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Original Article

Seroprevalence OF Antibodies against SARS-COV2

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ABSTRACT

Study Background: The novel corona virus (COVID -19) is a known virus in current pandemic. Clinical presentation ranges from asymptomatic to severe respiratory and multiple organ failure[2].Large number of SARS-COV2 cases are documented as asymptomatic or with mild illness. To determine the extent of infection, serological evidence of SARS-COV2 antibodies is warranted

Objectives of the Study: To study the Seroprevalence of covid-19 antibodies among hospitalized patients and to determine the cumulative immunity in hospitalized patients.

Methodology: This cross-sectional observational study was conducted at United hospital and Creek General Hospital, Karachi during the duration from December 2020 to February 2021. The study was approved by institutional ethical review board. Probability convenience method of sampling was used to collect blood samples. Patients of both genders having age group ranging from 1-80 years or above were included. These patients were asymptomatic and admitted due to non- COVID 19 reasons. Rapid test was used to detect SARS-COV2 IgM/IgG antibodies. Statistical analysis of data was performed by using SPSS-22. Age is organized into different subgroups. Chi-square test was applied to determine the significance between age groups and seropositive cases.

Results: 800 blood samples were collected from different age groups of hospitalized patients and analyzed. 18.8% (n=151) cases were observed seropositive. 25.7% (n=61) seropositive cases were in age group 16-30 years followed by 19.5% in age group from 61-75 years. Statistical significant correlation was observed among seropositive IgG cases and age group ranging between 16-45 in female and above 45 in males.

Conclusion: This study concluded 18.8% seroprevalence among asymptomatic admitted patients. The criteria of herd immunity is not fulfilled but it can be used for further research. The chance of seropositivity in females during reproductive age group is more as compared to other age groups.

Keywords: Serprevalence, COVID 19 , Antibodies, Rapid test, Asymptomatic

Introduction

The novel corona virus (covid-19) belongs to the family of SARS-COV2 is a widely known virus in recent pandemic and new mutations still remain a challenge to healthcare system.[2,6] Corona viruses are a group of RNA viruses with non-segmented, single stranded genome.[1,7] COVID-19 is a highly infectious disease which involves respiratory system of humans and animals in a range of complete absence of symptoms to severe respiratory distress syndrome, metabolic acidosis, coagulopathy and multi organ failure.[2,6] Most commonly observed clinical manifestation in our population include fever, dyspnea, cough, myalgia and GI symptoms.[6]

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However, the majority of individuals follow an asymptomatic course or suffer from very mild self-limiting disease (81%).[11] Only a small number of patients suffer from severe illness or critical consequences. The virus can be transmitted mainly through respiratory droplet infection. Both asymptomatic and symptomatic (and even pre-symptomatic) cases can transmit it and it has already affected millions of people around the globe.[4, 8]

Various diagnostic modalities are used for the detection of this virus, out of which viral ribonucleic acid detection by Polymerase Chain Reaction is considered the most reliable one. Other lab tests are also available and can predict the clinical outcome.[1, 3]Antibodies are produced as a result of exposure to viral antigens even in the absence of symptoms. The quantitative or qualitative analysis of these antibodies in serum or whole blood serves as an important diagnostic tool and helps to determine the immunity

against viral antigens.[8, 9] In comparison with other diagnostic measures like PCR or tissue cultures, Antigen-antibody test kits are the tools which are quite popular because they are easy to use, give rapid results and relatively inexpensive. Antibody assessment also helps to determine not only the active but also the convalescent and recovery phases of disease which aids in determination of the epidemiological spread and pattern of infection, effectiveness of safety measures practiced and assessment of herd immunity among asymptomatic population.[6, 9,10]

Most people infected with the virus develop antibodies specific to SARS-CoV-2 proteins approximately 1–2 weeks after the exposure. The IgM levels begin to rise in blood after one week of exposure to virus and it demonstrates the active phase of infection. The IgG levels appear after 14 days and may last for 6 months. Presence of both IgM and IgG levels indicate convalescent phase of disease while presence of IgG alone is representative of recovery phase/ indicates past exposure.[7,8] A sample however will be considered positive if it contains either IgM, IgG or both. Asymptomatic individuals should be evaluated by antibody testing to know about seroprevalence in community. Although difference in seroprevalence for anti-SARS-CoV-2 antibodies can be marked across the globe[6,8,9,10] The benefit of seroprevalence evaluation will be the assessment of herd immunity which can serve as a beneficial parameter to assess the need of vaccination. It will prevent the next wave of infection as well as serve as a guide about the need of further implication of social safety measures.[6]

Objective: The objective of study is to determine seroprevalence and to assess the cumulative immunity of COVID 19 among hospitalized patients. The study is designed to estimate asymptomatic carriers to limit the transmission of infection.

Materials & Methods

This was a cross-sectional observational study carried out at United hospital and Creek General Hospital Karachi from December 2020 to February 2021 after getting approval by institutional ethical review committee with reference to ERC number – UMDC/IRB- Ethics/2021/01/01/274. Sample size was calculated as 800 by using WHO calculator with a confidence level of 95% at 5% margin of error. As it is ongoing disease, so exact prevalence of disease is not known so we have taken estimated 50% prevalence as per statistical rule. Sampling technique used was probability convenience method. The inclusion criteria were following. The blood samples of both male and female patients admitted due to non- COVID reasons between the age group ranging from 1-80 years and above. The samples were collected from different wards of the hospital and analyzed for SARS-COV2 IgM /IgG antibodies. All the OPD patients less than 1 year of age and all the patients with positive COVID-19, PCR were excluded from the study.

Rapid Tests were performed using the serum of the participants with the help of High top SARS-COV2 IgM/IgG antibody rapid test kit.COVID-19 IgG/IgM. Rapid Test is a rapid chromatographic membrane-based immunoassay for the qualitative detection of IgG and IgM antibodies to COVID-19 is helpful in diagnosis of primary and secondary COVID-19 infections.[2,3,5]

After taking all the precautionary measures and consent from the patients, blood samples were collected. The whole blood samples were centrifuged to get serum. One drop of serum was added vertically into the sample well along with 2 drops of sample buffer. The test results were immediately observed within 15-20 minutes, When the sample contains the SARS-COV2 IgM antibody, it develops color at T2 line after forming complex which indicates a positive result. When the sample does not contain the SARS-COV2 IgM antibody, no complex can be formed at the T2 line, and no red band appears, which indicates a negative test.The performance characteristics of this test were evaluated as 94.15% sensitivity and 93.91% specificity. The test result of each participant was recorded and entered into the data. Data collected during the study period included patient's age, gender, ethnicity, profession, clinical history/diagnosis and ward name.

Data was analyzed by using SPSS-22. Age is arranged into different subgroups and correlated with positive cases and measures in terms of frequency. Chi-square test has been applied to determine the significant difference among different age groups. P value of <0.05 is considered significant.

Results

Total of 800 blood samples were collected from different age groups of hospitalized patients and analyzed. Out of 800 samples 18.8% (n=151) were seropositive and 81 %(649) were seronegative. Among 151 positive samples 63 were male and 88 were females as shown in figure-1.

The pie chart shows the breakdown of collected samples from different hospital wards. Out of 151 seropositive samples 32% (n=49) were collected from patients admitted to medicine ward followed by 30% (n= 45) and 20% (n= 30) from Obs/Gynae ward and surgery respectively.

Figure-1: Gender Wise Frequency of Seropositive Cases

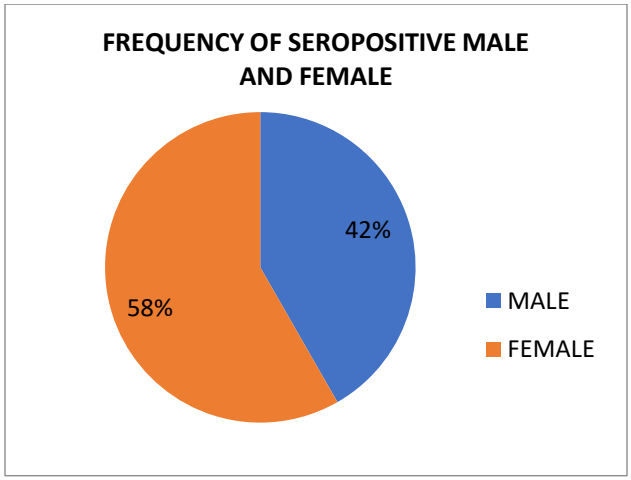

Figure-2: Percentage of Positive Samples from Different Wards

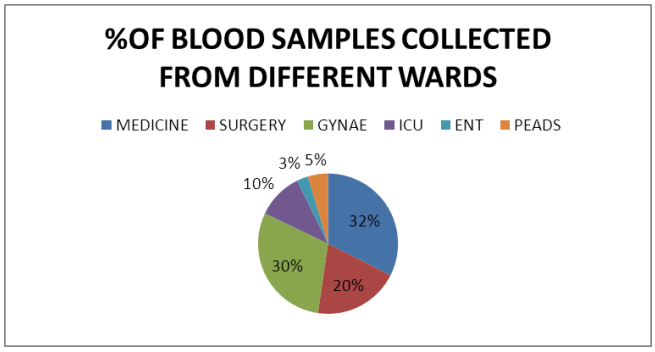

Table No: 1 showed results attained from initial analysis of blood samples from different age groups. The overall percentage of positive samples were 18.8% (n=151). Out of 151 seropositive samples 25.7% (n=61) were in age group 16-30 years. Surprisingly the second most seropositive age group is between 61-75 years of age group (19.5%).

Table-1: Frequency of Seropositive and Seronegative Cases in Different Age Groups

Sr. #	Age group (in years)	Seropositive	Seronegative	Total no samples
1	1-15	8 (13.%)	53(86.8%)	61
2	16-30	61 (25.7 %)	176 (74.2%)	237
3	31-45	39 (19%)	166 (81%)	205
4	46-60	24 (13.6%)	152 (86.3%)	176
4	61-75	17 (19.5%)	70 (80.4)	87
6	76-90	2 (6%)	32 (94%)	34
TOTAL		**151 (18.8%)**	**649 (81.1%)**	**800**

Among positive cases 23.17% blood samples were positive for IgM only 49.6% for IgG and 27.15% showed both antibodies as demonstrated in Table No 2.

Table No 2: Percentage Of Anitibodies Among Seropositive Cases

Antibodies	Number of cases	Percent
IgM	35	23.17
IgG	75	49.6
BOTH	41	27.15
TOTAL	**151**	**100**

Table No 3 demonstrated correlation between presence of antibodies IgM and IgG and age. To test the correlation Cross tab was performed and Chi-square test was applied. This revealed significant association among seropositive IgG cases and age group ranging between 16-45 years in female and above 45years in males(P= .006 < P= .05) . *P*- value of < .05 is considered as significant. In females 86% (n=37) of females showed positive IgG in age group ranging from 16-45 years. While 50% (n=16) of male above 45 years of age were observed IgG positive in contrast to 14% females above 45 years.

Table-3: IgM AND IgG Antibodies Association with Different Age Groups

Age in groups	IgM			IgG			Both		
	Male	Female	Chisqaure	Male	Female	Chisqaure	Male	Female	Chisqaure
1-15	1 (5%)	1 (6%)	**0.634**	2 (6%)	0 (0%)	**0.006**	3 (25%)	1 (3%)	**0.220**
16-30	6 (32%)	9 (56%)		9 (28%)	24 (56%)		2 (17%)	11 (38%)	
31-45	3 (16%)	1 (6%)		5 (16%)	13 (30%)		3 (25%)	14 (48%)	

46-60	6 (32%)	3 (19%)		7 (22%)	3 (7%)		2 (17%)	3 (10%)	
61-75	3 (16%)	2 (13%)		7 (22%)	3 (7%)		2 (17%)	0 (0%)	
76-90	0 (0%)	0 (0%)		2 (6%)	0 (0%)		0 (0%)	0 (0%)	
*. The Chi-square statistic is significant at the 05 level.									

Table no 4 represents different ethnicity groups with highest number 44.4% from Urdu speaking followed by 29% from Punjabi ethnicity. Statistical significance was not found.

Table-4: Frequency of Seropositive Cases in Different Ethnicity Groups

Ethnicity Groups		Frequency	Percent
1	SINDHI	28	18.5
2	URDU SPEAKING	61	40.4
3	PUNJABI	44	29.1
4	PATHAN	14	9.3
5	BALOCHI	4	2.6
TOTAL		151	100.0

Discussion

This hospital based research project was designed to study seroprevalence among hospitalized patients which demonstrated 18.8% seropositive cases. These admitted patients did not show any mild or severe COVID 19 symptoms and admitted because of other reasons. A study conducted in Karachi showed 36% seropositivity among general population[12]. Our results are nearer to studies conducted in Iran (22%)[13] and India (22%-33%)[14]. Our results are contradictory to western world showing 5.27% in France[15] , 5%in Spain[16] and about 1.1- 14.4% in USA[17,18]. The difference in seroprevalence may be due to a number of reasons such as different study population groups, variation in immune response, variation in antibody testing, difference in healthcare facilities and further unknown reason of increased seropositivity in this part of world[12]

Current study did not mention any significant difference in frequency of seropositive males and females. This is similar to other reported studies[19]. Present study witnessed highest number of positive cases in age group ranging between 15-45 (44.7%) years. Interestingly this is followed 19.5% seropositive cases in age group ranging from 61-75 years. The lowermost can be seen among children possibly linked to low expression of angiotensin converting enzyme 2.[20] A study conducted in Switzerland demonstrated 8-10% seroprevalence in age group between 25-40 years[8]. A probable justification of witnessing seropositivity in this age group of admitted patients is that majority of Pakistan population falls in the 15-40 years age group. Despite of safety measures, socio-cultural and behavioral differences make the younger people more exposed to the outside world hence more chances of infections. Additionally majority of our population belongs to middle –low socioeconomic status and they are living in close localities and have more social interaction with each other along with neglecting the precautions required for COVID 19[12]. Another possible aspect of this is different conspiracy theories in our part of world. Moreover prevalence in old age group may be associated with comorbidities and compromised nutritional status[12]

Current study revealed 23.17% IgM only, 49.6% IgG and 27.15% both. In present study 84% female having age group 16-45 years showed IgG. While 50% male above 45 years of age group showed IgG whereas only 14% females showed positive results. This demonstrated that these patients have developed immunity being an asymptomatic carrier. While a study conducted in tertiary care hospital India showed seropositive cases (22%) in patient age group 40-59[14]. A research in Lahore, Pakistan demonstrated 15.6% IgG in young police men[21]. Pinto.et al 2020 in Brazil demonstrated 11% IgM and 8.3% IgG and peak incidence in age group above 40[22]. A study in New York stated 14.8% seroprevalence in males which is more as compare to females[23]. Although in current study no significant difference was observed in IgM levels among males and females in different age groups. The variation in gender based immune response has been studied by scientists in various researches[24,25]. Ziegler et al 2017 studied the possible role of interferon and oestradiol in augmenting the adaptive response in reproductive age group during infectious disease[26]. A study conducted in U.S discussed the immunosuppressive role of testosterone concluded that decrease in testosterone levels would

enhance antibody production[27]. Furthermore the age related alterations in the immune system are also varies with gender[28]. However further investigations are needed to signify the importance of gender and age as a variable in research study. Moreover this would be helpful to understand the generalized public health response to pandemic COVID 19.

In our study 40.4% were Urdu speaking followed by 29% belongs to Punjabi ethnicity. Although, we did not observe any significance of seropositive cases among different ethnicity groups.

The strength of our study includes that few studies has been done among hospitalized patients and this study will help to identify the asymptomatic carriers in hospitals that would be helpful in limiting the exposure of health workers to COVID 19. Although the extent of antibody protection is still questionable, we recommend the continuation of research in this field and preventive measure against COVID 19.

The limitations of our study is that it was not a multicenter study so it will not be a true representation of seroprevalence among hospitalized patients and exact burden of the disease and immune status cannot be evaluated correctly. Quantitative analysis and PCR were not performed which could be another limitation of our study.

Conclusion

It is concluded that 18.8% of patients admitted to hospital due to non COVID reason showed seroprevalence. We concluded more seropositive cases in reproductive age group of females

Conflict of Interest:

There is no conflict of interest among the authors.

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C. Interpretation/ Analysis and Discussion