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Neutralizing antibody responses in asymptomatic close contacts of COVID-19 patients and in asymptomatic healthcare workers

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Abstract

The identification of novel antibodies that could neutralize SARS-CoV-2 is one of the novel approaches to use in combating COVID-19. This study aimed to explore the level of neutralizing antibodies (NAbs) in asymptomatic close contacts of COVID-19 patients and asymptomatic healthcare workers. *In vitro* qualitative detection of serum antibodies of participants from both populations was done using an anti-SARS-CoV-2 immunoassay. The study included 107 participants, of which 59.8% were healthcare workers and 40.2% were family contacts of confirmed COVID-19 cases. Their median age was 22 years. The percentage of positivity and median titer for NAbs were significantly higher among family contacts than mong healthcare workers (P = 0.013 and <0.001, respectively). We also measured C-reactive protein (CRP) levels and the median value of CRP was significantly higher in the family members who had been in contact with COVID-19 patients than in healthcare workers (P < 0.001). In the family contact group, there was a significant negative correlation between the absolute lymphocyte count and CRP (r = −0.409, P = 0.034). There was no significant correlation between neutralizing antibody titers and either CRP or absolute lymphocyte count (P > 0.05 for both). In conclusion, the indication of elevated NAb titers in asymptomatic family contacts could help lay the groundwork for further studies to explore the potential utility of these antibodies to provide future immunity from infection within a family as well as for potential use in general during passive antibody therapies for COVID-19 patients.

Keywords: COVID-19, neutralizing antibodies, asymptomatic contacts, healthcare worker, immunity.

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for coronavirus disease-19 (COVID-19), is a β-coronavirus.[1] It has four structural proteins, which are the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein

(N). The spike surface glycoprotein (S) is a type I transmembrane glycoprotein that mediates entry to target cells by interacting with the cell surface receptor angiotensin-converting enzyme 2 (ACE2) through its receptor-binding domain (RBD).[2]

The continual spread of COVID-19 (including its variants) during the current global pandemic highlights an urgent need to develop effective treatments or vaccines against SARS-CoV-2 infection. Among the many novel paradigms being generated toward that goal, the identification of novel antibodies to neutralize the virus is one of the approaches in the fight against COVID-19 [3]

Virus-specific neutralizing antibodies (NAbs), which are induced by infection or vaccination, can help to block viral infection later or during subsequent encounters with the pathogen.[4,5] In general, NAbs help confer immunity by deactivating viral access to receptors used to enter host cells and/or binding to viral capsids so as to block the key step of uncoating the viral genome.[6] The titers of NAbs were variable in different patients.[4]

NAbs to SARS-CoV-2 can be found in most infected persons 10–15 days after the onset of presenting symptoms [1]. Antibodies against different domains of the S protein, including S1, RBD, and S2, may all contribute to the neutralizing effects of these antibodies against the parent virus. [4] It is still unknown for how long antibody responses will be maintained after SARS-CoV-2 infection or whether they will prevent reinfection. [1] Long et al., 2020, studied 178 cases with confirmed infection. [7] Of these, 37 were asymptomatic individuals, diagnosed with reverse transcription-polymerase reaction (RT–PCR), as confirmed SARS-CoV-2 infections but without any relevant clinical symptoms during the preceding 14 days. The virus-specific IgG levels in the asymptomatic group (median 3.4) were significantly lower compared to those in the symptomatic group (median 20.5) during the acute phase. Forty percent of asymptomatic cases and 12.9% of the symptomatic cases became negative in the early convalescent phase. [7] Approximately 2.6% of close contacts of infected patients contracted COVID-19, with approximately half of them being either asymptomatic or having mild infections. [8]

To the best of our knowledge, no study to date has been undertaken in our country to determine levels of NAb in asymptomatic close contacts of COVID 19 patients or in asymptomatic healthcare workers. Accordingly, this study was designed to explore the levels of NAb in asymptomatic close contacts of COVID-19 patients and in asymptomatic healthcare workers. And to assess the C-reactive protein (CRP) and immune system cell levels in the study subjects.

Subjects and Methods

This is a cross-sectional analytic study, was carried out from May to July of the year 2020. It was designed to enroll close contact persons from the 21st day after the alleged contact with the COVID-19 patient. A total of 107 subjects were included. Of these, 43 subjects were close family contacts, 37 physicians and nurses working in isolation hospitals for COVID-19 cases, and 27 laboratory technicians.

Inclusion Criteria

Contacts aged > 18 years, asymptomatic close contacts of confirmed COVID-19 patients, apparently healthy (not complaining), and asymptomatic healthcare workers.

Exclusion criteria

Symptomatic close contacts, symptomatic healthcare workers, any close contacts with a malignant tumor, stroke, and cardiac, respiratory, gastrointestinal, and renal diseases.

The study protocol was reviewed and approved by the Committee of Medical Ethics of the Faculty of Medicine, Assiut University (July 2020). All participants gave their written consent to participate. The study was registered in clinical trial. gov. Number: NTC04444310. Date: 23/6/2020. https://clinicaltrials.gov/ct2/show/NCT04444310.

Sample collection and laboratory investigations

Fasting venous blood samples were collected under standardized conditions at the same time of day from all study participants. Each sample was divided into three parts. One part was

immediately used for a complete blood count (CBC), which was done on an ABX Pentra XL 80 hematology analyzer (HORIBA ABX, France). Another aliquot was used to measure the prothrombin concentration and international normalized ratio (INR) using a blood coagulation analyzer (Sysmex® CA-1500 System; Siemens, Germany). The final portion of the blood sample was allowed to clot, after which the serum was isolated. The serum was then divided into different aliquots to detect serum bilirubin, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), urea, creatinine, CRP, and ferritin, which were all measured using a chemistry analyzer (Cobas Integra 400 chemistry analyzer, Roche, Switzerland).

Neutralizing Ab Test

The presence of NAbs in the serum samples was measured using an electrochemiluminescence immunoassay (ECLIA) by an immunoassay analyzer (Cobas 411 immunoassay analyzer, Roche Diagnostic, Germany). Specifically, an Elecsys Anti-SARS-CoV-2 immunoassay kit (Roche diagnostics, Germany) was used for the *in vitro* qualitative detection of antibodies (including IgG) to SARS-CoV-2 in human serum, according to the manufacturer's instructions. Results were considered non-reactive when kit values were <1 and reactive at values of ≥ 1.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS-version 17, SPSS Inc., Chicago IL, USA). Because the continuous data were not normally distributed, they were all presented as the median and interquartile range, while categorical data were presented as frequencies and percentages. The Mann-Whitney U test was used for comparisons between continuous variables. Correlations were quantified using the Spearman correlation coefficient. The threshold for statistical significance was set at $P< 0.05$.

Results

Out of the 107 included participants, 64 (59.8%) were healthcare workers and 43 (40.2%) were family contacts of confirmed COVID-19 patients. Their ages ranged from 18 to 70 years, with a median value of 22 years. Approximately 1.9% of the study participants had a history of diabetes mellitus. There was no statistical difference between the median values of the duration of contact in both groups.

Blood chemistry and neutralizing antibody status

The laboratory data of the study groups are shown in Table 1. The percentage of positivity and median neutralizing antibody titer were significantly higher among family contacts than among healthcare workers (P = 0.013 and <0.001, respectively). Also, the median CRP level was significantly higher in the family contact group than in the healthcare workers group (P < 0.001).

Blood cell populations

There was no statistically significant correlation between neutralizing antibody titers and age (r = 0.030, P = 0.767). However, in the family contact group, there was a significant negative correlation between the absolute lymphocyte count and the CRP level (r = −0.409, P = 0.034) (Figure 1). There was no significant correlation between neutralizing antibody titers and either CRP levels or absolute lymphocyte counts (p > 0.05 for both).

Table 1. Laboratory data of the 107 study participants**.**

Variable	Healthcare workers (n = 64)	Family contact (n = 43)	*P*-value[a]
Hb (g/dl)			
Median	12.1	12.7	NS
Range	9.7-15	9.1-16	
WBCs (10^9/L)			
Median	6.1	7.4	NS
Range	4-13.2	3.7- 11.8	
PLT (10^9/L)			
Median	275	258	NS
Range	133-555	146-552	
Lymphocytes percentage			
Median	36.5	37	NS
Range	20-59	19-70	
Lymphocyte absolute count (10^9/L)			
Median	2.4	2.3	NS
Range	1-3.6	1.6-4.5	
CRP (mg/L)			
Median	3.3	4.7	<0.001*
Range	2-19	2.8-33.9	
Creatinine (µmol/L)			
Median	66	60.5	NS
Range	29- 160	38-132	
AST (U/L)			
Median	17	17	NS
Range	9-64	11-31	
ALT (U/L)			
Median	21	13	<0.001
Range	8-50	9-31	
Ferritin (ng/ml)			
Median	54.5	58.7	NS
Range	10.6-233	4.7-396.5	
LDH (U/L)			
Median	167	188	NS
Range	112- 264	91-303	
Neutralizing Antibody[b], n (%)			
Reactive	15 (23.4%)	20 (46.5%)	0.013
Non-reactive	49 (76.6)	23 (53.5)	
Neutralizing Ab titer			
Median	0.06	0.65	<0.001
Range	0.5-136.8	0.04-145	

[a] Mann-Whitney U test, which was used for comparisons between continuous variables.
[b] non-reactive < 1 and reactive ≥ 1. *P* value >0.05 is. Not significant (NS).
Hb: hemoglobin; g/dl: gram per deciliter; WBCs: white blood cells; µL: microliter; µmol micromole per liter. PLT: platelets; CRP: C-Reactive protein; AST: Aspartate Aminotransferase: ALT: Alanine Aminotransferase: LDH: Lactic dehydrogenase; U/L: unit per liter; ng/ml: Nanogram per milliliter.

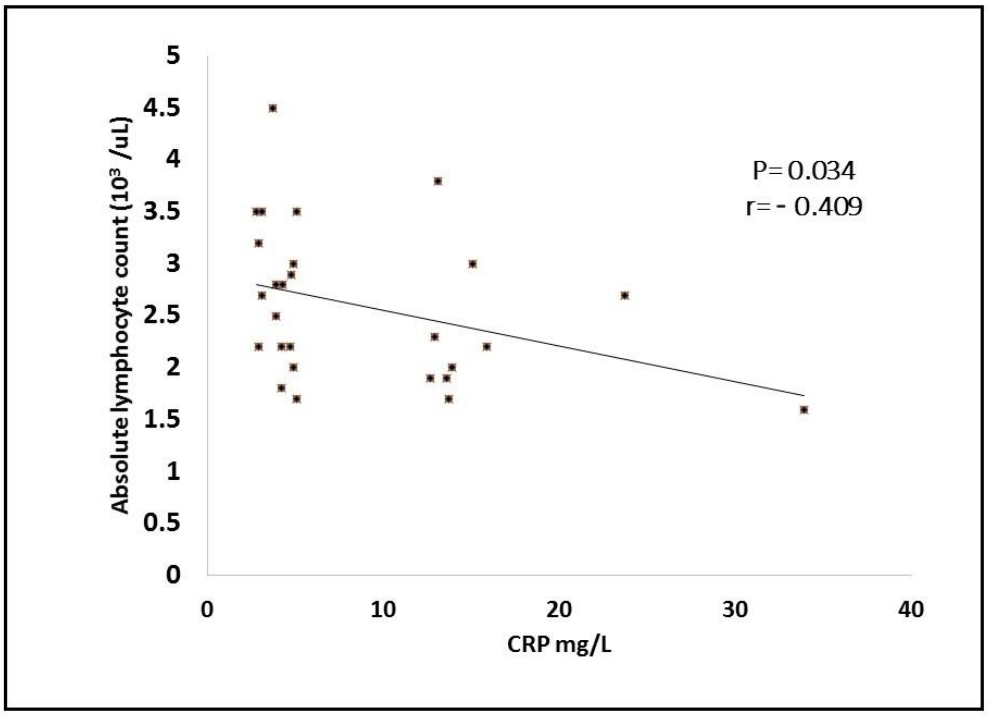

Figure 1. Correlation between CRP levels and absolute lymphocyte counts in the family contact group.

Discussion

Healthcare workers are susceptible to diseases due to their work and contact with patients in hospitals, especially as they are usually exposed to typical and atypical patients. During the COVID-19 pandemic, the follow-up of healthcare workers is vital because they can infect their family members, friends, and colleagues.[9] As such, one of the aims of this study was to assess antibody titers in healthcare workers in contact with COVID-19 patients.

Early screening and monitoring of healthcare workers can preserve their health and that of their contacts and other patients. During the early spread of COVID-19 in Wuhan, approximately 29% of patients infected with SARS- CoV-2 were healthcare workers who got infected in their respective hospitals.[10] Improving quality and frequency of screening among healthcare workers will help minimize the spread of the infection.[11]

In this study, NAb titers were used to monitor healthcare workers and healthy family members who had previously been in contact with confirmed COVID-19 patients. According to the results of this study, NAb titers were elevated in both asymptomatic groups. The levels observed among healthcare workers, in the present study, were in line with the results of a study by Dimeglio et al., 2020, in France, who found that only 3.2 % of healthcare workers developed total SARS-CoV-2 antibodies.[12]

The main results of this study provide some valuable insight on humoral immunity in asymptomatic close contacts of COVID 19 patients and in asymptomatic healthcare workers. Neutralizing antibody titers and CRP levels were higher among family contacts than among healthcare workers. There was no significant correlation between neutralizing antibody titers and either age, CRP levels, or absolute lymphocyte counts.

In 2020, the World Health Organization (WHO) reported that some governments had suggested that the detection of SARS-CoV-2 antibodies could be an “immunity passport” or what is known as the “risk-free certificate” that would enable individuals to travel or go to work

without fear of infection, as they are protected.[13] Also, Brouwer et al., 2020, reported that the antibodies that neutralize SARS-CoV-2 could be very important tools in the treatment of the disease. [14] They demonstrated that patients with high antibody titers had strong immune responses against the viral spike protein. The United States Food and Drug Administration (FDA) guidelines for convalescent plasma recommended target antibody titers of 160. [15]

Lee et al., 2020, reported that the neutralizing activity increased with time post-symptoms, reaching its peak 31–35 days after the onset of symptoms. They also reported that patients' sera contained high SAR- CoV-2 antibody levels.[16] Ko et al., 2020, in their study on neutralizing antibody production in asymptomatic patients compared to that in patients with COVID-19 pneumonia, found that the rate of antibody production was 100% in pneumonic patients, 93.9% in patients with mild symptoms, and 80% in asymptomatic patients. They reported that most asymptomatic and mild COVID-19 patients produced neutralizing antibodies. Although the titers may be lower than those of pneumonia patients, they can still be detected.[17] Wu et al., 2020, in their study of reversed patients, found that there was a correlation between NAb titers and age, lymphocyte counts, and blood CRP levels, which suggested that the interaction between the virus and host immune responses in cases of coronavirus infection should be explored to develop effective vaccines against the SARS-CoV-2 virus.[4]

This work may provide valuable insight into humoral immunity in asymptomatic close contacts of COVID 19 patients and asymptomatic healthcare workers. The neutralizing antibody titers and CRP levels were higher among family contacts than among healthcare workers. There was no significant correlation between neutralizing antibody titers and either age, CRP level, or absolute lymphocyte counts.

Regardless of the results, there were some key limitations to this study. The most important of these limitations is the small sample size. Further, no correlation between NAb titers and clinical characteristics associated with COVID-19 was found (which was expected, especially as all study subjects were asymptomatic). Next, no immune response, such as tests for SARS-CoV-2-specific memory B cells and memory T cells, was assessed.

In conclusion, NAb titers were high among asymptomatic family members that came in contact with known COVID-19 patients. This finding can help in laying the groundwork for future studies that will explore the potential for these antibodies to provide future immunity/protection from infection in uninfected family members. Further, those studies will explore the potential use of these NAbs in passive antibody therapies for COVID-19 patients.

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Author Contributions

AAM, shared in study design, writing the protocol, obtained ethical approval, shared in doing all laboratory work and in the writing of method section. AARMH, created the idea of the work, revised the protocol, shared in data collection, supervised the work and revised the manuscript. NAM, shared in writing the protocol, data collection from the participants, shared in literature review and in the writing of the manuscript. HAM, shared in the idea of the work, did the statistical analysis, and shared in the writing of the manuscript. HAY, shared in literature review and in the writing of the manuscript. AME, revised the protocol, supervised the laboratory work and revised the manuscript. AMA, shared in study design, writing the protocol, all laboratory work and in revising of method section.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Committee of Medical Ethics of the Faculty of Medicine, Assiut University (July 2020).

Informed consent

A signed consent form was obtained from each study participant.

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