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Screening of Anti-SARS-CoV-2 IgG and IgM Antibodies in Blood Donors: An Experience during Beginning of COVID-19 Pandemic

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Abstract

***Background**: The spread of COVID-19 has had a profound impact on the number of blood donations, on blood supplies, and on blood safety. It was critical to ensure that all donations from healthy and asymptomatic people are free from active COVID-19 infection as few people were ready to donate blood during this pandemic and patients requiring frequent blood transfusions should have safe blood. **Aim** of this study was to conduct screening of Anti-SARS-CoV-2 IgG and IgM Antibodies in healthy donors, to establish the magnitude of the threat that COVID-19 pandemic poses to blood safety.*

***Methods:** A prospective study was carried out on 857 donations performed in a tertiary care hospital-based blood centre. Anti-SARS-CoV-2 IgG and IgM Antibody detection in human blood was carried out using immunochromatography assay, as an additional test along with mandatory Infectious markers and positivity was estimated.*

***Results**: Mean age of all healthy donors tested during this period was 32.83 years with 98.5% male population. Seroprevalence of Anti-SARS-CoV-2 IgG antibody was detected in 6.3% cases and that of Anti-SARS-CoV-2 IgM antibody as 0.11%. Younger age group showed a higher seropositivity. Correlation of seroprevalence with ABO grouping was not found but E antigen seems to be a protective factor.*

***Conclusion**: Blood donors should be tested for Anti SARS Cov-2 IgG and IgM antibodies, as additional test along with other infectious markers, by a rapid, simple and highly sensitive test for safe blood supply till the time COVID-19 pandemic subsides.*

Keywords: *Coronavirus, COVID, Anti-SARS-CoV-2 IgG and IgM Antibodies, Blood Donors, E Antigen, Donor Screening.*

Introduction

The spread of COVID-19 has had a profound impact on the number of blood donations, on blood supplies, and on blood safety. The SARS-CoV-2 has a long incubation period, generally, 1-14 days; on average, 5-6 days; longest reported, 24 days [1] and causes asymptomatic infection in a large number of individuals [2], which poses huge challenges in the recruitment of blood donors, in blood collection and blood safety. The stringent social distancing measures have effectively contained the virus; however, these have also had a profound impact on the health system, including blood donation and supply, and clinical transfusion management. Contact with respiratory secretions from virus-infected individuals is currently known to be the main route of transmission, although there are reports of virus transmission via aerosol droplets and physical contacts. It is not known whether COVID-19 can be transmitted by blood transfusion. Respiratory viruses have never been reported to be transmitted through blood or blood components; therefore, any potential risk of transmission by transfusion of blood

collected from asymptomatic individuals is theoretical. [3] It is unclear how many people have contracted the causative SARS-CoV-2 unknowingly and are asymptomatic. Therefore, reported COVID-19 cases do not reflect the true scale of outbreak. If immune response to SARS-CoV-2 are similar when compared to other corona viruses, infected individuals may be less susceptible to reinfection for months to years, reducing the risk of severe COVID-19 and also limiting the possibility of spreading the virus.[4][5]Assuming that antibodies to SARS-CoV-2 are produced by the adaptive immune system in response to virus exposure, at least in the vast majority of cases, serology based tests for SARS-CoV-2 may be used to determine the extent of asymptomatic SARS-CoV-2 infections and to monitor the COVID-19 pandemic. For detection of SARS-CoV-2 antibodies, one of the earliest approved methods in India was indirect immunofluorescence assays (IFA). Seroprevalence of IgG antibodies against SARS-CoV-2 is an important tool to estimate the true extent of infection in a population. However, seroprevalence studies are scarce in South East Asian countries, including India, the second most populated country in the world, which makes 17.7% of world population and hence is expected to carry a high number of confirmed COVID-19 cases in the world.

Aims & Objectives

The purpose of this study was to conduct screening of Anti-SARS-CoV-2 IgG and IgM Antibodies in healthy blood donors who could be asymptomatic carriers also, to establish the magnitude of the threat that COVID-19 pandemic poses to blood safety and to assess that whether COVID-19 Antibody screening is feasible and cost effective.

Materials & Methods

A prospective study was conducted in a blood centre attached with a tertiary care hospital in capital city of India between May 2020 to July 2020, among healthy blood donors of 18 to 65 years of age and in good health, fulfilling the eligibility criteria for whole blood donation and single donor platelet (SDP) donations in accordance to the Drug & Cosmetics Act 1940 and rules 1945 therein (as amended on 11th March 2020). After obtaining written consent, 4 ml of blood was drawn. The blood was stored at 2• C to 8• C for up to three days if the tests could not be performed immediately. The blood samples attained room temperature prior to use. For the differential detection of Anti-SARS-CoV-2 IgG and IgM antibodies in human blood, an indigenously developed, rapid, qualitative immunochromatography in vitro assay (COVID 19 IgG/IgM RAPID TEST; Medsource Ozone biomedical Pvt. Ltd., India) was used having 98% specificity for IgM and 97.5% specificity for IgG. The principle of COVID 19 IgG/IgM RAPID TEST is an antibody-capture immunochromatography assay for the simultaneous detection and differentiation of IgM and IgG antibodies to COVID-19 virus in human serum, plasma and/or whole blood samples

Statistical Analysis

Statistics and data compilation and prevalence of antibodies is calculated using excel tool and chi square test to know the significance.

Results

Between May 2020 to July 2020, 857 blood donors were studied; 844 (98.5%) of whom were male and 13 (1.51%) were female donors. Of total 857 donors, 843 were voluntary whole blood donors and 14 were SDP donors. The mean age of the blood donors was 32.83 years. The Age and sex-wise distribution of blood donors is shown in Table 1.

Table1. *Age and sex-wise distribution*

Age group (in years)	Sex Distribution		Total
	Male	Female	
18-30	371	5	376
31-40	321	3	324
41-50	123	5	128
51-65	29	0	29

The ABO RH blood group distribution over different age group and sex is depicted in Table 2.

Table2. *ABO RH blood group distribution over age and sex groups*

ABO Blood group	Age group (in years)							
	18-30		31-40		41-50		51-65	
A	RH Positive	RH Negative	RH Positive	RH Negative	RH Positive	RH Negative	RH Positive	RH Negative
Male	77	4	74	3	28	0	4	0
Female	0	0	1	0	1	0	0	0
Total	**77**	**4**	**75**	**3**	**29**	**0**	**0**	**0**
B								
Male	116	9	112	8	41	3	14	0
Female	1	0	1	0	2	0	0	0
Total	**117**	**9**	**113**	**0**	**43**	**3**	**14**	**0**
AB								
Male	42	3	21	4	11	0	5	1
Female	3	0	1	0	0	0	0	0
Total	**45**	**3**	**22**	**4**	**11**	**0**	**5**	**1**
O								
Male	115	5	95	4	38	2	4	1
Female	1	0	0	0	2	0	0	0
Total	**116**	**5**	**95**	**4**	**40**	**2**	**4**	**1**

Of 857 donors tested, Anti-SARS-CoV-2 IgG antibody was detected in 54 (6.3%) donors and Anti-SARS-CoV-2 IgM antibody was detected in one (0.11%) donor. Single whole blood donor having Anti-SARS-CoV-2 IgM antibody in blood sample was having concurrent Anti-SARS-CoV-2 IgG antibody also. Out of 54 donors having Anti-SARS-CoV-2 IgG antibody, 53 were whole blood donors and one was SDP donor. The distribution of IgG and IgM antibodies in donors of various age groups is depicted in Table 3.

Table3. *Distribution of IgG and IgM antibodies in different age group*

Age group (in years)	Total No. of donors	IgG Antibody present	IgM Antibody present
18-30	376	27	0
31-40	324	17	1
41-50	128	8	0
51-65	29	2	0
Total	**857**	**54**	**1**

The distribution of ABO blood group in donors having Anti-SARS-CoV-2 IgG and IgM antibodies is shown in Figure 1.

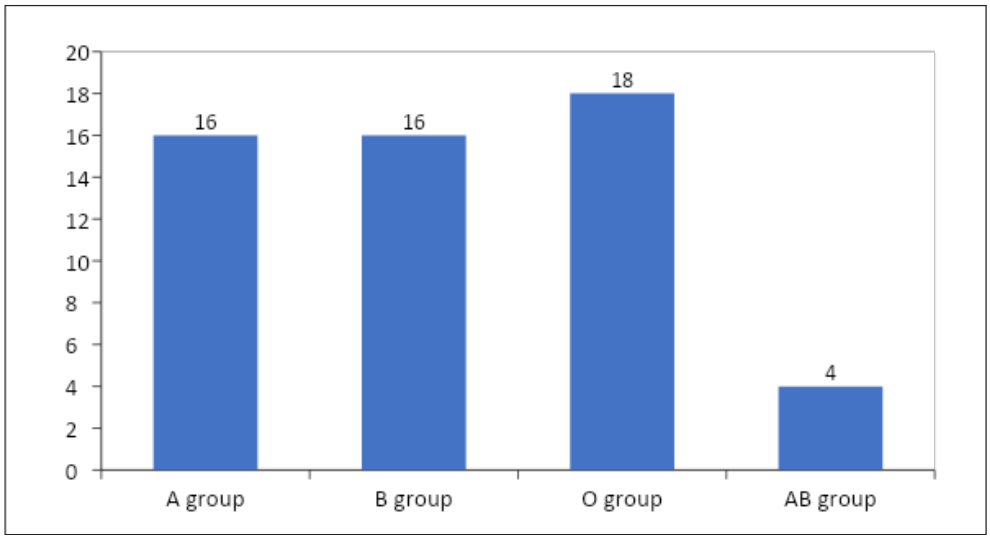

Figure1. *Distribution of ABO group in donors having Anti-SARS-CoV-2 IgG and IgM antibodies*

Rhesus (RH) and Kell phenotyping for all 857 donors was performed, of which 27 (3.15%) donors were found to be Kell antigen positive (K+), rest were cellino phenotype (Kell negative). All the 54 donors having Anti-SARS-CoV-2 IgG and IgM antibodies were found to be Kell negative. The distribution of RH phenotype in all 54 donors having Anti-SARS-CoV-2 IgG and IgM antibodies among different age groups is shown in Table 4. Study results are suggestive of association of RH phenotype CcDee and CCDee with the increased risk of SARS-CoV-2 infection, while the risk of having SARS-CoV-2 infection is found to be very low in RH phenotype ccDEe, ccddee, ccDEE, CcDEE ($P<0.001$). The results of our study are suggestive of higher incidence of SARS-CoV-2 infection in population having presence of C antigen and concurrent absence of E antigen in RH phenotype. This can be postulated as that E antigen is acting as a protective factor against COVID-19 infection. This hypothesis needs to be validated further by more studies.

Table4. *Distribution of RH phenotype in donors having Anti-SARS-CoV-2 IgG and IgM antibodies among different age groups*

RH phenotype/Age group (in years)	CcDee	CCDee	CcDEe	ccDEe	ccddee	ccDEE	CcDEE
18-30	11	10	3	1	1	0	1
31-40	5	6	1	0	0	1	0
41-50	9	2	1	0	0	0	0
51-65	0	2	0	0	0	0	0
Total	**25**	**20**	**5**	**1**	**1**	**1**	**1**

Discussion

The world is facing unprecedented challenges with communities and economies everywhere affected by the COVID-19 pandemic. The number of volunteer donors at places affected by the corona virus outbreak, such as the capital city of India, was greatly reduced. This has put a strain on the local blood supplies and jeopardised the blood stock needed for the clinical transfusion. The strictly implemented mobility constraints also led to a reduction in blood donation. It was recommended that donors travelling from, or residing in the regions hardest hit by corona virus should have their blood donation deferred for at least four weeks. Although the virus SARS-CoV-2 nucleic acid real-time polymerase chain reaction (RT-PCR) test has become the standard method for diagnosis of SARS-CoV-2 infection, there was an urgent need for an accurate and rapid test method to quickly identify a large number of asymptomatic carriers to prevent virus transmission. Testing of specific antibodies of

SARS-CoV-2 is a good choice for rapid, simple, highly sensitive diagnosis of COVID-19 in general population having exposed or asymptomatic carriers and in apparently healthy blood donors.

In our study, 6.3% showed seroprevalence of Anti-SARS-CoV-2 IgG antibodies and only 0.11% donors showed seroprevalence of Anti-SARS-CoV-2 IgM antibodies which is quite similar to other reports.[6,7] However, 6.3% seroprevalence of Anti-SARS-CoV-2 IgG antibodies in the population tested, demonstrated limited presence of potential antibody mediated immunity to SARS-CoV-2 at the time of the study. The single donor showing presence of Anti-SARS-CoV-2 IgM and IgG antibodies reflects recovery or later phase of Covid-19 infection exposure. It is widely accepted that Immunoglobulin M (IgM) provides the first line of defence during viral infections, before the generation of adaptive, high-affinity IgG responses that are important for long term immunity and immunological memory. It has been reported that after SARS infection, IgM antibody could be detected in patient's blood after 3 to 6 days and IgG could be detected after 8 days. [8,9] Since COVID-19 belongs to same large family of viruses as those that cause the MERS and SARS outbreak, it is assumed that its antibody generation process is similar and detection of Anti-SARS-CoV-2 IgG and IgM antibodies will be an indication of infection. Furthermore, detection of IgM antibodies tends to indicate recent exposure to SARS-CoV-2, whereas the detection of COVID-19 IgG antibodies indicates virus exposure sometime ago.

Antibodies were significantly more often detected in younger population group in this study (18-30 years), which might be related to age dependent social behaviours before social distancing was implemented. Our study further indicated that the prevalence of antibodies to SARS-CoV-2 is sex-independent throughout the age groups (18-65 years), though female donors in our study were only 1.5%.

Association of blood group with COVID-19 infection has also been described in recent studies. Individuals with blood group A have been found to be more at risk as compared to those with blood group O.[10,11]Although exact reason for this is unknown, but partly it could be due to the protective mechanism of circulating anti-A antibodies which inhibit the interaction of virus to its specific host ACE2 receptor. [12] However, in our study, O group blood donors were insignificantly more than A and B group blood donors (p= 0.339) (Figure 1). It was also found in our study that the commonest RH phenotype; CcDee, was having higher association with risk of having SARS-CoV-2 infection. This observation reflects to our earlier observation of commonest RH phenotype in our population in D positive donors being CCDee followed by CcDee. [13]The results of our study are suggestive of higher incidence of SARS-CoV-2 infection in population having presence of C antigen and concurrent absence of E antigen in RH phenotype. This can be postulated as that E antigen is acting as a protective factor against COVID-19 infection. This hypothesis needs to be validated further by more studies. To the best of our knowledge, no such correlation is observed in the literature. Kell antigen phenotype in seropositive donors is same as in our population observed in earlier study.[13]

Various studies are conducted globally to know the seroprevalence status in general population but a few studies are done on blood donors. Antibody testing on general population helps to know the asymptomatic carriers and help policy makers to decide the future course of action. Since RT- PCR test cost is much higher than rapid, immunochromatography test and it has many limitations. Infection-induced herd immunity might arise when enough people become infected and develop antibodies to the virus, in addition to people with non-antibody-mediated immunity or with SARS-CoV-2 pre-existing immune reactivity. [14, 15]Rapid immunochromatography test performed on human blood is very convenient and easy for sample collection and performance of test. This test can be used to detect seroprevalence in blood donors as well. With 6.3 % Anti-SARS-CoV-2 IgG seroprevalence and single case of Anti-SARS-CoV-2 IgM and IgG prevalence in blood donor, Covid-19 IgG/IgM test can be used as an additional test along with infectious markers till the time Covid-19 cases are under control with the advent of specific drugs to treat Covid-19 cases and vaccination to prevent the Covid-19 cases.

To conclude, blood donors should be tested for Anti-SARS-CoV-2 IgM and IgG antibodies as additional test along with other infectious markers, by a rapid, simple and highly sensitive test for safe blood supply till the time Covid-19 pandemic does not subside.

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