



## SARS- CoV2 Antibody Sero-Surveillance In The Whole Blood Samples Collected For Hemogram In A Tertiary Care Center

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### Abstract

The COVID-19 pandemic has resulted in more than 30 million confirmed cases in India. In the span of a few months, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified as the etiological agent of coronavirus Disease 2019 (COVID-19). Weeks later, viral diagnostic measures were deployed<sup>1</sup>. Precise diagnostic tests detect viral Nucleic acids; viral antigens or serological tests are required to affirm SARS-CoV-2 infection<sup>2</sup>. Chest computed Tomography (CT) or magnetic resonance imaging (MRI) confirm disease manifestations.<sup>2-3</sup>

A prospective study was conducted in the Department of Pathology, Osmania general hospital, Hyderabad, Telangana, India during June 2021 to July 2021 when 2nd wave had just started to recede, we evaluated 200 hospitalized patients blood samples collected for Hemogram with details given by the patient in a prescribed feedback form designed to collect information regarding patients occupation, exposure to this infection through family contact, previous history of confirmed infection of SARS COV-2, and details of vaccination received if any. The blood samples were tested for detection of IgM and IgG antibody positivity using immunochromatographic test. Finally the results were analyzed by standard statistical tools.

**Keywords:** IgM, IgG, hemogram, antibody, SARS COV-2, COVID-19

### Introduction

As of Sept 30, 2020, India reported the second highest number of COVID-19 cases in the world, amounting to nearly 6.3 million cases and more than 97000 deaths.<sup>4</sup>

The first nationwide SARS-CoV-2 sero-survey in India was done in May-June, 2020 when the entire country was under stringent lockdown, with the exception of conditional relaxation in areas deemed to be minimally affected<sup>5</sup>.

It found a low seroprevalence of 0.73% (95% CI 0.34-1.13) among the general adult population aged 18 years or older.<sup>6</sup> Seroprevalence of SARS-CoV-2 IgG antibodies in individuals aged 10 years or older was 6.6% (95% CI 5.8-7.4). Seroprevalence was

highest in urban slum areas followed by urban non-slum and rural areas.<sup>7</sup>

In this present study we aim at assessing seroprevalence level in inpatients admitted with varying co-morbid conditions in our institute by randomly screening blood samples collected for the purpose of hemogram.

### Materials And Methods:

The present study was conducted in the Department of pathology, Osmania Medical College, Hyderabad on blood samples submitted for hemogram during the period of March 2021 June to July 2021.

### Study design

Prospective study.

### Study period

The present study was conducted during June 2021 to July 2021.

Pre-designed feedback form in printed format given to patient or patient attender specified in annexure-1.

### Sample size

200 Patients.

### Sampling procedure

Data collected and compiled from the feedback form and from the records of Hematology section of Department of Pathology, Osmania general Hospital Hyderabad.

### Selection criteria

#### Inclusion Criteria

1. Patients with age more than or equal to 10 years and less than or equal to 75years.
2. Patients whose blood samples were submitted for complete hemogram evaluation.
3. Patients who had filled the feedback form true to their knowledge.

#### Exclusion Criteria

1. Patients with age less than 10 years and more than age of 75 years.
2. Patients with clotted blood samples.

### Interpretation of Results

#### Positive

- A) A distinct coloured line appears at the control region 'C' and at the test region 'IgG' and 'IgM'.



- B) A distinct coloured line appears at the control region 'C' and at the test region 'IgM'.



- C) A distinct coloured line appears at the control region 'C' and at the test region 'IgG'.

3. Patient samples who did not furnish the data in prescribed form.

### Procedure

The study was approved by the Ethical and Research Committee of Osmania general Hospital. During the study period, all patients presenting with and fulfilling the inclusion criterion were included in this study after obtaining informed written consent. All patients' blood samples were screened for IgM and IgG antibodies using ICMR approved card test.

### Antibody-based card testing

**Principle:** ImmunoQuick COVID-19 test is a Rapid, Qualitative, Immuno-chromatographic test for detection of IgM and IgG Antibodies to COVID-19 Virus in human serum/plasma/whole blood.

**Test kit:** Provided by Immuno-Science India Private Limited.

#### Procedure:

1. Allow the test device and sample reach to room temperature (20°C to 30°C) before opening the foil pouch.
2. Place test kit on flat surface.
3. Add one drop (10 µl) of serum or plasma sample or add two drops (20 µl) whole blood in well 'S' and add two drops (Approx. 60µl) of assay buffer in same well 'S'.
4. Interpret the result at 15 - 20 minutes.



### Negative

- Only one coloured line appears at the control region ‘C’ only.



### Invalid results

The test should be considered invalid if,

- A) no line appears at ‘C’ region, ‘IgG’ and ‘IgM’ region.



- B) No line appears at ‘C’ region and line appear at ‘IgM’ and ‘IgG’ region.



- C) No line appears at ‘C’ and at ‘IgM’ region and line appear at ‘IgG’ region.



### Data analysis:

The collected data were entered in a Microsoft Excel Sheet. Graphs and tables were generated using Microsoft Word and Microsoft Excel. Multivariable logistic regression model was applied to explore the effect of several epidemiological risk factors on seropositivity (Demographics: Age, Gender, Living region and Occupation). Data was analyzed using statistical software SPSS 23.0 and MS excel. Descriptive statistics are expressed in percentages. An appropriate test of significance like chi square test was applied to determine the significance between

parameters. A p value of  $<$  or equal 0.05 was considered as significant.

### Results

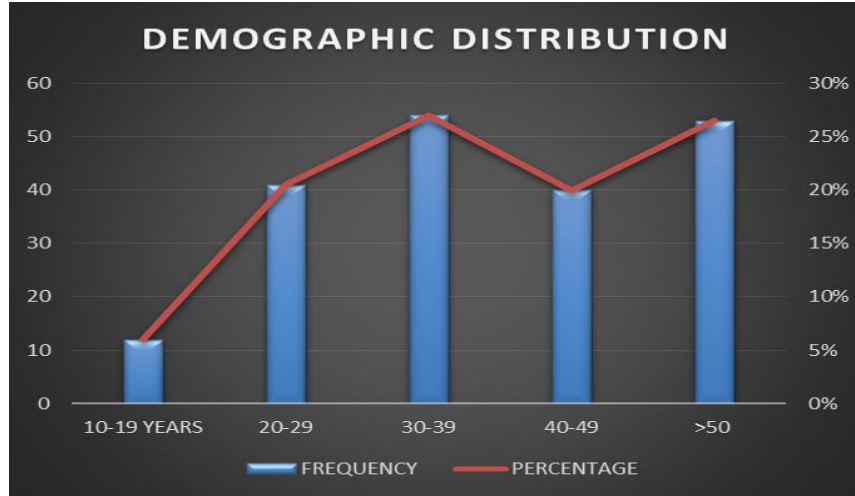
Out of 200 study participants 99 were male and 101 were female (Fig2).

Around 53% were urban localities and 47% were from rural localities (Fig3).

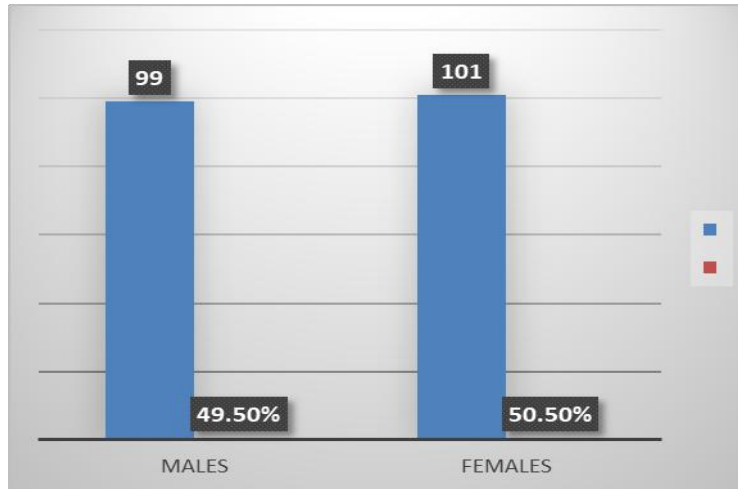
The mean age of the study population was  $39.2 \pm 14.88$  years with lowest age 10yrs and highest being 78yrs.

(Fig1)

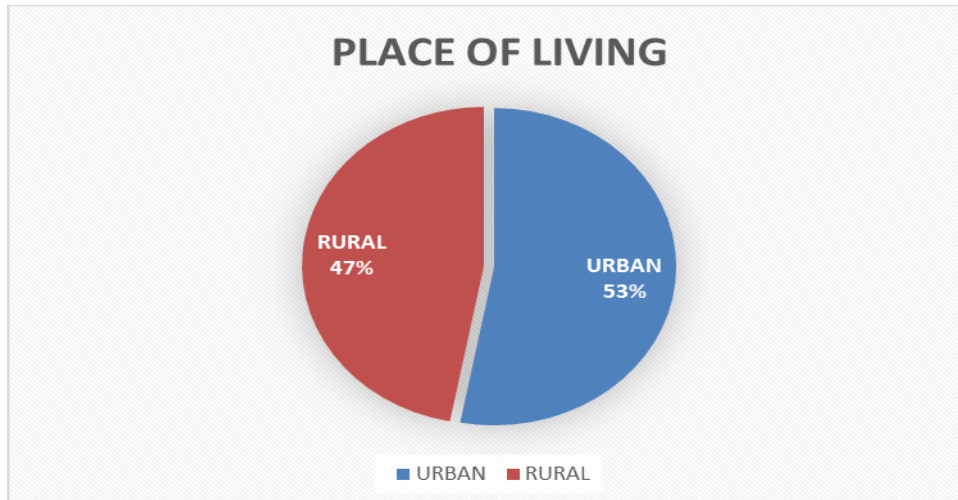
**Figure.1 Demographic Distribution Of Study Subjects**



**Figure.2 Gender Distribution Of Study Subjects.**



**Figure.3 Place Of Living Of Study Population**



**Table 1** The occupation among the subjects showed 43.5% were unemployed, 25.5% self-employed, 12.5% were students.

Occupation	frequency	percentage
Service	10	5%
Skilled	18	9%
Semiskilled	06	3%
Unskilled	03	1.5%
Self employed	51	25.5%
Unemployed	87	43.5%
Student	25	12.5%

Of all the 200 samples tested the known history of COVID was reported among 9 subjects (4.5%). 17 (8.5%) had exposure to COVID positive patients and 5 (2.5%) had history of travel. The immune status was noted for all and 19 (9.5%) were immunocompromised due to various reasons and with regard to vaccination against Covid only 5 (2.5%) were fully vaccinated (two doses), 14 (7%) were partially vaccinated (single dose) and majority of them 181(90.5%) were unvaccinated (Table 2,3).

**Table 2: Distribution of subjects according to past COVID history, exposure and travel**

Past COVID history	Frequency	Percent
Yes	09	4.5%
No	191	95.5%
<b>History of exposure</b>		
Yes	17	8.5%
No	183	91.5%
<b>History of travel</b>		
Yes	05	2.5%
No	195	97.5%

**Table 3: Distribution of subjects according to immune status and vaccination status**

Vaccination status		
Fully vaccinated	05	2.5%
Partially vaccinated	14	7%
Unvaccinated	181	90.5%

The underlying condition for which 200 patients had been admitted into the tertiary care center had been illustrated in table 4. Of all the causes hematological conditions were 31%.

**Table 4: Distribution of subjects according to reason for admission into tertiary care center.**

Condition	Frequency	Percent
<b>Medical</b>		
CVS	9	4.5%
CNS	8	4%
<b>GIT</b>	34	17%
<b>Renal</b>	11	5.5%
<b>Hematological</b>	62	31%
<b>Others</b>	23	11.5%
<b>Surgical</b>		
Traumatic	13	6.5%
Non traumatic	31	15.5%
<b>Obst. &amp; gynec.</b>	9	4.5%
Total	200	100%

The blood picture of the 200 patients had been illustrated in table 5. Normocytic blood picture constituted majority with 53% when compared to other types.

**Table 5: Distribution of type of blood picture in 200 sample examined.**

Blood picture	Frequency	Percent
Normocytic normochromic	53	26.5%
Normocytic hypochromic	53	26.5%
Microcytic	45	22.5%
Macrocytic	29	14.5
Dimorphic	20	10%
<b>Total</b>	200	100%

94 (47%) patient samples had thrombocytopenia. The mean neutrophilic lymphocyte ratio Was 7.14 with lowest value was 0.08 and highest was 37.42.

All the seropositive cases had been categorized into 3 categories

1. IgM positive
2. IgG positive
3. IgM and IgG positive.

#### 1. IgM positive cases (Table.6)

**Table 6. Number of cases positive for IgM antibodies**

IgM positive	Frequency	percentage
Yes	10	5%
No	190	95%

Of 200 samples tested for IgM antibody 10 (5%) were positive. The mean age was 36.47 (70%) out of 10 patients were from urban locality and male to female ratio was 1:1. Out of 10 cases positive 4 (40%) had known history of exposure and one case (1%) was fully Vaccinated with 2 doses of vaccine. 6 (60%) out of 10 cases had thrombocytopenia. The Mean N/L ratio was 3.7 among the positive IgM cases. When we observe demographic variables in relation to IgM positivity there was no significant P value noted (Table 7).

**Table 7: Logistic regression of seroprevalence rates according to socio-demographic characteristics**

IgM	Mean	SE	95 CI		P-value
			Lower	Upper	
<b>Age</b>					
10-19 years	17.96	7273.3	0.000	--	0.99
20-29 years	17.87	7273.3	0.00	--	0.99
30-31 years	17.55	7273.3	0.00	--	0.99
40-49 years	18.55	7273.3	0.00	--	0.99
>50 years	18.83	72.73	0.00	--	0.99
<b>Sex</b>	0.132	0.808	0.180	4.268	0.87
Place of living	0.178	0.716	0.293	4.865	0.80
<b>Occupation</b>					
Service	0.893	1.682	0.90	66.05	0.59
Skilled	18.88	9243.3	0.00	--	0.99
Semi-skilled	19.12	15868.5	0.00	--	0.99
Unskilled	18.27	20185.2	0.00	--	0.99
Self employed	1.079	1.649	0.13	8.604	0.51
Unemployed	0.535	1.544	0.28	12.07	0.72

## 2. IgG positive cases (Table.8)

**Table 8. Number of cases positive for IgG antibodies**

IgG positive	Frequency	percentage
Yes	51	25.5%
No	149	74.5%

Of 200 samples tested for IgG antibody 51 (25.5%) were positive. The mean age was 39.6. 27 (71%) out of 51 patients were from urban locality and male to female ratio was 1:0.9. Out of 51 cases positive 3 (5.8%) had known history of exposure and 2 cases (3.9%) were Fully Vaccinated with 2 doses of vaccine and 5 (9.8%) were partially vaccinated with one Dose. 17 (33.3%) out of 51 cases had thrombocytopenia. The Mean N/L ratio was 10.44 Among the positive IgG cases. 18 (35.2) out of 51 cases had normocytic blood picture. When we see socio-demographic variables in relation to IgG positivity P-value was found significant in relation of self-employed and those who are in service in government or private sector and in the patients of age group 40-49yrs (Table 9).

**Table 9: Logistic regression of seroprevalence rates according to socio-demographic characteristics**

IgG	Mean	SE	95 CI		p-value
			Lower	Upper	
<b>Age</b>					
10-19 years	0.53	1.42	0.104	27.953	0.70
20-29 years	0.65	0.59	0.218	2.206	0.53
30-31 years	0.732	0.527	0.171	1.351	0.48
<b>40-49 years</b>	<b>1.498</b>	<b>0.623</b>	<b>0.066</b>	<b>0.758</b>	<b>0.01</b>
>50 years	0.472	0.618	0.186	2.095	0.44
<b>Sex</b>	0.260	0.444	0.0544	3.095	0.55
<b>Place of living</b>	0.247	0.354	0.640	2.560	0.48
<b>Occupation</b>					
<b>Service</b>	<b>2.508</b>	<b>1.309</b>	<b>0.944</b>	<b>159.85</b>	<b>0.05</b>
Skilled	1.848	1.230	0.587	72.824	0.12
Semi-skilled	18.391	15838.4	0.00	--	0.99
Unskilled	1.778	7.715	5.917	0.205	0.30
<b>Self employed</b>	<b>2.270</b>	<b>1.172</b>	<b>0.973</b>	<b>96.25</b>	<b>0.05</b>
Unemployed	1.525	1.147	0.485	43.545	0.18

**3. IgM & IgG positive cases (Table.10)**

**Table 10. Number of cases positive for IgM & IgG antibodies**

IgM & IgG positive	Frequency	percentage
Yes	2	1%
No	198	99%

Of 200 samples tested for both IgM and IgG antibody 2 cases (1%) were positive. The mean age was 56.5, 2(100%) out of 2 patients were from urban locality and male to female ratio was 1:1. Out of 2 cases positive 1 (50%) had known history of exposure and 2(100%) out of 2 cases had thrombocytopenia. The Mean N/L ratio was 2.13. One out of 2 cases had Microcytic blood picture and another Macrocytic blood picture.



**Discussion:**

Conducting population-based sero-surveillance for severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) will estimate and monitor the trend of infection in the adult general population, determine the socio-demographic risk factors and delineate the geographical spread of the infection<sup>8</sup>. But when infection rates are low and in the resource limiting setting like our country with large urban to rural divide in health care facilities institute based sero-surveillance will help in having an estimate of active infection in community with the help of IgM positivity and community information of past infection and herd immunity due to vaccination by IgG positivity. The present study was undertaken to analyse sero-prevalence of IgM and IgG antibodies in patients admitted to a tertiary care centre for non-covid related conditions grouped as medical, surgical and obstetrics causes and given the sample of their blood for complete hemogram. So for this reason about 31% of samples are submitted for evaluation of underlying haematological condition like anaemia for evaluation or thrombocytopenia for evaluation followed by hemogram done as part of pre-surgical evaluation in 22% .

When we analyse demographic trends the significant finding with significant P value was overall IgG antibody positivity of about 25.5% with highest number of cases from urban areas (70% of cases) this was in concordance with the nationwide household sero-surveillance study conducted and published in lancet journal<sup>7</sup> which was conducted at same point of time in the previous year 2020 after recession of first wave of Covid-19 in India. According to it the higher seroprevalence in urban slum and non-slum areas observed in that study was consistent with that of other serosurveys in densely populated urban areas, where the prevalence ranged from 7.8% to 51.5%. The seroprevalence was also higher in slum areas of Mumbai (54.1%) compared with non-slum areas (16.1%) .

In our study significant statistical correlation was found in relation to urban self-employed persons and in persons in service indicating high prevalence rates among physically active urban population that are more susceptible for infection .

5% of IgM positivity among our study shows a risk of Covid infection among hospital admissions in a

tertiary care centre especially after an active wave, who had been admitted for non-covid conditions in general wards other than in covid wards. This emphasizes the need to screen non-covid patients soon after admission to prevent nosocomial infection among other patients and health care professionals working in that institution. This also avoids the centre being turned into epicentre for another epidemic.

The overall vaccination status in our study was only 4% which was explainable with the fact that in india vaccination drive was phasic with high priority given to health care workers and geriatric population during initial period of vaccine launch.

In our study the overall percentage of the seropositive patients with known history of exposure to contact cases was 4% signify the sub clinical nature of Covid infection in many in our community.

When we evaluate haematologically the predominant blood picture was normocytic which can be explained as many admit in tertiary care centre with chronic underlying conditions. The average N/L ratio in cases of active infection with IgM positivity was 3.7. In many studies, Multivariate analysis revealed an N:L ratio >3.7 was the major marker associated with severe forms Covid 19 infection<sup>9</sup> . N/L ratio of 10.4 among patients with IgG positive patients as it can be due to other underlying comorbid conditions. The presence of thrombocytopenia upto 60% of IgM positive patients was high when compared Wool G.D etal. Study which indicated thrombocytopenia in 5–41.7% of COVID-19 patients with the incidence varying according to disease severity<sup>10</sup>.

**Conclusion:**

Sero-surveillance studies aimed to assess community prevalence is done by state and central health authorities when prevalence rates are high, But when prevalence rates are low hospital based sero-surveillance is required to assess the active infection rate in chronic ill patients who are admitted in a tertiary care center with other Co-morbidities through IgM positivity rate. This also helps us to inform local bodies for contact tracing as already done in cases of Vector Borne diseases in our country. It also gives an estimate of risk of Covid 19 infection to hospital staff working in Non covid wards through patients admitted for other conditions in tertiary care centers. The IgG positivity rate indicates the amount of herd

immunity and post vaccination status in our community. Hospital based fever, RTI cases IgM sero-surveillance and its incorporation into integrated disease surveillance programme is the only future cost-effective modality to detect the infection early to mark the containment zones, endemic areas and prevent future epidemics of SARS COV-2 in our country. The Indian Council of Medical Research (ICMR), therefore, proposes to establish a community-based district-level sero-surveillance system to monitor the transmission of SARS-CoV-2 infection in the general population. The initial survey would serve as a baseline to determine the seroprevalence of SARS-CoV-2 Infection in the community and in high-burden cities as well, while the subsequent rounds would help to monitor the trends of infection in the community<sup>8</sup>.

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