

## Coronavirus Pandemic

# Impact of SARS-CoV-2 RNA titer on the level of IgG antibodies in recovered patients with COVID-19 disease

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### Abstract

**Introduction:** It is important to better understand the presence of neutralizing anti-SARS-CoV-2 antibodies in the population, as they potentially prevent (re)infection.

**Aim:** To correlate the Cycle threshold (Ct value) of SARS-CoV-2 and its impact on specific Anti-SARS-CoV-2 IgG titer, to reveal the effect of age and disease severity on antibodies titer.

**Methodology:** A total of 153 infected participants laboratory-confirmed COVID-19 cases 4-11 months ago, aged 18-85 years old (mean = 43.58, SD ± 15.34) were enrolled in the study. They have not received any COVID-19 vaccine. A questionnaire was prepared including demographic data, age, gender, residence, and symptoms severity they suffered. Five mL of venous blood was taken from each participant to measure SARS-CoV-2 IgG antibodies against the receptor binding domain (RBD) by (VIDAS SARS-COV-2 IgG - Biomerieux kit). Ct values were measured by qRT-PCR (BIO-RAD-CFX96) kit which detected two virus genes, namely (RdRp-N genes).

**Results:** Lowest Ct values were detected significantly in age group 50-59 and 70-85 respectively. The highest mean of IgG was detected in age groups 70-85 and 50-59, and was found to be significantly correlated with disease severity. There is a direct relationship between Ct values and the titer of specific IgG, as increasing in viral load is associated with a higher level of antibodies. Antibodies were detected several months after infection with the highest mean after 10-11 months.

**Conclusions:** Specific Anti-viral IgG are significantly associated with increasing age and disease severity, and the direct relation of IgG with viral load. Antibodies are detected several months post-infection but their protective efficacy is controversial.

**Key words:** SARS-CoV-2; Ct value; serum IgG.

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### Introduction

Coronaviruses have existed and replicated for thousands of years and continue to do so [1]. The virus SARS coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), resulted in a worldwide pandemic [2]. Globally, as of 17 June 2022, the confirmed cases of COVID-19 were 535,863,950, with 6,314,972 estimated deaths, and the number of vaccine doses administered has reached 11,902,271,619 according to WHO. In Iraq, between 3 January 2020 and 22 June 2022, there have been 2,333,443 confirmed cases of COVID-19 with 25,229 deaths, reported to WHO. As of 20 June 2022, a total of 18,589,241 vaccine doses have been administered [3].

Coronaviruses mutate rapidly as it is generally known, and have the ability to cross the species barrier and adapt to various epidemiological circumstances [4].

Despite of that the initial target of SARS-CoV-2 is the respiratory system, new fact show that COVID-19 also influence the vascular system, producing thrombotic microangiopathy and coagulation in multiple organs, counting the lungs [5-8]. Therefore, it is not shocking, that individuals who already have hypertension, cardiovascular disease, or other comorbidities are at higher risk [9].

Higher levels of neutralizing antibodies are produced in relation to a very effective T-cell response. Unlike B cell epitopes which need a fixed position, T cell epitopes can be found everywhere in a viral protein. In the instance of SARS-CoV, the antibody profile of this virus produces IgM and IgG, and at a later phase, sero-conversion has been identified that is mediated by the helper T cells. The helper T cell also plays a role in isotype switching [10,11].

IgG has been found to last for a longer time than IgM, which suggests that IgG may be a powerful protective antibody during the infection as IgM vanishes at the end of week 12 but IgG has been found to last longer [12].

The host immunological response to SARS-CoV-2 infection can be used to indirectly detect COVID-19 infection. A serological analysis is specifically essential for patients with mild to moderate infection who may present later, beyond the first two weeks of their illness, Serological diagnosis also is a crucial tool for determining the prevalence of COVID-19 in a population and identifying those who are immune and potentially "protected" from infection. Total antibodies are the most accurate and early serological sign, and their levels start to rise the second week after symptoms start. Despite the fact that IgM and IgG ELISA results can be positive as early as the fourth day after the onset of symptoms, the levels are higher in the second and third weeks of illness [13]. Evidence indicates that antibody development following infection likely confers some degree of immunity from subsequent infection for at least 6 months [14].

This study aimed to: Correlate between respiratory syndrome coronavirus 2 (SARS-CoV-2) viral loads and specific serum-antibodies (immunoglobulin IgG) among confirmed patients by RT-PCR, to detect the impact of viral load represented by Ct on the immune response. To reveal the relationship between IgG titer with age and disease severity as well as to find out whether the titer of IgG remains protective and after how many months to elicit the possibility of reinfection.

**Methodology**

A total of 153 out of 185 invited individuals agreed to participate in this cross-sectional study to estimate the SARS-CoV-2 IgG titer, all the participants had tested positive for COVID-19 by real-time reverse transcription-polymerase chain reaction (RT-PCR) of their nasopharyngeal swabs at least 4-11 months prior

to the study. All participants have not received any dose of any COVID-19 vaccine.

A well-designed questionnaire was prepared and filled by all participants including demographic data, age, gender, residence, and symptoms they suffered during infection (fever, dry cough, shortness of breath, muscle ache, sore throat, headache, fatigue, loss of taste, lethargy of body, diarrhea, and stomach ache) and if they need oxygen supplementation at home or required hospitalization. The exact time that relapsed past infection was also recorded.

Then 5 mL of venous blood was taken from each participant to measure SARS-CoV-2 IgG antibodies against the receptor binding domain (RBD) of the spike protein by automated machine (VIDAS SARS-COV-2 IgG instrument- Biomerieux kit) according to manufacturer’s instructions. The cut-off value for antibody response was 1, above which the sample was considered positive [15].

Cycle threshold Ct values measured by qRT-PCR (BIO-RAD-CFX96 Real-Time using SARS-CoV-2 Nucleic Acid Detection Kit (PCR-Fluorescent Probe Method) which detected two genes of the virus namely (RdRp-N genes) of all participants were gathered from the central lab and COVID-19 centre at Duhok, and was considered as representative of the viral load [16].

Ethical Approval: This research was approved by the research ethics committee at the directorate general of health with reference No. 21122021-12-4 in December-2021. Informed consents were taken from all participants.

*Statistical analysis*

Collected data were entered into Microsoft Excel, then transferred to SPSS version 26, for analysis. The variables were described by their range, mean, standard deviation, and 95% confidence interval. Differences in means were analyzed by the one-way analysis of variance test, then if the overall *p* value was statistically significant, the sidak posthoc test was used for intergroup comparisons. Scatter diagram and

**Table 1.** Ct by age group.

Age (years)	Group	No. of participants	Ct				Group significantly different from*
			Range	Mean	SD	95% CI for Mean	
18 – 29	a	27	16.80 - 38.00	28.64	5.57	26.43 - 30.84	d
30 – 39	b	45	19.00 - 36.00	28.18	3.24	27.21 - 29.15	d
40 – 49	c	30	19.00 - 36.00	27.66	4.99	25.79 - 29.52	d
50 – 59	d	23	16.44 - 30.30	23.28	3.76	21.66 - 24.91	a, b, c, e
60 – 69	e	18	20.00 - 36.00	28.61	4.73	26.25 - 30.96	d
70 – 85	f	10	19.00 - 37.00	25.10	5.36	21.26 - 28.94	
Total		153	16.44 - 38.00	27.27	4.79	26.51 - 28.04	

Overall *p* < 0.001 (based on one-way analysis of variance); \**p* < 0.05 based on Sidaktest for multiple comparisons; SD: Standard deviation; CI: Confidence interval.

correlation coefficients were used to examine the association between the inverse of CT (1/CT) and IgG titer. The level of statistical significance was set at  $p < 0.05$ .

**Results**

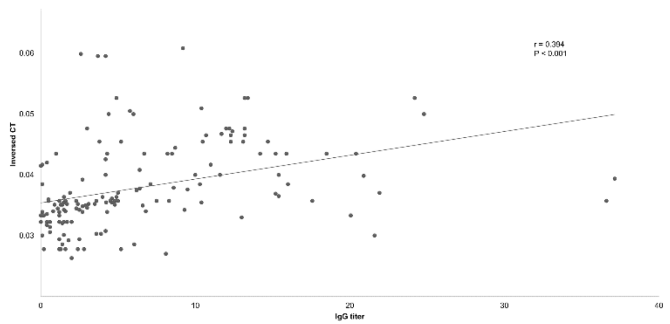
This study enrolled 153 participants (74 male and 79 female) with ages ranging from 18-85 years -old (mean 43.58, SD ± 15.34) all had been infected once with SARS-CoV2 4-11 months ago but were not -vaccinated with any COVID-19 vaccine. All were found positive for Anti SARS-CoV-2 IgG when examined.

They were classified into different age groups, as shown in Table 1. The lowest Ct value mean (the highest viral load) was found in the age group (50-59 years old) 23.28-which is significantly different from all other age groups with  $p < 0.001$ . This is followed by the age group (70-85 years old) with a mean Ct value of 25.10.

Comparison of IgG (Anti RBD antibodies) mean among different age groups revealed the highest value of 16.60, and -9.89 in age groups (70-85), and (50-59) respectively. A high significant variation ( $p < 0.001$ ) was detected in relation to the other age groups (Table 2).

There was a significant association in symptoms by IgG titer as shown in Table 3. The mean for IgG titer for those with asymptomatic, mild, moderate, and severe were 2.10, 6.67, 10.55, and 22.02, respectively which indicates a significant correlation between the

**Figure 1.** Scatter diagram (correlation) of inverse of CT (1/CT) and IgG titer.



symptom’s presentation at infection with Anti SARS-CoV2 IgG ( $p$  value  $< 0.001$ ).

To understand the influence of Ct value at the time of infection on the level of Anti RBD IgG of the participants, a scatter diagram was constructed (Figure 1) that shows that there is a direct relationship between the Ct values and the titer of Anti SARS-CoV-2 RBD IgG, as increasing in viral load is associated with a higher level of antibodies.

The participants were classified into several groups according to the time lapsed after infection. The mean IgG level of all participants was 6.38, the highest number of participants were found 6- 7 months post-infection (85) as shown in Table 4, with a mean of 6.55. There was no significant correlation in IgG titer level with timing ( $p = 0.427$ ).

**Table 2.** IgG titer by age group.

Age (years)	Group	No. of participants	IgG titer				Group significantly different from*
			Range	Mean	SD	95% CI for Mean	
18 – 29	a	27	0.01 - 10.40	2.88	2.64	1.83 -3.92	d, f
30 – 39	b	45	0.01 - 17.57	4.09	4.83	2.64 - 5.54	d, f
40 – 49	c	30	0.01 - 21.60	6.32	6.50	3.90 - 8.75	f
50 – 59	d	23	2.60 - 37.14	9.89	7.34	6.71 - 13.07	a, b, f
60 – 69	e	18	0.40 - 36.60	7.32	8.19	3.25 -11.40	f
70 – 85	f	10	6.70 - 24.80	16.60	6.62	11.86 -21.34	a, b, c, d, e
Total		153	0.01 - 37.14	6.38	6.82	5.29 - 7.47	

Overall  $p < 0.001$  (based on one way analysis of variance); \* $p < 0.05$  based on Sidaktest for multiple comparisons; SD: Standard deviation; CI: Confidence interval.

**Table 3.** Symptoms by IgG titer.

Symptoms	Group	No. of participants	IgG titer				Group significantly different from*
			Range	Mean	SD	95% CI for Mean	
Asymptomatic	a	40	0.01 - 14.70	2.10	2.93	1.17 - 3.04	b, c, d
Mild	b	95	0.01 - 37.14	6.67	6.02	5.45 - 7.90	a, d
Moderate	c	12	0.10 - 20.90	10.55	6.72	6.27 - 14.82	a, d
Severe	d	6	11.60 - 36.60	22.02	9.11	12.46 - 31.58	a, b, c,
Total		153	0.01 - 37.14	6.38	6.82	5.29 - 7.47	

Overall  $p < 0.001$  (based on one way analysis of variance); \* $p < 0.05$  based on Sidaktest for multiple comparisons; SD: Standard deviation; CI: Confidence interval.

**Discussion**

SARS-CoV-2 emerged in the year 2019 and many parameters related to the virus are not standardized yet, but they are used as substitutions to reflect some aspects of the viral replication. Although the Swabbing method is not standardized and there is no cut-off value for Ct to estimate the infectivity and outcome of infection, Ct value is used to reflect the viral titer or load measured by real-time RT-PCR to measure the patient’s infectivity so the lower value represents the higher viral titer. Positive results of rRT-PCR for SARS-CoV-2 might not always correlate with the degree of infectiousness and may need a reference standard curve [17]. In this study Ct values of COVID-19, patients were obtained and when applied to different age groups we found significantly lower Ct values among age groups 50 -59 and above 70 years old. Some studies have found a similar demonstration of Ct values. Maltezou *et al*, [18]-in Greece and Mishra *et al* [19] in India found lower Ct values in older people than younger ones while other studies demonstrated higher Ct values among patients older than 80 years old [20]. To have high viral titer in old people is logical considering the high rate of death among them. On the other side high Ct values in older people in other studies had been explained by the fact that low viral load is enough to cause severe disease in this age group in addition to the comorbidities they suffer from. Controversy in demonstrating the correlation between the age of patients and Ct values may be attributed to different sample sizes of different studies and fluctuation of immune response even within the same age group.

A detailed understanding of immune responses following SARS-CoV2 infection will enable better treatment and diagnostic procedures, as well as the development of successful vaccines that will help to control the global COVID-19 pandemic. In this regard, it is important to better understand the presence of neutralizing anti-SARS-CoV-2 serum antibodies in the population, as they potentially prevent (re)infection and might be a treatment option. Several studies elicit evidence that antibody production after SARS-CoV-2 infection protects from re-infection for a minimum of 7

months [21]. IgG titer was measured in this study and revealed higher levels in those above 70 years old followed by the age group 50-59 which is an interesting finding since the immune response of age extreme has lower capacity to respond to infections in general.

Several pieces of evidence accumulated suggest that the response of specific antibodies against the virus may be different in different age groups [22], with a potential impact on clinical presentation and outcome of infection.

Yang *et al* [23] found that SARS-CoV-2 IgG antibody production was distinctly different in children, adolescents, and different age groups of adults which may reveal different aspects of immune response to SARS-CoV-2 according to age. Gültekin [24] discovered higher antibody levels in the age group >55 years old. Another study found a high level of protection against reinfection in the old age group. Thus, findings suggest that the differences in the clinical manifestations of COVID-19 in pediatric patients compared with adult patients could be partly due to age-related immune responses. Several studies have demonstrated that older people are more susceptible to emerging viral infections which is attributed to changes in the immune system both innate and adaptive related to age including immune senescence [25-27]. Since many studies demonstrated the different concentrations of antibodies among different age groups, finding a high level of antibodies in old age recovered people may explain their ability to overcome the infection without complication. On the other hand, there is a strong correlation between IL-6 levels in the serum and the upcoming respiratory failure in infected patients so the impact of inflammatory response with high IL6 level should be calculated as responsible for complications [28]. Further work is mandatory to reveal the threshold titer level of Anti SARS-CoV-2 IgG in the blood that may interfere with reinfection.

The relationship between Anti-SARS-CoV-2 RBD and inversed Ct values was direct, so an increase in viral load is associated with a higher level of antibodies. Anti-SARS-CoV-2 Antibodies occurs at 7–11 days after exposure to the virus in general or may need more

**Table 4.** IgG titer level by timing.

IgG timing	No. of participant	IgG titer			
		Range	Mean	SD	95% CI for Mean
4 - 5 months	22	0.10 - 21.92	6.43	5.87	3.83 - 9.03
6 - 7 months	85	0.01 - 36.60	6.55	6.93	5.06 - 8.05
8 - 9 months	41	0.10 - 37.14	5.47	7.20	3.20 - 7.75
10 - 11 months	5	2.40 - 15.90	10.72	5.08	4.41 - 17.02
Total	153	0.01 - 37.14	6.38	6.82	5.29 - 7.47

Overall  $p = 0.427$  (based on one way analysis of variance).

days to be detected. IgG antibody tests are considered as evidence of previous infection [29]. Research has revealed that many asymptomatic patients had a low titer of viral load in the nasal swab, which is attributed to a high level of local IgA, thus preventing the development of a high level of IgG [30], which may need exposure to abundant volume of viral antigens in the nose with a suitable time to elicit good humoral immune response [31].

Wellinghausen *et al.* [30] explained the lower SARS-CoV-2 IgG in asymptomatic patients by the lower viral load demonstrated by high Ct values. In addition, Appak *et al.* [32] concluded that higher Anti-SARS-CoV-2 antibodies are correlated with lower Ct values. This is in accordance with our results that the level of anti-RBD-spike antibodies correlates positively with presenting symptoms.

The essential question which remains to be elicited is for how long the Anti-SARS-CoV-2 antibodies after natural infection are protective, and to know the titer threshold that is enough to prevent reinfection. We found antibodies in recovered patients after several months and the highest mean value was for a group of 10 months after infection. Despite that exact length of immunity acquired by natural infection is still unknown, neutralizing antibodies level against SARS-CoV-2 spike protein were detectable for at least five months after primary infection [21].

Hansen *et al.* [33]-demonstrated that immunity to protect against reinfection is strong and persists for more than 10 months following primary infection, while Chen *et al.* [34] concluded that the protective efficacy of naturally acquired IgG is 84% he raised the attention that chance of reinfection with SARS-CoV-2 in people with positive IgG increases with time but slowly. Another study measured antibodies, memory B cells, and T cells to SARS-CoV-2 and found that 95% of recovered patients retained immune responses for 8 months [35].

The essential question that remains to be elucidated is for how long these antibodies are really effective in preventing reinfection.

#### Limitations of the study

The main obstacle was the recruitment of recovered individuals to share in this study mainly old age participants and the absence of a standardized cut-off value for Ct to estimate the infectivity and outcome of infection.

## Conclusions

We have found that Ct values are significantly lower with higher viral load in older age groups mainly 50-59 and above 70 years old. An interesting result was a high mean of IgG titer among the old age group (70-85) while the lowest mean was among the young age group (18-29) years old, in addition significantly higher level of IgG was found in moderate and severe infections. A direct relationship was detected between humoral immunity represented by IgG titer and inversed Ct values, so those with higher viral load at initial diagnosis will gain more antibodies. High IgG titer was detected 11 months post-infection but it is controversial regarding its protective efficacy. Differences in these titers and IgG levels in some age groups should be noted, which would deserve further investigations in search of explanations for this finding.

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