk was compared between young, mobile population (18-45 years) and middle age, elderly (>45 years) people, odds ratio of 1.04 (0.8 – 1.29) indicated that the risk of catching COVID-19 infection was not different between these two ages groups, review of seventy studies on isolated effect of age showed no evidence of specific age threshold at which the risk accelerates considerably [23], findings of which is similar to our study.

The chi-square test was applied for goodness to fit with the ABO blood grouping system and seropositivity suggested that the seropositivity was distributed statistically significantly different across all four blood groups ($p<0.001$),highest being in AB and least among O blood groups, studies have been demonstrated association of blood group with COVID 19 infections including severity in terms of hospitalization and outcome [2425], findings of this study reiterates the higher susceptibility of AB group but could not show any statistical association between RH groups as the risk ratio of 0.9 (0.71-1.8) suggested that there is no statistically significant difference for COVID-19 infection between RH positive and RH negative blood donors.

First-time donors were more susceptible for COVID-19 infection, especially during pandemic days. The risk ratio calculated was 1.3 (1.16-1.46) suggesting higher risk among first-time donors as compared to regular donors. Similar risk was reported in the study conducted by Raouf, May [26]

The correlation coefficient between the number of cases being reported in district (RTPCR positive) using active surveillance methods by health authorities and the sero-positivity tested among healthy voluntary blood samples at the same time period turned out to be 0.738 suggesting strong positive correlation between the spread of infection in community and its reflection in blood bank, this suggests continuity between Period of Pre-Pathogenesis and Period of pathogenesis [27]. It also clarifies the much-disputed term “community transmission”.

When positivity was observed in the city from August 2020 to May 2021 a statistically significant ($p <0.001$) variation in seropositivity was observed across months suggesting presence of active transmission of COVID-19 in the community.

Ultimately the SARS-CoV-2 has transformed itself as a pandemic in a few months has suggested as having higher communicability although much less than measles, where R0 of measles has been reported as high as 12-18 by systematic review and meta-analysis study [28], whereas for COVID has reached its peaked at 3.4 as reported by modelling studies in India [29].

CONCLUSION

We found high sero-positivity by presence of IgG antibodies against SARS-CoV-2 among healthy voluntary blood donors in second largest blood bank of south Gujarat, just six months to one year of index case, which showed proportionately similar trend of positive cases detected by RTPCR test through active surveillance. Individuals with AB blood group had higher susceptibility as compared to other blood groups like O, A and B but Rh positivity did not show any difference in susceptibility to COVID-19 infection.

PUBLIC HEALTH IMPLICATION

Early containment measures and quick action by health authorities of the district limited spread of disease through Identification of symptoms, contact tracing by active surveillance and isolation by establishment of COVID-19 dedicated hospitals and isolation centers was possible for symptomatic cases but missing out huge proportion of asymptomatic ones.

Passive surveillance such as this can be valuable tool for timely monitoring, planning interventions for the policy makers. we urge policy makers to strengthen hospital-based surveillance for real time insights and act accordingly.

LIMITATION OF STUDY

The 30-day asymptomatic specific to COVID-19 criteria for healthy voluntary blood donors was chosen as per the Drug and Cosmetic Act guidelines revised for

COVID-19 during the study period. However, IgG antibodies can be positive for a period of as long as 90 days.

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