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Serological Investigation of COVID-19 Antibodies in Armenia

Inessa Nazaryan, Shahane Mnatsakanyan, Narek Pepanyan

EcoSence LLC, Yerevan, Armenia
Email: info.heq@gmail.com

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Abstract

Background: In December 2019 in Wuhan China the new coronavirus outbreak emerged and quickly spread in all parts of the world resulting to more than 500,000,000 infection cases and around 6,200,000 deaths. The global incidence of the infection is still growing as well as number of deaths. COVID-19 is a new virus, therefore not much is known about the immune response of infected organism, which is crucial not only for vaccination policy development, but also for identification of public health strategies. **Aim:** Current research aims to describe COVID-19 IgG levels depending on symptoms, antibiotic and antiviral medications intake history, existing chronic condition and smoking status during March-December of 2020 in Armenia. Furthermore, the study aims to help elucidate the fraction of asymptomatic or presymptomatic/subclinical infections in the population and understand the main risk factors for infection complication. **Methodology:** The cross-sectional study with convenience sampling of individuals who turned to "EcoSense" laboratories to be tested for COVID-19 IgG were examined. The NovaTec SARS-CoC-2 (COVID-19) IgG COVG940 96 Determinations ELISA test kits were used. The questionnaire was filled regarding the COVID-19 status, symptoms, exposure history, disease history, pre-existing chronic conditions, medication and vaccination history. The descriptive as well as multivariate analysis was performed. **Results:** Overall 1573 testing was performed 837 of subjects agreed to participate in the interview. 24.1% of participants had laboratory confirmed COVID-19 but by the time of interview were already recovered. 212 (25.3%) participants had positive IgG levels, among 126 (15.1%) participants IgG levels were in the grey zone. Out of PCR confirmed cases only 58.7% had positive IgG levels and 3.9% IgG level was in the grey zone. Headache was the most common symptom among participants (37.2% among all participants and 53.1% among participants who previously had positive COVID-19 PCR test). The second most common symptom was anosmia (23.7% among all participants and 48.9% among participants who previously had positive COVID-19 PCR test).

5.4% of participants mentioned previous hospitalization due to COVID-19, 71 (8.5%) mentioned being diagnosed with pneumonia and 24 (2.9%) participants mentioned being admitted to ICU, 20 (2.4%) mentioned receiving oxygen therapy and 4 (0.5%) of the participants mentioned receiving an artificial ventilation of lungs. There was a weak correlation between symptom sum score and IgG titers. The Correlation coefficient was 0.273, $p < 0.05$, $R^2 = 0.075$. The linear regression analysis was also performed. The obtained results indicate that the number of symptoms patients have is a significant predictor for IgG level $F(1, 711) = 57.45$, $P < 0.01$, $R^2 = 0.075$. **Conclusions:** Our study reviled that around half of PCR confirmed COVID-19 patients do not have positive titer for IgG, most importantly the number of symptoms is a weak predictor for IgG levels, which contradicts the existing misassumption regarding severity of clinical manifestation of COVID-19 and post-infection immunity.

Keywords

Coronavirus, IgG, Immunity, Seroprevalence

1. Introduction

A novel Coronavirus, severe acute respiratory syndrome (SARS-CoV-2) has emerged in December 2019 in Wuhan China and caused a human pandemic resulting to more than 500,000,000 infection cases and around 6,200,000 deaths [1] [2] [3]. The global incidence of the infection is still growing as well as number of deaths [3]. Although molecular diagnostic tests were developed rapidly, serological studies are still lacking and yet extremely needed [4]. According to WHO currently there is a need to conduct seroprevalence studies in different countries, which will allow to make inferences for future strategic decisions and understand the real incidence of infection in different countries [5]. Currently seroprevalence studies are initiated in many countries [4] [5] [6]. Serological assays are approved by FDA and EUA and are widely used [4] [6]. In all countries the initial epidemiological surveillance was focused on symptomatic cases, so the proportion of asymptomatic cases to the pandemic is not well studied [2] [5]. PCR test has an ability to detect the virus from upper respiratory system mainly for during 2 weeks of viral shedding, after which the virus can be detected with serological methods only [5] [7] [8]. Coronavirus antibodies usually become detectable after 1st week of symptom onset [4] [5]. There are various theories regarding the fact that during the third week the infectiousness declines as patient becomes immune, however additional studies are needed [5] [7]. In Armenia the first case of coronavirus was confirmed on the 1st of March 2020, after which the daily incidence has been rising daily [9]. There is a need to understand IgG prevalence among population taking into account severity of infection, medication taken during treatment and other factors in anamnesis, to be able to make in-

formed decisions regarding public health prevention strategies [5] [7] [8]. This study will serve as a basis for understanding of kinetics of IgG titer changes in blood of population may provide valuable information not only for policy makers but also for scientists working on development of vaccine and testings [10]. [11] There is a controversial opinion in literature regarding smoking status and COVID-19, therefore in the scope of current study, the difference in hospitalization status among smokers and non-smokers was also explored, as several studies published previously highlighted the protective effect of smoking status in the likelihood of being hospitalized with COVID-19 [12] [13].

2. Objectives

Current research aims to determine the IgG antibody levels following COVID-19 infection. Nevertheless, the study will help to elucidate the fraction of asymptomatic or pre-symptomatic/subclinical infections in the population and understand the main risk factors for infection complication.

Research also aims to explore the difference of antibody titers among symptomatic vs. asymptomatic patients. The difference of antibody titers will also be explored from the prospective of antiviral drugs, antibiotics taken and vaccination anamnesis. The multivariate analysis will be conducted.

One of the research questions will be related with smoking status and coronavirus infection complications.

The study will also explore how the pre-existing chronic conditions are related to the infection complications.

Current research aims to describe COVID-19 infection among large sample of population. This study will also serve for the baseline information to further evaluate the difference of the symptoms during different periods of pandemics caused by various mutated strains.

Starting from very early stages of coronavirus pandemics various assumptions existed regarding natural immunity [14] [15] [16] [17] [18]. We hope to be able to answer the question about the determinants of natural immunity. We assume that many countries may do similar studies, however this study will be able to describe the situation among Armenian patients. The gathered data will give us opportunity to make preliminary analysis on various associations of IgG titers and different factors, so it will help to elucidate some important gaps in existing knowledge and generate hypothesis for future research.

The study was approved by Ethics Board of "EcoSense" diagnostic medical center. All participants were given an informed consent.

3. Research Methodology

The cross-sectional study was performed with convenience sampling. The exclusion criteria was having COVID-19 positive test during last 14 days prior participation in the study. 1573 individuals who turned to any of the branches of "EcoSense" laboratories were offered to participate in the study by taking a

blood sample and filling the interviewer administered questionnaire regarding the COVID-19 status, symptoms, exposure history, disease history, pre-existing chronic conditions, medication and the vaccination history (see **Appendix**). 837 agreed to participate in the study. The total duration of the study was 12 months. On the time of testing all the participants were recovered in less than 3 months period.

The high-quality reagents manufactured in Germany (NovaTec) were used. The testing was performed in "EcoSense" diagnostic center in capital of Armenia: Yerevan. As the center also has 7 functioning branches in regions of Armenia as well as in the Republic of Artsakh the laboratory services are accessible not only for participants living in Yerevan, therefore it was possible to achieve a good geographic representativeness.

EcoSense is the only diagnostic center in Armenia having ISO 9001:2015 international certificate issued by TÜVRheinland (ID 9108658675) in 2020.

Blood samples were centrifuged and sent to the central laboratory in Yerevan. The ELISA equipment (Thermo Scientific Multiskan FC) has been used for analyzing. The data has been entered to SPSS and analyzed. The following ELISA test kits have been used NovaTec SARS-CoC-2 (COVID-19) IgG COVG940 96 Determinations. Descriptive, correlation as well as linear regression analysis was used to answer the research questions. Data was entered and analyzed using statistical package SPSS 22.

4. Results

Overall 1573 testing was performed 837 of subjects agreed to participate in the interview. 57.5% were females. 24.1% of participants had laboratory confirmed COVID-19 but by the time of interview were already recovered. 212 (25.3%) participants had positive IgG levels, among 126 (15.1%) participants IgG levels were in the grey zone. Out of PCR confirmed cases only 58.7% had positive IgG levels and 3.9% IgG level was in the grey zone.

4.9% of participants was previously symptomatic, however were not tested to confirm COVID-19 infection. Out of all previously symptomatic but non-confirmed cases 31.5% had positive IgG levels.

Table 1 illustrates the prevalence of symptoms among all participants, among participants with previously confirmed PCR test and participants with positive IgG titer.

10.5% of patients had some level of symptoms persistence during participation in the study. 13.7% of participants with positive IgG titer did not have any symptoms, 3.8% had only loss of sense of taste and anosmia.

Headache was the most common symptom among participants (37.2% among all participants and 53.1% among participants who previously had positive COVID-19 PCR test). Second most common symptom was anosmia (23.7% among all participants and 48.9% among participants who previously had positive COVID-19 PCR test).

Table 1. Symptom's frequencies.

Symptoms	% From all participants n = 837	% From previously PCR+ participants n = 143	% From all IgG positive participants n = 212
Fever more than 38˚C	25.9	44.8	40.1
Subjective fever feeling	33.8	51.0	41.9
Chills	23.5	35.7	33.9
Myalgia	35.8	51.7	46.7
Rhinorrhea	20.6	25.2	23.6
Sore throat	32.1	35.6	30.7
Cough (newly started or worsening of chronic cough)	28.9	40.6	31.6
Shortness of breath	18.5	32.2	26.4
Nausea/vomiting	15.2	25.2	22.2
Headache	37.2	53.1	42.5
Abdominal pain	14.3	20.9	18.4
Diarrhea	19.6	34.9	28.3
Loss of sense of smell or taste	23.7	48.9	44.3
Conjunctivitis	4.7	4.9	3.8
Other	4.1	6.3	0.5

5.4% of participants mentioned previous hospitalization due to COVID-19, 71 (8.5%) mentioned being diagnosed with pneumonia and 24 (2.9%) participants mentioned being admitted to ICU 20 mentioned receiving oxygen therapy and 4 (0.5%) of the participants mentioned receiving an artificial ventilation of lungs. 26 of the participants mentioned being pregnant gestation age varied from 6 - 35 weeks.

19.4% of participants mentioned being a smoker. 6.8% of them previously had positive COVID-19 PCR test. However, among all smokers only 14.2% had positive IgG, 13.0% were in the grey zone.

41.1% of patients mentioned taking antibiotics during last one year period and 30.5% mentioned history of taking antiviral medication.

The symptoms quantity was summed up to a symptom score. In order to identify the association between symptoms quantity and IgG levels the correlation and linear regression analysis were performed. There was a weak positive correlation between symptom sum score and IgG titers. The Correlation coefficient was 0.273, $p < 0.05$, $R^2 = 0.075$. Table 2 presents linear regression analysis results.

The overall regression model was significant $F(1, 711) = 57.45$, $P < 0.01$, $R^2 = 0.075$. The obtained results for the linear regression analysis indicate that the number of symptoms patients have is a significant predictor for IgG level.

Table 2. Linear regression analysis.

Model Summary				
Model	R*	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.273[a]	0.075	0.073	3.369

[a]Predictors: (Constant), IgG1. *R is a correlation between predicted values and observed values.

ANOVA[a]						
Model		Sum of Squares	Df*	Mean Square	F**	Sig.
1	Regression	652.125	1	652.125	57.450	0.000[b]
	Residual	8070.638	711	11.351		
	Total	8722.763	712			

[a]Dependent Variable: symptomsum; [b]Predictors: (Constant), IgG1; *Df is Degree of Freedom; **F test of a null hypothesis.

Coefficients[a]						
Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.221	0.198		11.248	0.000
	IgG1	0.115	0.015	0.273	7.580	0.000

[a]Dependent Variable: symptomsum.

5. Discussion

Unfortunately, there are no other seroprevalence studies to our knowledge conducted in Armenia, so we could compare our results. However, it was obvious that the numbers of COVID-19 cases are much greater than it was reported, as 31.5% had positive titer without previously having a positive COVID-19 test. This finding is comparable to the studies done in other countries [14] [15].

The prevalence of symptom persistence is relevant to the existing studies [19]. Though it is difficult to evaluate as different participants participated in testing in different time interval after recovery. Further studies are required to assess the exact burden of persistent symptoms and factors associated with those symptoms.

According to our findings the most common symptom among participants was headache which contradicts other studies where the most common reported symptom was fever [8] [20] [21].

Current study has several limitations. The main limitation is sampling by convenience design, as our sample is consisting of individuals who turned to examination voluntarily, so it may consist of individuals who have previously had symptoms and are concerned about COVID-19. There is an overrepresentation of female participants in our study. Our study also cannot be quite generalized

for vulnerable social groups of population, as the data was collected from the tests performed for payment. Even though the samples were arriving from different parts of Armenia, however there is no even distribution from each region as the majority of participants are from Yerevan. Therefore, the study results cannot be generalized to entire Armenia. Another important limitation is the fact that the evaluation of symptoms is based on self-reported data, which may involve some recall bias.

Scaling up the population wide serological testing in Armenia can help with evidence based public health decision making [15].

For further research we recommend to perform a follow up study and to assess antibody kinetics over time and the incidence of reinfection with COVID-19 in the year of 2021 in cases of non-vaccinated as well as vaccinated individuals. The effects of smoking status, as well as different chronic conditions on COVID-19 shall be studied further.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

COVID19 SEROLOGYCAL INVESTIGATION QUESTIONNAIRE N

1. Date ___ / ___ / 202__		2. Patient name	
3. DOB ___ / ___ / _____		4. Gender (*mark*) ☐ male ☐ female	
5. Address		6. Phone number	
7. Patient's current status		○ Unknown ○ Laboratory confirmed (PCR positive) ○ Laboratory non-confirmed (PCR negative) ○ Under investigation ○ Under treatment ○ Treated ○ Previously had symptoms ○ Other (*please specify*)_____	
8. If laboratory confirmed (PCR), please mention the confirmation date		___ / ___ / 202__	
9. If patient was recovered, please provide date for negative PCR test		___ /___ / 202__	
10. Did you have contact with confirmed case of coronavirus?		☐ **Yes**	☐ **No** ☐ **Unknown**
11. If yes, please provide the date of last contact		___ / ___ / 202__	
12. Is the patient medical worker		☐ **Yes**	☐ **No** ☐ **Unknown**
During illness did patient have the following symptoms?			
13. Fever 38˚C and higher		☐ **Yes**	☐ **No** ☐ **Unknown**
14. Subjective fever feeling		☐ **Yes**	☐ **No** ☐ **Unknown**
15. Chills		☐ **Yes**	☐ **No** ☐ **Unknown**
16. Muscle pain/myalgia		☐ **Yes**	☐ **No** ☐ **Unknown**
17. Rhinorrhea		☐ **Yes**	☐ **No** ☐ **Unknown**
18. Sore throat		☐ **Yes**	☐ **No** ☐ **Unknown**
19. Cough (newly started of worsening of chronic cough)		☐ **Yes**	☐ **No** ☐ **Unknown**
20. Shortness of Breath		☐ **Yes**	☐ **No** ☐ **Unknown**
21. Nausea/vomiting		☐ **Yes**	☐ **No** ☐ **Unknown**
22. Headache		☐ **Yes**	☐ **No** ☐ **Unknown**
23. Abdominal pain		☐ **Yes**	☐ **No** ☐ **Unknown**
24. Diarrhea (≥3 loose/looser than normal stools/24hr period)		☐ **Yes**	☐ **No** ☐ **Unknown**
25. Loss of sense of taste or smell		☐ **Yes**	☐ **No** ☐ **Unknown**
26. Conjunctivitis		☐ **Yes**	☐ **No** ☐ **Unknown**
27. Other (*please specify*)			
28. Do you currently have symptoms?		☐ **Yes**	☐ **No** ☐ **Unknown**
29. If no, please provide the date of last symptoms		___ / ___ / 202__	
30. Were you hospitalized?		☐ **Yes**	☐ **No** ☐ **Unknown**
If the answer is "no", please go to the question 36			
31. If yes, please provide the date of your hospitalization		___ / ___ / 202__	
32. Were you diagnosed with pneumonia?		☐ **Yes**	☐ **No** ☐ **Unknown**

Continued

33. Were you treated in intensive care unit?	☐ **Yes**	☐ **No**	☐ **Unknown**
34. Did you receive oxygen therapy	☐ **Yes**	☐ **No**	☐ **Unknown**
35. Did you receive mechanical ventilation of lungs	☐ **Yes**	☐ **No**	☐ **Unknown**
Pre-existing medical conditions?			
36. Chronic Lung Disease (asthma/emphysema/COPD	☐ **Yes**	☐ **No**	☐ **Unknown**
37. Diabetes Mellitus	☐ **Yes**	☐ **No**	☐ **Unknown**
38. Cardiovascular diseas	☐ **Yes**	☐ **No**	☐ **Unknown**
39. Chronic Renal disease	☐ **Yes**	☐ **No**	☐ **Unknown**
40. Chronic Liver disease	☐ **Yes**	☐ **No**	☐ **Unknown**
41. Immunocompromised Condition	☐ **Yes**	☐ **No**	☐ **Unknown**
42. Other chronic diseases (*please mention*)			
43. If female, currently pregnant	☐ **Yes**	☐ **No**	☐ **Unknown**
44. If yes, please mention gestation weeks			
45. Do you smoke?	☐ **Yes**	☐ **No**	☐ **Unknown**
46. If yes, please mention how many cigarettes a day? _____			
47. (if 45 is "no") Are you a former smoker?	☐ **Yes**	☐ **No**	☐ **Unknown**
48. Did you have acute respiratory infection during last one year?	☐ **Yes**	☐ **No**	☐ **Unknown**
49. Did you take antibiotics during last one year?	☐ **Yes**	☐ **No**	☐ **Unknown**
50. Did you take antiviral drugs during last one year?	☐ **Yes**	☐ **No**	☐ **Unknown**
51. Did you receive influenza vaccine during last one year?	☐ **Yes**	☐ **No**	☐ **Unknown**
52. Did you receive all the vaccines indicated in national immunization plan (MMR, OPV, BCG, DPT and etc.)	☐ **Yes**	☐ **No**	☐ **Unknown**
53. Can we also contact you months later and invite you for additional testing to identify the titer of antibodies in your blood?	☐ **Yes**	☐ **No**	☐ **Unknown**
THANK YOU FOR TAKING YOUR TIME AND ANSWERING THIS QUESTIONNAIRE			
Name of person filling the questionnaire_____**Signature**_____			