

# Serum IgG Profile in Convalescent COVID-19 Patients in Different ABO Blood Grouping System: A Retrospective Study

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## ABSTRACT

**Introduction:** The plasma collected from the recovered Coronavirus Disease 2019 (COVID-19) patients is used therapeutically in serious COVID-19 patients as a life saving procedure. Serum Immunoglobulin G (IgG) estimation is a routine procedure followed in these voluntary donors. In this routine procedure, authors have come across patients with particular blood group having more chances of seronegativity for COVID-19 IgG antibody. This is the reason why the present study was conducted.

**Aim:** To find out the variable IgG response in recovered COVID-19 patients with different types of clinical manifestations along with the possible relation with different types of blood groups of ABO system.

**Materials and Methods:** This retrospective study was conducted in plasma bank of Veer Surendra Sai Institute of Medical Science and

Research, Burla, Odisha, India, from 1<sup>st</sup> August to 15<sup>th</sup> December 2020. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) specific IgG antibody level was estimated quantitatively using Chemiluminescence Immunoassay (CLIA) method. Patients with IgG level more than 1.4 were taken as positive.

**Results:** This study had estimated IgG level during screening of 440 voluntary plasma donors. Out of these, seropositivity was found in 354 patients. Patients with blood group O and AB were having seropositivity rate more than that of other ABO blood groups, whereas seronegativity percentage was more in blood group A patients.

**Conclusion:** There was a non specific relation between severity of symptoms and detectable development of IgG antibody in recovered corona patients. Also, blood group A patients develop less likely IgG antibody against COVID-19.

**Keywords:** Chemiluminescence immunoassay, Plasma donor, Severe acute respiratory syndrome coronavirus 2

## INTRODUCTION

COVID-19 is presently active as a pandemic worldwide causing remarkable morbidity and mortality. On 30<sup>th</sup> January 2020, it has been declared as a Public Health Emergency [1]. It had started to spread from China in the month of December 2019 to more than 200 countries throughout the globe. The mode of spread is human to human transmission. The causative agent is a Betacoronavirus named SARS-CoV-2. The clinical picture of this viral disease varies from fatal manifestations to asymptomatic. The symptoms includes sore throat, fever, cough, myalgia, sorethroat, headache, diarrhoea, anosmia, skin patches, loss of taste sensation, pneumonia, etc. Till January 2021 total 8,87,82,137 patients with confirmed SARS-CoV-2 and 22,47,395 death tolls has been reported worldwide by WHO. In severe cases, difficulty in breathing requiring mechanical support, multiorgan damage and death can occur [2]. Old age group people and those with other co-morbid conditions like diabetes, Chronic Obstructive Pulmonary Disease (COPD), malignancy, obesity and other heart diseases are vulnerable to COVID-19 infection. Many patients with mild symptoms recover quickly with/without symptomatic therapy.

Sometimes, severe inflammatory response called as Cytokine Storm may develop leading to lethal injury to different vital organs [3]. Out of all the SARS-CoV-2 patients only 80% show mild symptoms. SARS-CoV-2 enters into the human cells binding with the Angiotensin Converting Enzyme (ACE-2) receptor with the Receptor Binding Domain (RBD) of its spike protein [4]. The humoral immune response which develops against S Protein antigen may neutralise the virus [5]. For knowing seroprevalence of the disease usually SARS-CoV-2 and Nucleoprotein (N) Protein specific IgG antibodies are taken into consideration. For diagnosis of COVID-19 clinical manifestations, some laboratory tests and Computed

Tomography (CT) thorax are considered. The gold standard for confirming COVID-19 is Real Time Polymerase Chain Reaction (RT-PCR) test which detects presence of viral Ribonucleic Acid (RNA) in nasopharyngeal/oropharyngeal swabs. But this test needs to be done in sophisticated Bio Safety Level (BSL) -2/3 laboratories and also it is time consuming [6]. Sometimes, there can be false negative result due to inadequate sample collection. So, in a mass scale for quick diagnosis, many depends on antigen tests.

After SARS-CoV-2 infection, first antibody to develop is IgM within 3-6 days and thereafter, IgG develops within eight days. Therefore, recently serological estimation of IgG/IgM is taken into considerate for rapid and cost effective diagnosis along with antigen testing.

It has been observed that adequate serological response develops within 30 days of appearance of symptoms and persists for a limited period, in some cases, the IgG level remains in the peak level up to 80-90 days and starts waning thereafter [7].

In this study, the serum IgG level has been estimated in a cohort of recovered SARS-CoV-2 patients who were having severe/mild symptoms and who have voluntarily come for donating convalescent plasma. Possible correlation of these groups of patients with different ABO blood groups has been attempted to be established as ABO blood group system is the commonest to be adopted and clinically significant.

## MATERIALS AND METHODS

This retrospective study was conducted in plasma bank of Veer Surendra Sai Institute of Medical Science and Research (VIMSAR), Burla, Odisha, India over the period from 1<sup>st</sup> August to 15<sup>th</sup> December 2020. The sample size was based on the number of voluntary plasma donors. All the corona patients were having some symptoms/were

asymptomatic during their hospital stay or home quarantine period. This protocol has been adopted as per Indian Council of Medical Research (ICMR) and State Government Guidelines issued from time to time [8].

**Inclusion criteria:** Patients within age group between 18 to 65 years; having weight more than 50 kg and no co-morbid condition; unmarried/nulliparous females and those who fulfilling the primary screening criteria for blood donation were included.

**Exclusion criteria:** Patients less than 18 and more than 65 years; having weight less than 50 kg and those with comorbid conditions; patients not fulfilling the primary screening criteria for blood donation were excluded.

All the eligible convalescent COVID-19 patients who have recovered either from the hospital or from home quarantine and have been discharged/declared to go outside and have crossed 28 days and within 60 days were taken into consideration after taking prior written consent from the donor. In the plasma bank of VIMSAR, Burla, Odisha, India, all the samples of these donors were taken for Complete Blood Count (CBC), Transfusion Transmissible Infection (TTI), screening, grouping, X-matching, allo-antibody screening, serum protein estimation as well as for estimation of SARS-CoV-2 specific IgG antibody. The exclusion and inclusion criteria for selection of COVID-19 patients were followed as per ICMR guidelines [8]. Before coming for screening, all these recovered patients were subjected for RT-PCR test by collecting their nasopharyngeal swabs. All the persons with RT-PCR negative results were called for secondary screening.

For estimation of IgG specific antibody, the samples were sent periodically to ICMR, Bhubaneswar, Odisha and the assay was done by CLIA method using Abbott Architect i 100SR machine. The cut-off value for taking positive was more than equal to 1.4 index [10].

SARS-CoV-2 specific IgG antibody levels were quantified using clinically validated and widely used commercial serological assay (Architect, Abbott laboratories) detecting antibodies against the spike and nucleocapsid protein.

## STATISTICAL ANALYSIS

Statistical data were analysed by using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) statistical software 22.0. The data was calculated in percentage format and were analysed.

## RESULTS

In this study, over a period of about four and half months screening for IgG was done in 440 voluntary donors who have recovered from COVID-19 infection and voluntarily gave plasma for donation. Out of these 330 patients suffered from fever, 20 patients suffered from cough, 150 patients suffered from myalgia and 50 patients suffered from loss of taste/smell sensation. Thirty patients suffered from dyspnoea, 22 patients suffered from pneumonitis and 18 patients were asymptomatic. Out of the total patients, 260 patients were having more than one symptom as shown in [Table/Fig-1].

Symptoms	Symptomatic patients (n)	Percentage (%)
Fever	330	75
Cough	20	4.5
Myalgia	150	34.09
Loss of taste/smell	50	11.36
Dyspnoea	30	6.81
Pneumonitis	22	5
Asymptomatic	18	4.09
More than one symptoms	260	59.09

[Table/Fig-1]: Demographic and clinical features of patients.

On estimation of IgG antibody against COVID-19 in all these 440 patients, 354 patients showed positive value (cut-off value more than 1.4 AU/ml). Out of which most of the donors were with AB+ve (90.62) and O+ve (82.58) bloodgroup having seropositivity more than the average i.e., 80.45%. But donor having blood group A+ve (25.33%) and B+ve (33.33%) were having more seronegativity percentage than average i.e., 19.54%. The distribution of antibody positivity is different in different blood group patients, which has been depicted in [Table/Fig-2].

Blood group	Rh	Total cases	Ab Positive	Ab Negative	% Ab Positive	% Ab Negative
O	Positive	155	128	27	82.58%	17.42%
	Negative	02	02	00	100%	00
A	Positive	81	60	21	74.07%	25.93%
	Negative	02	02	00	100%	00
B	Positive	160	127	33	79.37%	20.63%
	Negative	06	04	02	66.67%	33.33%
AB	Positive	32	29	03	90.62%	9.38%
	Negative	02	02	00	100%	00
Total		440	354	86	80.45%	19.55%

[Table/Fig-2]: Relation between patient's bloodgroup and COVID-19 antibody (Ab) IgG.

## DISCUSSION

The anti-spike protein in COVID-19 SARS-CoV-2 are Anti-Receptor Binding Domain (RBD), Anti-S1 and Anti-S1/S2 proteins. As per Director General of Health Services (DGHS) and Central Drugs Standard Control Organisation on 1<sup>st</sup> July 2020 for use of convalescent plasma therapy, neutralising titre of donor plasma should be above the specific threshold. If this facility is not available, then plasma IgG titre (against S-protein RBD) above 1:640 should be taken as criteria. In another guideline by ICMR on 17.11.2020 they have advised regarding required concentration of anti-SARS IgG concentration to be in the titre of 1:640 (ELISA), or 13 AU/mL (CLIA) or titre of 1:80 Plaque Reduction Neutralisation Test/ Micro Neutralisation Test (PRNT/MNT) [8].

As per the abbot medical team and the publication it was observed that the value above cut-off will correspond to neutralising antibody titres of 1:80 (PRNT/MNT) as per guidelines and a cut-off value 4 will correspond to neutralising antibody titre of 1:160. The conclusion is strictly based on the supporting documents and publications in various reputed journals [9].

Apart from classical clinical presentation, many depend on the antigen test in addition to serological detection of IgG and IgM for diagnosis of COVID-19. In convalescent patients, first the serum IgG level is estimated which can be tested by several methods like lateral flow immunoassay CLIA and ELISA. In present study, the serum samples were sent to Regional Diagnosis Centre (ICMR) at Bhubaneswar for estimation of IgG level and it was estimated by CLIA principle [10].

In SARS-Cov-2 infection in early stage within 3 days, IgM develops and attains peak level in 2-3 weeks and then declines. Thereafter, IgG level starts appearing and remains usually with a remarkable level till 45-60 days. SAR-2 is a beta corona virus.

In this study, no relation of IgG level against SARS-CoV-2 was found with that of severity of the clinical manifestations. In the course of the disease, cytokine storms with severe immune dysfunction and other co-morbid conditions may be other contributory risk factors [11]. In some other studies, they have found that quantitative result of antibody detections are associated with the severity of COVID-19 and therefore, may help in predicting the outcome of the disease [12]. So to conclude, it can be opined that anti-SARS-CoV-2 antibody level is found to be within a remarkable level in this group of patients with different clinical presentations [12].

It has been observed by some studies that the specific IgG is detected in SARS-CoV-2 patients as early as four days after the onset of illness [13]. Also, some researchers have found that the antibody level in SARS-CoV-2 patients is not influenced by co-morbid conditions like diabetes mellitus, hypertension and cases with immunosuppression/immunomodulating agents [10]. The early appearance of specific antibody IgG in some SARS-CoV-2 patients may be due to possible prior infection of SARS-CoV-2 in these patients in the epidemic period but is extremely unlikely [6]. RT-PCR from the nasopharyngeal secretion has been found to be both sensitive and specific for the diagnosis of COVID-19 in early phase of illness [14]. Premkumar L et al., examined 77 samples for RT-PCR test and found that, nine days after onset of symptoms 98% patients had a positive IgG response with a specificity of 100% [15]. As per a study by Long QX et al., virus specific IgG levels in the asymptomatic group were lower than in the symptomatic group [16].

Sensitive and specific serological diagnostic modalities may be followed to estimate the prevalence of COVID-19 infection in a population to determine the level of herd immunity either following infection or in the population after vaccination. These data will be very useful for seroepidemiological studies as well as for vaccine trial. This study also opines that not all COVID-19 patients develop detectable IgG and it is vital for interpretation of COVID-19 seroprevalence survey. If in future a large group of such plasma donors will be followed-up, it may give clearer picture about the relation of IgG level with specific ABO blood group system. This study also shows that even after 90 days of appearance of symptoms, some patients develop detectable level of IgG in their body, though this group has not been included in this cohort group [17].

### Limitation(s)

There are certain limitations in this study. As this study group was limited in number it requires further study with a large group to arrive at any conclusion. The nature of and timing of serum sample collection differs from patient to patient. Detectable IgG may have appeared before samples meaning that the exact timing of seroconversion is not certain.

### CONCLUSION(S)

All COVID-19 patients do not develop detectable level of IgG using validated commercial method. The severity of clinical picture of the COVID-19 patients also is not directly proportional to the level IgG in the serum. In this study, it has been seen that though the percentage of corona patients with blood group O was the highest but on the

contrary, it has been found that the seronegativity in these group of patients were less than that found in patients with blood group A. The more incidence of seronegativity in patients with blood group A needs further studies for arriving at any conclusion.

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