

Sero-Prevalence of Anti SARS-COV-2 Antibodies with Impact of Age, Gender and Severity of the Disease: A Hospital Based Study: COVID-19

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ABSTRACT

Objective: To determine the sero-prevalence of neutralizing antibodies levels against SARS-COV 2 with impact of age, gender and severity of the disease.

Methodology: A cross sectional study was conducted in department of Pathology from 1st July 2020 to 15th August 2020. A total of 109 respondents attended the laboratory for knowing their anti SARS-COV-2 antibodies levels irrespective of age and gender. Antibodies levels were measured by electro-chemiluminescence immunoassay using Roche Cobas E411 Chemistry Analyzer.

Results: The mean age of the subjects was 35+13 years while mean cut off antibodies levels of patients with standard deviation was 15.74+7.18. Out of total, 82(75.2%) were males and 27(24.8%) were females. 91(83.5%) of the respondents were in age below 60 years and 18 (16.6%) were above 60 years. The mean antibodies level were higher in patients with age < 60 y and in patients who had symptoms in the recent past during the pandemic (17.5+3.93, p=0.03) and (36.51+4.41, p=0.001) respectively. Similarly a statistically significant inverse correlation was observed between antibodies levels and age of patients (p=0.03, r=-0.204). Among the asymptomatic/mildly symptomatic patients, 10/67, had yielded antibodies with a seroprevalence of 14.9% while all PCR positive cases (100%) recovered from COVID-19 had developed antibodies after 3 week of being reported negative.

Conclusion: A low mean neutralizing antibodies levels production against SARS-COV-2 had a sero-prevalence of 14.9% in asymptomatic non-PCR-tested population. All positive patients who recovered had developed antibodies after 3 weeks of being reported negative. Age<60 years, female gender and patients who had symptoms matching the COVID-19 during the pandemic irrespective of their viral profile produces higher level of antibodies. Male gender and patients with age > 60 years showed low levels of antibodies.

Keywords: Anti SARS-COV-2 antibodies, COVID-19, Pandemic.

INTRODUCTION

Covid-19 pandemic has resulted in change in life style across the globe with clear declination in the economy to run official business. Pakistan is not an exception. People especially the salaried official and daily wagers are more concern about, when to return to work and earn the live hood for their families. One of the necessity for return to work in major commercial sectors is to certify, by doing their antibodies level to check whether they are immune to the pandemic and are being non-infectious.^{1,2} This way they can join duties in a responsible and safe manner. Many believe that by knowing the antibodies level on larger scale could

help making evidence based decision both at individual and social levels. In Dec 2019 many cases with pneumonia of unknown etiology were reported from the metropolitan city of Wuhan, China that was later on named COVID-19 by the World Health Organization.³ To date many patients have been reported with COVID-19 to have symptoms of fever, cough, dyspnea, loss of taste and smell and myalgia.⁴ By date 21st August, Pakistan data from government sources declares 291,588 confirmed cases with 6219 deaths. Out of the total confirmed cases, 273,5793 patients are recovered so far and 1316 cases are under treatment in high dependency units countrywide.⁵ After an infection the body produces pathogen specific immunoglobulin, this feature is

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called immunological memory and body do retain memory of the previous infection.

Antibodies testing are done to detect immunoglobulins that are produced in human blood in response to infection with COVID-19. However the issue is the time period that for how long these antibodies are effective to protect an individual against the COVID-19. The antibodies production is related to the severity of infection and reportedly last for 3 months after the onset of first symptoms.⁶ The use of both IgM (produced in early infection) and IgG (produced in late stage) are advantageous to protect the body from infection.⁷

In case of COVID-19 the IgM detection threshold stands for 14-21 days after the onset of symptoms while IgG having long half life and its production continues to rise for 28 to 35 days after the symptoms and are detectable for months after the resolution of infection.^{1,6,7}

The IgG antibodies production in mild, moderate and severe COVID, differs in gender groups. Antibodies levels in female gender tends to be high as compared to males which may account for different outcome of COVID.⁸ Regarding the impact of age in COVID-19, we have been able to publish a study in Microbes and infectious disease (MID) where it was observed that the rate of infectivity and exposure was 2 times higher in people with age <55 years (**p=0.005, OR=2.01**) while the case fatality was three times more in patients with age >55 years (**p=0.001, OR=3.16**).⁹ One of the possible explanation would be that people with age less than 55 years has strong immune system to respond by producing the high titer of the neutralizing antibodies as compared to the patients with age >55 years.

Present study was designed as to determine the mean neutralizing antibodies levels, impact of age and gender and on production of neutralizing antibodies levels and to predict the severity of the disease, the respondent had in the recent past along based on the neutralizing antibodies level.

METHODOLOGY

This cross sectional study was conducted in the department of Pathology on 109 respondents, who attended the Laboratory for measuring their anti-SARS-COV-2 antibodies levels, from 1st July 2020 to 10th Aug, 2020. A total of 109 tests were done, out of which 42 were those patients who were reported negative on PCR, with a gap of 3 weeks.

Sample size of 109 was calculated on the assumption that the anticipated proportion of the sero-prevalence of IgG against corona virus as 10%¹⁰ with Absolute precision of 5%,

Confidence level of 95%.

All patients were randomly selected irrespective of age and gender with non-probability convenient sampling. Ethical Approval was taken from ethical review board of Nowshera Medical College Nowshera Via letter No 123/ERB/NMC Dated 12-6-2020. Verbal consent was taken from all the respondents, that their confidentiality shall be maintained.

The inclusion criteria was; patients who were reported positive previously on PCR, and to present a negative PCR done 3 weeks before. Similarly patients who were not previously tested or had no confirmatory evidence of sero-conversion, but were advised antibodies test by the consultants of the hospital were also included.

The exclusion criteria was; positive COVID-19 patients with duration less than three week of being reported negative for corona virus.

3ml of the blood was collected in Gel tube under aseptic conditions. Antibodies level was measured by electro-chemiluminescence immunoassay using Roche Cobas E411 Chemistry Analyzer for which commercial kits of Roche diagnostics were used as per the instructions of the manufacturer.

Data was uploaded in the SPSS version 25. Numerical variables like age and anti SARS-COV-2 antibodies levels were presented as mean with standard deviation and range. Categorical variables like age categories, gender and clinical presentation were presented with percentages. Independent t test was used to determine the difference in mean antibodies level in different groups/categories like gender, age categories and categories of clinical presentation.

Spearman's correlation was used to determine the correlation of quantitative variables i.e antibodies and age of patients.

RESULTS

The mean age of patients with standard deviation was 35+13 years while mean antibodies cut off values of patients with standard deviation was 15.74+7.18.(Table 1). Regarding the frequency of different variables we observed that 82(75.2%) were males and 27(24.8%) were females. 91(83.5%) of the respondents who attended the department for antibodies testing were in age below 60 years. Forty two (38.5%) of the tested population had done their prior PCR test and were confirmed positive cases with recovery. Out of total, 67(61.46%) were asymptomatic since the beginning of the epidemic irrespective of their viral profile. (Table 2)

Table 1. Frequency of different variables

2.1. Gender			
	Frequency	Percent	Cumulative Percent
Male	82	75.2	75.2
Female	27	24.8	100.0
Total	109	100.0	
2.2. Age groups			
	Frequency	Percent	Cumulative Percent
Age<60 y	91	38.5	83.5
Age>60 y	18	61.5	100.0
Total	109	100.0	
2.3 PCR status			
	Frequency	Percent	Cumulative Percent
PCR Done	42	75.2	38.5
PCR not done	67	24.8	100.0
Total	109	100.0	
2.4. Clinical presentation/History			
	Frequency	Percent	Cumulative Percent
Asymptomatic History	66	60.6	60.6
Symptomatic on history	43	39.4	100.0
Total	109	100.0	

Table 2. Antibodies levels in difference in dichotomous variables

3.1. Age Group Statistics				
Age categories	Number of patients	Mean	Std. Deviation	p-value
Antibodies	Age<60 y	91	17.50	0.034
	Age>60 y	18	6.87	
3.2. Gender Group Statistics				
Gender	Number of patients	Mean	Std. Deviation	p-value
Antibodies	Male	82	16.23	0.678
	Female	27	14.29	
3.3. Clinical presentation				
Clinical History	Number of patients	Mean	Std. Deviation	p-value
Antibodies	Asymptomatic	67	22.22	0.001
	Symptomatic	42	36.51	

The difference in mean antibodies level of the respondents in age <60 years and age >60 years was statistically significant and patients in age less than 60 years reported with high antibodies level (17.5+3.93,p-0.03). However the difference in mean antibodies level in gender groups was not statistically significant (p-0.67). A higher mean antibodies levels were reported in patients who were clinically symptomatic during the pandemic irrespective of knowing their viral status (36.51+4.41, p-0.001) (Table 3). It was observed that a statistically significant inverse correlation was observed between antibodies levels and

age of patients (P=0.03, r: -0.204). This phenomenon explain the fact that patient in younger age produces high titer of antibodies as compared to the aged patients. Hence the younger age people confer more resistance to the virus. (Table 4).

DISCUSSION

By detecting the antibodies level we thought to help administration to make wise, evidence based decisions regarding reverting back to normalization of routine duties to the pre-COVID era and to know about the level of

Table 3: Spearman's correlation between quantitative variables i.e age and antibodies levels.

		Mean	Antibodies	age
Spearman's (rho)	Antibodies	Correlation Coefficient	1	-.206*
		Sig. (2-tailed)	.	0.032
		N	109	109
	Age	Correlation Coefficient	-.206*	1
		Sig. (2-tailed)	0.032	.
		N	109	109

*Correlation is significant at the 0.05 level (2-tailed)

immunity of healthcare workers, as more than 90% of the studies population belonged to the employees of Qazi Hussain Ahmed Medical Complex Nowshera. Decision based on facts and figures supporting the vulnerable groups (male patients and age > 60 years), give peace of mind to physician and surgeons working emergency and outdoor patients department to deal with COVID-19 patients in elective settings.¹¹

Ten (10/67) asymptomatic non-PCR-tested target populations had yielded antibodies production with a sero-prevalence of 14.9%. They were not aware of their viral status and had sufficient neutralizing antibodies against COVID-19. Our findings are slightly higher than the Leung GM¹² who reported 10% sero-prevalence among the asymptomatic non tested population, for SARS-COV-2 in their study. The mean antibodies cut off values of patients with standard deviation was 15.74+7.18 in our study. A higher antibody titre was directly associated with a worse clinical classification (p=0.006) that was consistent with an understating that patients with severe disease have a higher antibody titre in their plasma to neutralize the antigenic virus, along with high levels of other inflammatory markers like d-dimers, ferritin and C reactive proteins.¹³ Out of total 42 were reported positive by PCR and were tested for antibodies level after 3 week. All of the recovered patients had sufficient antibodies levels 36+13. A study from Iraq has reported 91% of the population who were previously infected and had developed antibodies to Corona virus.¹⁴ The difference in mean antibodies level of the respondents in age <60 years and age >60 years was statistically significant and patients in age less than 60 years reported with high antibodies level (17.5+3.93,p=0.03). The CDC report from China reported 80% of the casualties (deaths) due to COVID-19 were in the adults aged >60 years as compared to 0.1% in person aged < 19 years, that identifies the immunity gap.¹⁵ A statistically significant downhill correlation was observed between antibodies levels and age of patients (P=0.03, r: -0.204). There has been

a relationship between age and related morbidity and mortality in COVID-19. As the young people morbidity is less severe due to their good immune status.¹⁶ Study from China has reported a high rate of severity of infection with high relative mortality risk of (rr-0.48) in an increase in age.¹⁷ It was noted from a trial conducted in Wuhan, China that individual with prolonged viral shedding were older patients (> 65 years, IQR 63-70 years) versus patients 55 years inter-quartile range (IQR 44-68 years) with a significant p-value of 0.05. Similarly those patients had other co-morbidities as well. The IgG antibodies levels were significantly lower in those patients showing their weak immune status and less ability to defend.¹⁸

In present study a higher mean antibodies levels were recorded in patients who were clinically symptomatic during the pandemic irrespective of knowing their viral status (36.51+4.41, p=0.001). The IgG antibodies were significantly higher in asymptomatic confirmed COVID-19 cases and in patients with mild symptoms. However the IgG levels were lower in severe infection and in critically ill patients who had recovered, this could be because of high disease activity and compromised immune system.¹⁹ No significant difference can be predicted in probability of surviving in both the genders based on antibodies levels to correlate it with the severity of infection (Fig-1). However in age > 60 years, the probability of surviving drops vertically due to production of very low neutralizing antibodies and hardly they could report antibodies cut off values above 30 in this study. This low level of antibodies production again is in concordance with the observation made by Hou H et al¹⁹ who correlated these low level of antibodies production with high activity of the disease and compromised immunity in these people at extreme of age.

CONCLUSION

We concluded that low mean neutralizing antibodies levels production in our population confers a low immunity and that's why the researcher are afraid of a second wave of the

emergence of COVID-19. The seroprevalence in asymptomatic cases was 14.6% while it was 100% for patients recovered from COVID-19. Patients with age <60 years, female gender and patients who had symptoms matching the COVID-19 during the pandemic irrespective of their viral profile had higher level of antibodies. Male gender and patients with age >60 years produces low levels of antibodies and are more susceptible to morbidity and mortality associated with COVID-19.

There were some limitations to the study, we could not followed these cases from the start, we could not record the IgM levels and the time period for conversion/replacement of IgM with IgG. Similarly we could not follow these patients to check for how long the peak values of IgG could sustain to confer immunity duration.

RECOMMENDATION

Therefore future studies should concentrate on the sustainability of IgG to determine the duration of immunity that antibodies confer against COVID-19.

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