Kinetics of Covid-19 antibodies in terms of titre and duration among healthcare workers: A longitudinal study

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ABSTRACT

Background. Most individuals with Covid-19 infection develop antibodies specific to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, the dynamics of these antibodies is variable and not well-studied. We aimed to determine the titres of naturally acquired antibodies over a 12-week follow-up.

Methods. We recruited healthcare workers who had tested positive on a specific quantitative reverse transcription-polymerase chain reaction (qRT-PCR) for SARS-CoV-2, and then tested for the presence of immunoglobulin G (IgG) antibody against the same virus at baseline and again at 6 and 12 weeks. The antibody titre was determined by a semi-quantitative assay based on signal/cut-off ratio. Healthcare workers with antibody positivity were divided into those with high titre (ratio $\geq$ 12) and low titre (< 12). Their demographic details and risk factors were surveyed through a Google form and analysed in relation to the antibody titres at three time-points.

Results. Of the 286 healthcare workers, 10.48% had high antibody titres. Healthcare workers who had tested positive by qRT-PCR and those who had received the Bacille Calmette–Guérin (BCG) vaccination or other immune-boosters had a higher frequency of high antibody titres. While there was a significant decline in antibody titres at 6 and 12 weeks, 87.46% of individuals positive for IgG antibody persisted to have the antibody even at 12 weeks.

Conclusion. Healthcare workers who tested positive for SARS-CoV-2 on qRT-PCR had a high positivity for the specific antibody, which continued to express in them even at 12 weeks. Further follow-up is likely to enhance our understanding of antibody kinetics following SARS-CoV-2 infection.

Natl Med J India 2022;35:201–5

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[**To cite:** Goenka MK, Goenka U, Patil VU, Das SS, Afzalpurkar S, Jajodia S, *et al*. Kinetics of Covid-19 antibody in terms of titre and duration among healthcare workers: A longitudinal study. *Natl Med J India* 2022;**35:**201–5.]

INTRODUCTION

The ongoing Covid-19 was declared a pandemic in March 2020. We still do not have a clear understanding regarding the immunological response of the host to this novel coronavirus. However, it is well known that antibodies including immunoglobulin G (IgG), IgM and IgA are generated in most patients in response to Covid-19 infection within 1–3 weeks.[1–6] The humoral immune response may be variable in terms of magnitude and duration. The quantity of antibodies in plasma may be the determinant of its protective capability and the utility of convalescent plasma as a treatment.[7] Experience with another coronavirus has shown that the humoral immune response is variable.[8–11] While antibody to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and Middle East respiratory syndrome are known to persist even up to 3 years, those against human alpha and beta coronaviruses usually wane before 12 weeks.[9,12–14] Data suggest that there is rapid disappearance of IgM antibody following Covid-19 infection.[15] Durability of IgG antibody response to Covid-19, however, has not been evaluated well.[3,4,15–19]

We conducted a seroprevalence study of Covid-19 among healthcare workers (HCWs) in our hospital, and analysed those seropositive (IgG Ab) for Covid-19.[20] It is believed that detectable IgG Ab imparts protective immunity from re-infection to that particular individual. However, it is unclear whether the antibody response is related to disease severity.

We aimed to determine the magnitude and durability of naturally acquired Covid-19 antibodies over a 12-week follow-up period. Our primary objective was to compare groups with high and low titres of antibodies to independent variables among Covid-19 cases. The secondary objective was to determine the relationship between various risk factors of Covid-19 infection and the antibody response.

METHODS

Study population

The study was approved by the internal ethical committee of the institute. Subjects included HCWs from our hospital who had

IgG Ab against Covid-19 detected between July and September 2020.

We evaluated the level of IgG in HCWs who had tested positive for SARS-CoV-2 by qRT-PCR. These HCWs were tested either due to symptoms suggestive of Covid-19 disease or close contact with positive cases. The HCWs were then requested to come for a follow-up antibody testing at 6 weeks (±3 days) and again at 12 weeks (±3 days).

HCWs were divided into three groups based on the risk of exposure to Covid-19-positive patients:

1. High risk: Those who were working or had worked in a Covid-19 ward or an intensive care unit and those regularly involved in the testing or investigating a Covid-19 patient.
2. Intermediate risk: Those not belonging to either high- or low-risk groups, i.e. HCWs who are managing patients or performing procedures on patients not diagnosed or suspected to be having Covid-19. These included, but were not limited to, staff working in emergency, aerosol-generating facilities and outpatient services.
3. Low risk: Those who had no direct contact with the patients or their belongings, e.g. staff belonging to the administrative cadre, human resource department and marketing.

Covid-19 antibody testing

Antibodies to Covid-19 were tested in the plasma of participants using the enhanced chemiluminescence method (Vitros ECi, Ortho Clinical Diagnostics, New Jersey, USA) on a luminometer. Signal-to-cut-off ratio (S/Co) was used to semi-quantitatively categorize patients with antibodies to Covid-19 as high titre or low titre at a cut-off value of 12 (≥12 categorized as high titre).[7,21]

Profile questionnaire

All participants were also sent a questionnaire on Google forms either through a registered phone number or email address. This form collected variables that included demographic and clinical data. The form had 26 questions with multiple-option answers requiring either single or multiple replies. Survey questions were divided into three categories: (i) demographic details of participants; (ii) details of job profile; and (iii) medical history including symptoms or diagnosis of Covid-19 (by qRT-PCR).

Statistical analysis

Participants with high antibody titre were compared with those with low antibody titre in terms of various parameters including qRT-PCR positivity, age, gender, occupation, blood group, history of smoking, Bacille Calmette–Guérin (BCG) vaccination and comorbid conditions. During 6- and 12-week follow-up testing, the number of HCWs becoming negative for IgG Ab against Covid-19 was also recorded.

The data were compiled and later analysed by the software SPSS version 22.0 (IBM Inc, Chicago, Illinois, USA). The Chi-square test was used for comparisons of antibody titres. The McNemar test and Paired *t*-test were applied to compare baseline and follow-up antibody titres. Binary logistic regression was used to determine the strength of predictors. For all tests, confidence interval and p value were set at 95% and <0.05, respectively.

RESULTS

We recruited 286 HCWs who tested positive for SARS-CoV-2 IgG Ab. These included 129, who had tested positive during our seroprevalence study among HCWs but had never been positive by qRT-PCR for SARS-CoV-2. The other 157 HCWs had tested positive for antibodies 2–3 weeks after having been qRT-PCR-positive for SARS-CoV-2. Five HCWs who were qRT-PCR-positive did not have any detectable IgG SARS-CoV-2 antibodies even on sequential testing and hence were excluded from the study (Fig. 1).

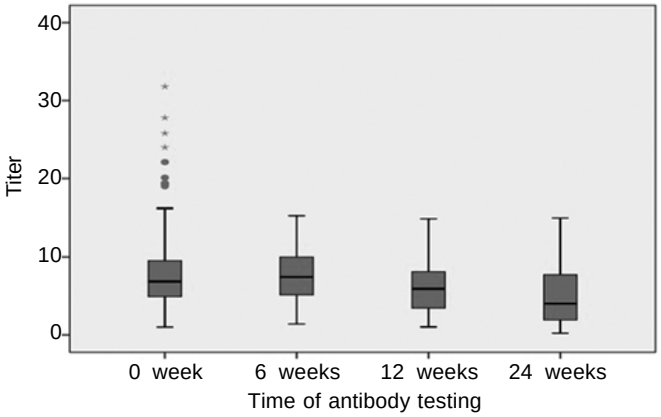

FIG 1. Study flowchart and result in the form of IgG Covid-19 antibody positivity

Table I compares the various parameters in these two groups at baseline.

There was a greater probability of high titre in those who had earlier received BCG vaccination than those who had not (15.0% *v.* 7.2%, p=0.034). Similarly, individuals receiving immune boosters had 14.7% incidence of high titre compared to 6.3% without a history of intake of immune boosters (p=0.021). qRT-PCR-positive HCWs had 24.26% times higher odds of high antibody titre than those not testing positive (p=0.002, Table II).

Thirty-seven (23.57%) Covid-positive patients required hospitalization, and among them only 4 (10.8%) had high antibody titres (Table III). Low oxygen/acute respiratory distress syndrome (ARDS), need for hospitalization, duration of hospitalization and requirement of treatment with steroids and/or antiviral drugs did not have any significant association with antibody titre (Table III).

Follow-up at 6 and 12 weeks

Of the 286 participants, 28 dropped out of the study at 6 weeks and a further 17 dropped out at 3 months (Fig. 2). Thus, 258 HCWs were tested for IgG Ab against Covid-19 at 6 weeks, while 232 HCWs were re-tested at 12 weeks. Only 9 of 258 (3.49%) become negative for antibody at 6-week follow-up, and 9.05% (21 of 232) become negative for antibody at 12 weeks. Interestingly, the 30 individuals, who became undetectable for the antibody during follow-up belonged to the low-titre group.

Among 232 patients evaluated at 12 weeks, the titres at 0 week, 6 weeks and 12 weeks are given in Table IV and shown in Fig. 3. The decrease in titre between the three time-periods was significant (p=0.0001, Wilcoxon signed Rank test). The symptomatic group had significantly high antibody titre not only at baseline but also on 6-week and 12-week follow-up (Table V).

Antibody response among the seropositive group (after excluding RT-PCR-positive patients) was compared at 6 weeks and 12 weeks from the baseline (Table VI). There was no significant drop in the mean antibody levels during follow-up

TABLE I. Comparative assessment of high and low titres of antibodies at baseline according to independent variables

Independent variable	Antibody titres		p value	Total, *n* (%)
	Low (<12), *n* (%)	High (≥12), *n* (%)		
qRT-polymerase chain reaction				
Positive	128 (81.5)	29 (18.5)	0.001*	157 (54.9)
Not positive	128 (99.2)	1 (0.8)		129 (45.1)
Age group (years)				
<30	95 (96)	4 (4)	0.058	99 (34.6)
31–40	113 (86.9)	17 (13.1)		130 (45.5)
41–50	41 (85.4)	7 (14.6)		48 (16.8)
51–60	7 (77.8)	2 (22.2)		9 (3.1)
Gender				
Men	86 (90.5)	9 (9.5)	0.693	95 (33.2)
Women	170 (89)	21 (11)		191 (66.8)
Occupation				
Administration	10 (83.3)	2 (16.7)	0.217	12 (4.2)
Dietician	12 (85.7)	2 (14.3)		14 (4.9)
Consultant doctor	3 (60)	2 (40)		5 (1.7)
Non-consultant doctor	16 (80)	4 (20)		20 (7)
Front office staff	8 (80)	2 (20)		10 (3.5)
Housekeeping	74 (94.9)	4 (5.1)		78 (27.3)
Laboratory assistant/pharmacist	21 (84)	4 (16)		25 (8.7)
Nurse	64 (90.1)	7 (9.9)		71 (24.8)
Technician	25 (92.6)	2 (7.4)		27 (9.4)
Ward executives	12 (92.3)	1 (7.7)		13 (4.5)
Others	11 (100)	0		11 (3.8)
Blood group				
A	47 (92.2)	4 (7.8)	0.496	51 (17.8)
Non-A	209 (88.9)	26 (11.1)		235 (82.2)
Smoking				
No	202 (89.8)	23 (10.2)	0.777	225 (78.7)
Yes	54 (88.5)	7 (11.5)		61 (21.3)
Diet				
Non-vegetarian	249 (89.2)	30 (10.8)	0.359	279 (97.6)
Vegetarian	7 (100)	0		7 (2.4)
Bacillus Calmette–Guerin vaccination				
No	154 (92.8)	12 (7.2)	0.034*	166 (58)
Yes	102 (85)	18 (15)		120 (42)
Comorbid conditions				
Absent	221 (90.9)	22 (9.1)	0.06	243 (85)
Present	35 (81.4)	8 (18.6)		43 (15)
Use of immune boosters				
No	134 (93.7)	9 (6.3)	0.021*	143 (50)
Yes	122 (85.3)	21 (14.7)		143 (50)
Allergic disorders				
Yes	230 (90.2)	25 (9.8)	0.278	31 (10.8)
No	26 (83.9)	5 (16.1)		255 (89.2)
Total	256 (89.5)	30 (10.5)		286 (100)

*Statistically significant difference, Chi-square test

after 6 weeks (p=0.9, Wilcoxon signed ranks test). However, there was a significant drop in antibody titre in the third time-period, i.e. 12 weeks in comparison to the first titre (p=0.05). Similarly, there was a drop in mean antibody levels after 12 weeks in comparison to the levels after 6 weeks (p<0.0001).

DISCUSSION

We have shown that patients with PCR positivity had a higher chance of a high antibody titre. A study by Long *et al.*[22] from China has also shown that IgG Ab level in symptomatic Covid-19 patients was much higher compared to that in the asymptomatic group. However, high titre in our study did not correlate with severity of disease as evidenced by need for target therapy, oxygen and duration of hospitalization. This is somewhat different from the experience by Seow *et al.*[16] who did notice a relationship between antibody titre and severity of disease. The reason for low antibodies among seropositive cases could be a reflection of the time duration between exposure and time of testing.

Our study also shows a higher antibody response in patients who had received BCG vaccination in childhood and 'immune-boosters'. Sharma *et al.*[23] had reported the mortality to be lower and recovery rate to be higher in countries that have BCG vaccination in their universal health programme. Malik *et al.*[24]

TABLE II. Binary logistic regression analysis with antibody titre as the dependent variable

Independent variable	Odds ratio	Confidence interval	p value
Polymerase chain reaction test			
Positive	24.26	3.227–182.467	0.002
Negative*			
Bacillus Calmette–Guerin vaccination			
Received	2.28	1.008–5.136	0.048
Not received*			
Use of immune boosters			
Yes	2.27	0.971–5.326	0.059
No*			

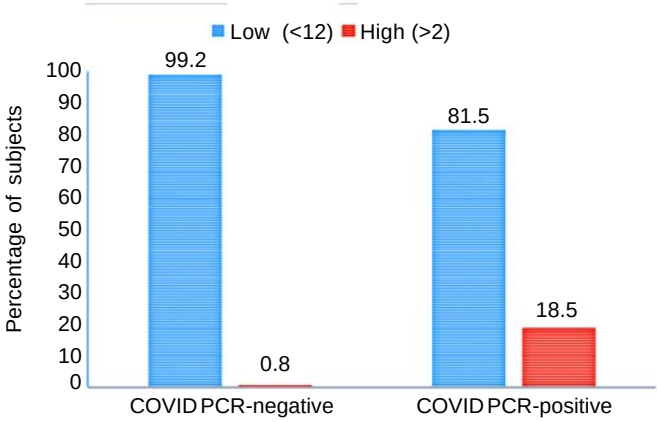

FIG 2. Comparative assessment of high and low titres of antibodies according to Covid-19 positivity

TABLE III. Comparative assessment of high and low titres of antibodies according to independent variables among polymerase chain reaction-positive Covid-19 healthcare workers

Independent variable	Antibody titres, *n* (%)		p value	Total *n* (%)
	Low, *n* (%)	High, *n* (%)		
Low oxygen or acute respiratory distress syndrome				
No	122 (80.8)	29 (19.2)	0.234	151 (96.2)
Yes	6 (100)	0		6 (3.8)
Hospitalization				
No	95 (79.2)	25 (20.8)	0.17	120 (76.4)
Yes	33 (89.2)	4 (10.8)		37 (23.6)
Duration of hospitalization (weeks)				
<1	59 (75.6)	19 (24.3)	0.3	78 (49.7)
1–2	40 (85.1)	7 (14.9)		47 (29.9)
2–3	19 (86.4)	3 (13.6)		22 (14)
>4	10 (100)	0		10 (6.4)
Treatment given				
No	61 (74.4)	21 (25.6)	0.074	82 (60.3)
Yes	47 (87)	7 (13)		54 (39.7)

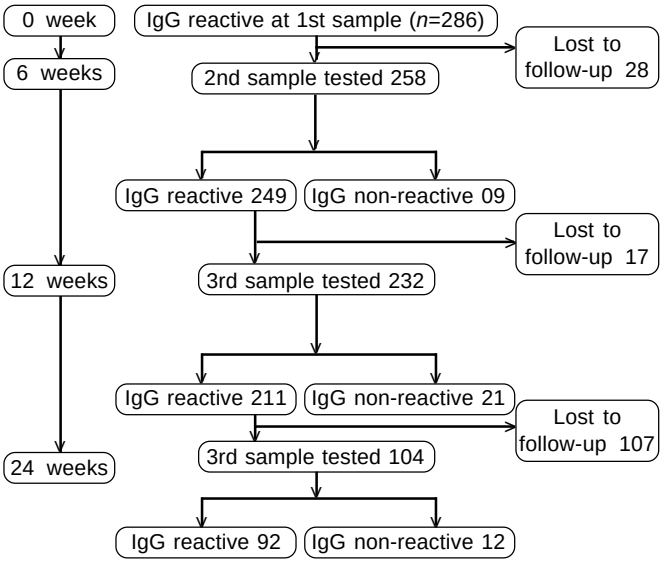

FIG 3. Follow-up Covid-19 IgG titres at 0, 6 and 12 weeks

TABLE IV. Follow-up coronavirus disease IgG titres at 0, 6 and 12 weeks among 232 healthcare workers

Statistic	0 week	6 weeks	12 weeks
Mean (SD)	7.80 (5.83)	7.02 (3.85)	5.73 (3.93)
Median (range)	6.78 (1–31.8)	6.82 (1.06–19.10)	5.04 (0.27–17.80)
Variance	34.00	14.82	15.48

SD standard deviation

TABLE V. Comparison of antibody response between symptomatic and asymptomatic reverse transcription-polymerase chain reaction-positive patients

Time	Number of patients	Symptomatic	Asymptomatic	p value	Test
0 weeks	Number of patients	111	23		
	Mean (SD)	10.59 (7.01)	8.04 (6.40)	0.035	Mann–Whitney U
	Median (range)	9.06 (1.13–31.80)	7.44 (1.60–29.20)		
6 weeks	Number of patients	111	23		
	Mean (SD)	8.59 (3.63)	5.65 (2.82)	<0.0001	Independent samples *t*-test
	Median (range)	8.77 (1.02–19.10)	5.16 (1.11–11.00)		
12 weeks	Number of patients	99	19		
	Mean (SD)	7.56 (3.58)	5.04 (2.99)	0.004	Mann–Whitney U
	Median (range)	7.10 (1.81–15.90)	4.45 (1.03–11.80)		

explained the role of BCG vaccination and its immunological effects. A few studies have evaluated the immune-boosting role of vitamins such as D, C, E, zinc, selenium and omega-3 fatty acids in the prevention and treatment of Covid-19 by improving immunity, in general.[25,26] More work, however, is needed to establish these relationships.

Our study shows that 87.46% of subjects with IgG Ab against Covid-19 continue to have antibodies at 12 weeks. However, as shown in earlier studies from the UK[16] and China,[22] the antibody titre decreased during follow-up. The decrease or disappearance was more likely in those who had low initial antibody titre both in our data and earlier reported series. Seow *et al*.[16] noted that individuals with high peak ID50 (serum dilution that inhibits 50% infection) for neutralization maintained high neutralizing antibody titre for longer period. Ibarrondo *et al*.[18] and Bruni *et al*.[19] have shown rapid decaying of anti-SARS-CoV-2 antibodies in persons with mild Covid-19.

We used Vitros anti-SARS-COV-2 IgG assay, which targets the S1 spike protein.[27,28] Compared to other coronaviruses, S1 protein is more specific and unique to SARS-CoV-2.[29,30] Chemiluminescence-immunoassay used in our study has been shown to be superior to the ELISA method.[31]

Our limitations include the modest sample size and not measuring the exact quantity of the specific IgG antibody in plasma.

Conclusion

Our study shows that IgG Ab to SARS-CoV-2 was higher in HCWs who had tested positive for SARS-CoV-2 by qRT-PCR than in those in whom antibodies were detected during the seroprevalence study. Moreover, HCWs having received BCG vaccination and administered immune boosters had higher antibody titres. We also noted that most participants continued to have antibodies even at 12 weeks, though the titre showed a significant decline. Further follow-up is needed to clearly illustrate the kinetics of antibody response in Covid-19.

ACKNOWLEDGEMENT

We thank Mr Saurav Barman for helping in the compilation of data.

Conflicts of interest. None declared

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