Original Article

A Case Control Study of COVID-19 and its Association with Antibody Titre

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Abstract

Background : Antibodies are detected in the blood of people who are tested after infection; they show the body's efforts to fight off a specific infection.

Aims and Objectives : To know the difference in the antibody titre between cases and control. To correlate the time interval between development of disease and antibody titre test and the number of days of symptoms with antibody titre in COVID-19 positive participants

Materials and Methods : A case control study was done at University Health Centre of the Maharaja Sayajirao University of Baroda. SARS CoV-2-specific IgG antibody titre was measured in 100 case and 100 controls. Correlation coefficient and chi square test was applied for statistical analysis.

Result : 82% cases and 36% controls were seropositive for IgG Ab titre against COVID-19.97% cases had mild symptoms and only 3% had moderate symptoms. The mean and SD of symptoms was 8.4 days (SD-6.7). The correlation between time interval of development of disease and Ab titre test done with antibody titre was found to be inverse with r=-0.3810, P=0.0001, (95%CI = -0.5372 to -0.1995). The correlation between number of days of symptoms present with antibody titre was positive with r= 0.2254, P=0.0242, (95% CI= 0.03029 to 0.4039).

Conclusion : About one-third of controls were seropositive for IgG antibody titre against COVID-19 without any sign and symptoms of COVID-19. The correlation between time interval of development of disease and Ab titre test done with antibody titre was inverse with low negative correlation and negligible correlation was found between number of days of symptoms with antibody titre.

Key words : Case Control Study, Antibody Titre, IgG, COVID-19, Symptoms.

COVID-19 is the disease caused by a new Corona virus called SARS-CoV-2. WHO first learned of this new virus on 31 December, 2019[1].

Antibodies are detected in the blood of people who are tested after infection; they show the body's efforts to fight off a specific infection. In general, a positive antibody test is presumed to mean a person has be eninfected with SARS-CoV-2, the virus that causes COVID-19, at some point in the past. Antibodies usually start developing within 1 to 3 weeks after infection[2].

Antibodies to SARS-CoV-2 demonstrate infection when measured at least 14 days after symptom onset[3].

Two studies published demonstrate that COVID-19

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Editor's Comment :
- Asymptomatic infections are common in COVID-19.
- As the duration of illness increases, the antibody titre decreases.
- Immunity is attained as a result of COVID-19 infection.

immune-responses last as long as 8 months, although the authors focus on different reasons. The first study, published in *Science Immunology*, followed as mall cohort of Australians from day 4 to day 242 after infection. All patients demonstrated the presence of memory B-cells, immunecells that "remember" viral proteins and can trigger rapid production of antibodies when re-exposed to the virus as long as 8 months safter initial infection. The second study investigated antibody responses in 58 confirmed COVID-19 patients in South Korea 8 months after asymptomatic or mild SARS-CoV-2 infection, finding high rates of serum antibodies. These results, published in *Emerging Infectious Diseases*, are contradictory to both the first study's antibody data and previous research that showed antibodies waning after 20 days, but theauthors suggest that variations in immunoassay test characteristics and manufacturing may be responsible for the difference[4].

AIMS AND OBJECTIVES

(1) To know the difference in the antibody titre between cases and controls.

(2) To correlate the time interval between development of disease and antibody titre test with antibody titre in COVID-19 positive participants.

(3) To correlate the number of days of symptoms with antibody titre in COVID-19 positive participants

MATERIAL AND METHOD

A case control study was carried out at University Health Centre of the Maharaja Sayajirao University of Baroda. The study was conducted among residents of University campus including staff members and students studying and residing at university. Those who were COVID-19 positive and minimum 30 days have elapsed from the date of RTPCR/RAT testing were selected as cases. Those who were COVID-19 test negative or those who had never tested for COVID-19 and never developed any COVID related symptoms were selected as controls.

Each participant was explained the purpose of the study and only those who signed the consent form was included in the study. Blood sample for IgG antibody titre was collected by a trained laboratory technician. Total study duration was 13 months, January, 2021 to January, 2022. The blood sample was collected solely for the purpose of measurement of IgG antibody of COVID-19. Report of each participant was given and explained to them.

Out of 200 participants, 100 were cases and 100 were controls. Pilot study was done for calculation of sample size. In this pilot study, the Odds of having antibody in COVID-19 positive patients was 3 times higher as compared to non-COVID-19 patients. At 95% CI with power-90%, ratio of cases and controls was 1:1, proportion of exposure in control-20% and in case -42% and using Open Epi software[5] the sample size came 100 in each group. So, we took sample size of 100 cases and 100 controls.

Questionnaire : Apre-tested, semi-structured questionnaire was used to collect data on COVID-19 diseasere garding Socio-demographic details of the participants, co-morbidity of the patients, knowledge of hand hygiene, social distancing, any ayurvedic medicine or homeopathic medicine intake, chemoprophyl axis of HCQ, visit to public places like hotels, malls, parks, had been in social gatherings like party, marriage, any travel history outside city, state or country, the manner, place and type of masks used by them.

ANALYSIS

Data entry was done in Microsoft Excel 2007 and analyzed by using MedCalc software version 12.5.0.0[6]. Descriptive analysis was used to describe the distribution of all variables in cases and control group. Odds ratio was calculated for presence of specific SARS CoV-2 IgG antibody among cases and controls with 95% confidence interval. Correlation was assessed for the time interval between development of disease and antibody titre test with antibody titre in COVID-19 positive participants. Correlation was also assessed for the number of days of symptoms with antibody titrein COVID-19 positive participants. Correlation coefficient was calculated for the same. Chi square was calculated to see the significance of factors associated with presence of antibody in controls.

Ethics : The study was approved from the Ethical Institutional Ethics Committee for Biomedical and Health Research (IECBHR) of the institute. After explaining the participants in their local language about the study, its purpose and confidentiality of information a written consent was taken from them before starting interview.

Data Confidentiality : The study participants were assured that the information they shared would be kept strictly confidential and would be used only for research purposes and underno circumstances was shared with anyone in a manner which could identify any of the participant.

COVID-19 antibody IgG was measured by fully automated 2-step sandwich immuno-assay using indirect chemiluminescent technology (<1.0 index is non-reactive and $\geq$1.0 index is reactive).

OBSERVATIONS

200 (100 cases and 100 controls) participants (mean age and SD of cases - 42.97±15.91 years, controls- 37.53±14.52 years) were included. Among cases 46% were males and among controls 43% were males. 68% cases and 44% controls were educated more than or equal to graduation (Table 1).

Table 2 showed, there was statistically significant difference observed in education (p-value- 0.042),

Table 1 — Socio-demographic profile of the participants (N=200)

Variables	Case (n=100)	Control (n=100)	Total (n)(%)
Age group (years) :			
>45 years	49	28	77(35.5%)
≤45years	51	72	123(61.5%)
Age (Mean ± SD)	42.97±15.91	37.53±14.52	
Gender :			
Male	46	43	89(44.5%)
Female	54	57	111(55.5%)
Education :			
More than or equal to Graduation	68	44	112(56%)
Less than Graduation	32	56	88(44%)
Occupation :			
Business / self employed	5	8	13(6.5%)
Government service	46	42	88(44%)
Private service	8	6	14(7%)
Home maker and retired	24	21	45(22.5%)
Student	17	23	40(20%)

travel history (p-value- 0.0064), mask use (p-value-0.0010), technique of wearing mask (p-value- 0.182), knowledge of hand hygiene (p-value- 0.0032) between two groups of controls, one with antibody titre present (n= 36), and other with no antibody titre (n=64).

Out of 100 cases 82 cases were seropositive and 18 cases were seronegative for COVID-19 IgG antibody and among controls 36 were seropositive. The crude odds ratio was 8.09 which shows that the odds of antibody presence in cases was 8 times more in comparison to controls, with CI=4.21-15.56. We found χ^2=43.73 and p-value=<0.0001, which shows that this association was statistically significant (Table 3).

97% cases had mild symptoms and only 3% had moderate symptoms. The mean and SD of symptoms was 8.4 days (SD-6.7).

Table 4 shows that 73% cases of COVID-19 had fever as presenting complain, 33% had fatigue. Loss of taste and smell was present in 30% patients of COVID-19, cough in 26%, Sore throat in 22%. 18%, 13% and 10% had experienced myalgia, diarrhoea and rhinorrhoea respectively. Breathlessness was present in 10% of cases.

6 out of 100 cases were asymptomatic. In 2 patients had symptoms which lasted for about 30 days complaining loss of taste and smell and fatigue as symptoms which persisted for long time.

The correlation between time interval of development of disease and Ab titre test done with antibody titre was found to be inverse with r= -0.3810, P=0.0001, (95%CI=-0.5372 to -0.1995). This means that with increase in duration there was decrease in antibody titre, which showed that the finding was statistically significant.

Table 2 — Factors associated with antibody presence among controls

Parameters	Antibody present n=36(%)	Antibody absent n=64(%)	χ^2	p-value
Age (in years) :				
>45	12 (33.33%)	16(25%)	0.793	0.372
≤45	24 (66.67%)	48(75%)		
Gender :				
Male	23 (63.89%)	34(53.12%)	1.089	0.2966
Female	13 (36.11%)	30(46.88%)		
Education :				
More than or equal to Graduation	11 (30.56%)	31(48.43%)	4.126	0.042
Less than Graduation	25 (69.44%)	33(51.56%)		
Travel History :				
Yes	8 (22.22%)	32(50%)	7.407	0.0064
No	28 (77.78%)	32(50%)		
Visit outside the home :				
Never/sometimes	13 (36.11%)	21(32.81%)	0.1117	0.7381
Daily	23 (63.89%)	43(67.19%)		
Social gathering :				
Yes	11 (30.56%)	18(28.13%)	0.0661	0.7970
No	25 (69.44%)	46(71.87%)		
Mask use :				
Regular	25 (69.44%)	61(95.31%)	10.746	0.0010
Irregular	11 (30.56%)	3(4.69%)		
Technique of wearing mask :				
Correct	29 (80.56%)	61(95.31%)	5.5748	0.0182
Incorrect	7 (19.44%)	3(4.69%)		
Knowledge of hand hygiene :				
Correct	20 (55.56%)	53(82.81%)	8.684	0.0032
Incorrect	16 (44.44%)	11(17.19%)		
Maintaining social distance :				
Yes	30 (83.33%)	61(95.31%)	2.706	0.099
No	6 (16.67%)	3(4.69%)		
Self-medication of homeopathic or ayurvedic medicine :				
Yes	3 (8.33%)	4(6.25%)	0.0003	0.9869
No	33 (91.67%)	60(93.75%)		
Home remedies :				
Yes	9 (25%)	13(20.31%)	0.295	0.587
No	27 (75%)	51(79.69%)		
HCQ prophylaxis :				
Yes	1 (2.78%)	4(6.25%)	0.0822	0.774
No	35 (97.22%)	60(93.75%)		
Comorbidity :				
Yes	6 (16.67%)	17(26.6%)	1.274	0.2590
No	30 (83.33%)	47(73.4%)		

Table 3 — Status of antibody presence in cases and controls (n same as percentage as n= 100 for both cases and controls)

Antibody	Cases (100)	Controls (100)	Total
Present	82	36	118 (59%)
Absent	18	64	82 (41%)
OR = 8.09, CI = 4.21-15.56, χ^2 = 43.73, p-value = <0.0001			

After categorizing the participant of COVID-19 into 3 groups according to time interval of development of disease and Ab titre test done.

A=<90 days; B=90-180 days; C=>180 days. We

Table 4 — *Cases present with specific symptoms*	
Symptoms	Number of cases (n=100)
Fever	73 (73%)
Fatigue	33 (33%)
Loss of taste and smell	30 (30%)
Cough	26 (26%)
Sorethroat	22 (22%)
Myalgia	18 (18%)
Diarrhoea	13 (13%)
Rhinorrhoea	10 (10%)
Breathlessness	10 (10%)
Expectoration	03 (03%)
others	10 (10%)
Asymptomatic	06 (06%)
*Multiple answers possible	

observed the mean and SD of titre level in each group to be A(n=53)=11.9(SD-10.3); B(n=25)=11.4(SD-10.0); C(n=21)=2.4(SD-3.5).

The correlation between number of days of symptoms present with antibody titre was positive with r= 0.2254, P=0.0242, (95% CI= 0.03029 to 0.4039). This means that with increase in number of days of symptoms, there was increase in antibody titre. This shows that the finding was statistically significant.

DISCUSSION

This study proposed to determine the antibody titre difference in cases and controls, correlation of time interval of disease development and antibody titre test done with antibody titre in COVID-19 positive patients and correlation of number of days of symptoms with antibody titre in COVID-19 positive patients.

In our study we had taken 100 cases and 100 controls. We found 36 participants among controls to be seropositive for COVID-19 antibody.

In the present study 82% COVID-19 patients were positive for IgG serum antibody to COVID-19 and 36% controls were also positive.

The reasons were irregular use of mask (11%), incorrect hand wash technique (16%) travel history (28%) and in correct maskuse technique (7%). They were exposed to Corona virus due to above mentioned reasons but were asymptomatic and produced antibody to COVID-19.

A study conducted by Caturegli G, MateriJ, Howard BM, Caturegli P showed similar result as 83.33% of cases of COVID-19 were positive for serum antibody but no control had serum antibody[3].

One study conducted in Ahmedabad by Shilpa Yadav,

21% of those who had recovered from COVID-19 were positive for IgG antibody titre while in our study 82% were positive for the same[7].

Similarly, a study was done in Karnataka by G. Babu which showed that 16.8% of post COVID19 patients were positive for IgG antibody titre wherein our study showed that 82% were positive for the same[8].

The correlation between time interval of development of disease and Ab titre test done with antibody titre was found to be inverse and statistically significant. It was observed that as time elapsed after infection with COVID-19 there was decrease in antibody titre.

A study conducted by Levi R, et al showed that the antibody response persists for at least 8-10 months[9].

After categorizing the participant of COVID-19 into 3 groups according to time interval of development of disease and Ab titre test done.

A=<90 days; B=90-180 days; C=>180 days. Here we had observed that the mean of antibody titre of group A and group B was similar equal to 11 and mean of antibody titre of group C was significantly low and it was around 2. It was observed that there was low level of antibody titre when sample was taken after 180 days had elapsed from the day of RAT/RTPCR positive for COVID-19.

A study was conducted by F Javier Ibarrondo, et al which showed that levels of antibody against COVID-19 decreases dramatically over 3 months of infection while in our study we had observed that levels of antibody decreases dramatically over 6 months of infection[10].

Initially we had planned to check the Ab titre for COVID-19 for each case at different points of time from the time of COVID-19 diagnosis (30-90 days, 90-180 days, >180 days). However due to ethical issues this could not be done as it was unethical to ask the cases to refrain from vaccination just for the purposes of this study.

In the present study, there was statistically significant positive correlation of number of days of symptoms with antibody titre which showed that with increase in number of days of symptom there would be higher serum antibody titre. In our study there was negligible correlation.

Similarly, a study conducted by Files J, et al showed that symptom duration correlates with S-specific IgG antibody (r = 0.4); there was low positive correlation[11].

A study conducted by Young MK, et al showed that

IgG antibodies were most positively correlated with days from symptoms onset with an r value of 0.4 while in our study there was positive correlation but with R value of 0.22[12].

In our study, we found that 73% had the chief complain of fever with second most common complain of fatigue and loss of taste and smell with 33% and 30% respectively. Cough was present in 26% of cases, diarrhea (13%) Sore throat (22%) and least common was expectoration in 3% of cases.

A study conducted in China by Guan W, *et al* the predominant symptom was fever as was present in 43.8% of the patients on admission but developed in 88.7% during hospitalization. The second most common symptom was cough (67.8%). Nausea or vomiting (5.0%) and diarrhea (3.8%) were uncommon[13].

Another study conducted in tertiary care of Delhi by Jugal Kishore *etal* observed fever (51.4%) as the most common complain of COVID-19 patients followed by cough (41.7%), breathlessness (28.1%),headache (25.2%) and nausea/vomiting (20.3%)[14].

The study was limited only to the University Health Centre, which represents patients visiting to that centre only. And due to fear of COVID-19 limited number of patients used to come. Those who had history of moderate orsevere COVID-19 and had any comorbidity avoid to come to health centre unless there was any emergency. Therefore, data for moderate and severe categories were missed, which could have improved the results.

The strength of the study was that we have quantified the actual IgG titre value of all the participants instead of doing only presence and absence of IgG antibody (seroprevalence).

CONCLUSION

82% cases and 36% controls were seropositive for IgG Ab titre against COVID-19.

Asymptomatic and healthy seropositive controls for COVID-19 IgG Ab, who were undetected may actas superspreaders for COVID-19. This highlights the need for continuation of self-protective measures like proper and regular mask wearing and maintaining social distance.

The correlation between time interval of development of disease and Ab titre test done with antibody titre was inverse with low negative correlation.

Negligible correlation was found between number of days of symptoms with antibody titre.

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