Seroprevalence of Severe Acute Respiratory Syndrome-Coronavirus-2 Immunoglobulin M and Immunoglobulin G Antibodies among the Population of Koya University

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***Abstract*–Coronavirus is a pandemic disease. In most cases, the exact infection rate cannot be determined as not everybody can be tested for the virus, even though some of them carry the virus silently. Therefore, detection of antibodies of this virus is more practical to give us a better clue about the rate of infection because the asymptomatic people can be tested too. The serological detection of anti-Severe Acute Respiratory Syndrome-Coronavirus (SARS-COV-2) antibodies among asymptomatic and moderate symptomatic individuals gives us the vital point to understanding the prevalence rate of COVID-19 among the population. Total of (436) volunteers were participated, (96) from teaching staff, (172) employee, and (168) students. Anti-SARS-COV-2 immunoglobulin G (IgG) and Immunoglobulin M (IgM) were detected in the serum by ELISA technique, and complete blood count was performed for all participants. The number of seropositive of anti-SARS-COV-2/ IgG was (159), whereas IgM was (66). The highest prevalence rate of IgG detected among participants with family member infected with coronavirus (42.7%). Total WBCs count significantly increased among IgM positive participants. Many asymptomatic people were infected with coronavirus, which lead to more spreading of the virus among the population. Therefore, mass screening of the population for specific antibody against coronavirus is important to reduce the infection rate.**

***Index Terms* — Anti-severe acute respiratory syndrome-coronavirus-2 Antibody, Coronavirus, Coronavirus disease-19, Immunoglobulin G, Immunoglobulin M.**

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I. Introduction

As a zoonosis that has spread to several million human beings and probably to domestic and wild animals, the world has witnessed waves of coronavirus infection threatening the global catastrophe of Severe Acute Respiratory Syndrome (SARS) in 2002–2003 and Middle East Respiratory Syndrome in 2011 (MERS). In both cases, newly described coronaviruses of the genus Beta-coronavirus with zoonotic origins were causative agents. In Wuhan, Hubei, China, an outbreak of another coronavirus that causes pulmonary disease has been announced in late of 2019.

The disease officially called Coronavirus Disease 2019 (COVID-19), SARS-COV-2 (etiological agent of COVID-19) genome sequences analysis demonstrated close to the genomic structure of both MERS and SARS-COV (Wu, et al., 2020; Azkur, et al., 2020). The recent study done by Dimonte et al. (2020) illustrated that the phylogenic tree analysis by sequencing the amino acids associated with the structural proteins of the novel coronavirus (nCOV) almost indistinguishable from the SARS-COV, which founded among the different area (Dimonte, et al., 2020).

COVID-19 is transmissible between humans through nasal particles or close contact in both clinical and subclinical persons (Lauer, et al., 2020). The first case of COVID-19 recorded in Iraq, on February 24, 2020, was registered in Najaf province for a student who had travelled from Iran, followed by four cases of one family members in the Kirkuk zone on February 25, all of whom had travelled to Iran, by April 16, 2020, the number of reported cases rose to 1415, with 78 deaths (Sarhan, et al., 2020).

The serological analysis is available to be applied commercially based on Enzyme-linked immunosorbent assay (ELISA) and other techniques, mainly designed to examine anti-spike (S) and anti-nuclear (N) antibody in the blood that synthesized by B-lymphocytes after induced by viral particles (S and N) because of their high antigenic properties (Li et

al., 2005). Anti-S immunoglobulin M (IgM) antibodies can be detected in proximity about 5–12 days after the symptoms appear among individuals who suffered from COVID-19, whereas immunoglobulin G (IgG) and IgA capable of detected in the sera of patients after a fortnight (Wölfel, et al., 2020). Immunoglobulin detection against SARS-COV-2 in the serum samples of individuals shows they had been infected at a particular stage during the outbreak. As a result, serologic analyses could be used to achieve people-based assessments of infection, including individuals who also had asymptomatic or moderate illnesses or who were not ever examined although having symptoms. Mainly the reported cases focused on symptomatic persons and those who have been in contact with the confirmed COVID-19 individuals and came back from an epidemic area. In general, used reverse transcription-polymerase chain reaction (RT–PCR) method for detecting the genome of the virus is a highly sensitive technique. Sadly, a vast population of asymptomatic and subclinical infected persons cannot be diagnosed based on molecular biology strategy (Huang, et al., 2020). The existence of anti-SARS-CoV-2 antibodies in a community may provide a better realistic prediction of the cumulative prevalence of SARS-CoV-2 infection than the viral examination because immunoglobulin against the virus, predominantly IgG, can persist for a prolonged period after the viral infection is eliminated (Xu, et al., 2020).

The benefits of seroprevalence investigation focus on their efficiency in evaluating the degree of asymptomatic exposure among patients and identifying high-risk classes. The study aimed to establish the prevalence of SARS-CoV-2 (IgG and IgM) antibodies in Koya University among academic staff, employers, and students.

II. Materials And Methods

A. Study design and Participants

This study was performed at Science and Health Research center/Koya University/Erbil/Iraq from January 5 to March 10, 2021. It is a cross-sectional study which involved a random selection of (436) participants, (96) from teaching staff, (172) employee, and (168) students. Bodyweight was one of the parameters represented by body mass index (BMI) (BMI=kg/m2 where kg is a person's weight in kilograms and m2 is their height in meters squared). The participants classified as smokers according to the WHO's Smoking and Tobacco Use Policy; a smoker is someone who smokes any tobacco product, either daily or occasionally.

B. Sample Collection and Examination

We collected 5 ml venous blood, 2 ml were put in a lavender tube (Biozek-BE320030) for complete blood count, and 3 ml were put in a gel bottom tube (Biozek-BVG419020) to obtain serum. The blood in the gel bottom tube was centrifuged at (5000 RPM) for 5 min. The obtained serum was divided into two-part and stored in a 1.5 ml Eppendorf test tube, then preserved at -80 °C in (GLF-6483) and defrosted upon testing. Complete Blood Count (CBC) was performed using (Mythic 18). COVID-19 IgM and IgG were evaluated using (SARS-Cov-2 IgM Capture ELISA kit 96t Cat. No: 7424-96) and (SARS-Cov-2 IgG Capture ELISA kit 96t Cat. No: 7324-96), respectively, according to manufacturer's instruction. The test performed using (BIOTEK-ELX800) reader. The result of anti-SARS-COV-2/IgM and IgG was calculated using the cutoff index (COI) method. The result was marked positive if COI > 1.1 and negative if COI < 0.9.

C. Questionnaire and Statistical Analysis

To determine the seroprevalence of COVID-19 at Koya University, a questionnaire was designed. The questionnaire was involved asking some categorical (Yes and No) response. Analysis of data was performed using Statistical Package for the Social Science (SPSS) Version 25.0. Quantitative data, the test of significance used for comparing differences in means, were made by t-test, whereas for qualitative data, the difference in proportions was tested by using Chi-square (X2) test. P-value ≤ 0.05 was considered statistically significant.

III. Results

The study was carried out among (436) participants who compromised academic staff (22%), employed (39.5%), and (38.5) students, and the highest (44.7%) were at the age range of (24≤) years. The mean age of the participant was (30.61 ± 10.95) years. More than half (64%) were male. 16.3 had a smoker, and only (4.4%) person consume alcohol. The residence was predominantly home (67%), compare to accommodation which was (33%), with approximately half of them wearing masks regularly (50.5%). Mostly the blood group of volunteers was O positive (34.4%) and followed by blood group B plus (27.3%). The BMI of 211 participants out of the total were normal, whereas (12.8) members of participants were obese, shown in Table I.

The highest cases of IgG anti-SARS-COV-2 were detected in obese individuals (42.9%), and IgM type antibody found among normal body weight (16.6%). The Chi-square analysis shows no significant differences ($P > 0.05$). Table II illustrated that the prevalence of IgG antibody significantly higher among people who had blood B+ types (50.4%) ($P < 0.05$). Simultaneously, there was a non-significant difference in IgM anti-SARS-COV-2 antibody among blood groups ($P > 0.05$). The risk of Coronavirus (COVs) infection increased highly significantly among participant's that had a family history infected by SARS-COV-2 (42.7%) in comparison to those who did not their family members suffered by COVID-19 ($P < 0.01$).

Furthermore, regularly wearing a face mask reduces the rate of transmission of viral infection. The data revealed seropositivity of anti-SARS-COV-2 IgG significantly declined among individuals who wear a face mask daily (30%), ($P < 0.01$), in comparison to other classes that did not wear it on a daily basis was 43.1%. Recent infection of COVs, which capable determined by anti-SARS-COV-2 IgM antibody significantly lower in participants was (3.1%) who have a chronic disease ($P < 0.05$). There is no significant difference in seropositivity prevalence rate in terms of alcohol genders, consumption, smoking, and occupations, as shown in Table II.

Table I
Socio-demographic Attributes among study Samples

Variables	Frequency	%
Age Group		
24≤	195	44.7
25–34	104	23.9
35≥	137	31.4
Gender		
Female	157	36
Male	279	64
Total	436	100
Residency		
Home	292	67
Accommodation	144	33
Occupation		
Teacher	96	22
Employed	172	39.5
Student	168	38.5
BMI Categories		
Underweight	9	2.1
Normal	211	48.4
Overweight	160	36.7
Obesity	56	12.8
Smoking		
No	365	83.7
Yes	71	16.3
Alcohol consumption		
No	417	95.6
Yes	19	4.4
Wearing mask		
No	216	49.5
Yes	220	50.5
Blood types		
A+	105	24.1
B+	119	27.3
O+	150	34.4
AB+	28	6.4
A-	14	3.2
B-	7	1.6
O-	12	2.8
AB-	1	0.2
Total	436	100

t-test explains that, total white blood cells (WBCs) count significantly decreased ($P < 0.05$) in the blood sample of seropositive for IgG participants (6.29 ± 1.44*103/μl) compare to seronegative individuals (6.67 ± 1.61*103/μl), in contrast, IgM positive blood sample, total WBCs counts dramatically increased (7.01 ±1.74 *103/μl) in compare to non-detected IgM blood samples, which was 6.45 ± 1.51*103/μl. Absolute granulocyte counts significantly decreased among seropositive IgG individuals whereas increased among IgM seropositive samples ($P < 0.05$), vice versa, monocyte and lymphocyte percentage significantly reduced during IgG positive and elevated among IgM positive blood specimens ($P < 0.05$). Other blood parameters such as total erythrocyte count, hemoglobin concentration, platelet count, and indexes show no significant differences among blood samples, as shown in Table III.

Among all the samples, the overall of frequency of IgM positivity was (15.1%), whereas the IgG was (36.5%), whereas only 20 participants (4.6%) were positive for both SARS-Cov-2 immunoglobulins, IgG and IgM depending on the Cutoff Index (COI) as shown in Table IV.

IV. Discussion

Coronavirus disease 2019 (COVID-19) is a pandemic disease which has a significant effect on global economy and other sectors of life. Educational institutes form schools to universities were obligated by the governments to close their doors at the beginning of the pandemic in fear of spreading the virus within the community. In Kurdistan, Iraq, Kurdistan Regional Government (KRG) put tight restrictions for travelers coming to the region and announce safety measures and raising awareness for its residence. One of the restriction measures was closing all states and private schools and universities in the region from February 26 until May 2, 2020 (Merza, et al., 2020). Koya University was one of the state Universities that closed due to the Coronavirus pandemic. However, Koya University was the first university to open its door on October 4, 2020, after releasing the restrictions by the government and brought students to the university accommodation and campus.

Since the beginning of COVID-19 outbreaks, many questions were asked about controlling the spread of the virus; one of the classical ways of controlling any infection in the community is by gaining herd immunity, how long does it take for the herd immunity to reach the protection level this depends on different regions (Britton, Ball and Trapman, 2020). These results represent the first seroprevalence data to be conducted within Kurdistan regional government universities in Iraq. However, a larger scale community-based seroprevalence study will give a better representative picture in this area.

In the present study, the overall seroprevalence of anti-SARS-COV-2 antibodies, IgG and IgM was (36.6%) and (15.2%), respectively. Therefore, measuring the amount of seropositivity, as recommended by the WHO, will notify the proportion of people positive for anti-SARS-CoV-2 antibodies in the community and thereby show the transmission rate over time (WHO, 2020b). Furthermore, the degree of infection in a society depends primarily on communication encounters and population size; it is crucial to assess the proportion of potentially healthy and protected persons in communities with different exposure levels (Shakiba, et al., 2020).

Over 14 day duration, 30-40% among all infected persons by SARS-COV-2 are thought to be asymptomatic (Oran and Topol, 2020). According to the Centers for Disease Control and Prevention, 35% of COVID-19 patients are asymptomatic, and 40% of infection happens before symptoms appear (CDC and Prevention, 2020). Therefore, mass screening aims to determine and isolate victims and tracing their associations, which are essential to minimizing the spread of coronavirus (Hellewell, et al., 2020).

During this asymptomatic stage of infection specific antibody will form against the virus and the first IgM antibodies can be detected as early as 3 days after infection, which serve as a first line of defense. Then its

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Table II
Association between Socio-demographic Attributes with Anti-SARS-COVID-2 Antibodies

Variables	IgG				*P* value	IgM				*P* value
	Negative		Positive			Negative		Positive		
	No.	%	No.	%		No.	%	No.	%	
Gender										
Female	97	61.8	60	38.2	0.569	134	85.4	23	14.6	0.83
Male	180	64.5	99	35.5		236	85.6	43	15.4	
Age group										
24≤	130	66.7	65	33.3	0.221	161	82.6	34	17.4	0.35
25–34	68	64.5	36	34.6		88	84.6	16	15.4	
35≥	79	57.7	58	42.3		121	88.3	16	11.7	
BMI										
Underweight	9	100	0	0.0	0.08	8	88.9	1	11.1	0.538
Normal	131	62.1	80	37.9		176	83.4	35	16.6	
Overweight	105	65.6	55	34.4		135	84.4	25	15.6	
Obesity	32	57.1	24	42.9		51	91.1	5	8.9	
Occupation										
Teacher	57	59.4	39	40.6	0.446	82	85.4	14	14.6	0.159
Employed	115	66.9	57	33.1		152	88.4	20	11.6	
Student	105	62.5	63	37.5		136	81	32	19	
Blood groups										
A+	70	66.7	35	33.3	0.029*	91	86.7	14	13.3	0.69
B+	59	49.6	60	50.4		105	88.2	14	11.8	
O+	105	70	45	30		122	81.3	28	18.7	
AB+	19	67.9	9	32.1		22	78.6	6	21.4	
A-	11	78.6	3	21.4		13	92.9	1	7.1	
B-	4	57.1	3	42.9		6	85.7	1	14.3	
O-	8	66.7	4	33.3		10	83.3	2	16.7	
AB-	1	100	0	0.0		1	100	0	0.0	
Residency										
Home	184	63	108	37	0.749	235	86.6	39	13.4	0.139
Accommodation	93	64.6	51	35.4		117	81.3	27	18.8	
Family member infected by COVID-19										
No	152	69.7	66	30.3	0.007*	187	85.8	31	14.2	0.593
Yes	125	57.3	93	42.7		183	83.9	35	16.1	
Chronic diseases										
No	257	63.8	146	36.2	0.619	338	83.9	65	16.1	0.03*
Yes	19	59.4	13	40.6		31	96.9	1	3.1	
Wearing Mask										
No	123	56.9	93	43.1	0.003*	182	84.3	34	15.7	0.72
Yes	154	70	66	30		188	85.5	32	14.5	
Smoking										
No	225	61.6	140	38.4	0.063	309	84.7	56	15.3	0.78
Yes	52	73.2	19	26.8		61	85.9	10	14.1	
Alcoholism										
No	264	63.3	153	36.7	0.425	325	84.4	65	15.6	0.18
Yes	13	68.4	6	31.6		18	94.7	1	5.3	
Total	277	63.4	159	36.6		369	84.4	66	15.2	

* Indicate significant levels ($P \leq 0.05$)

level will decline, and IgG antibody will form. This will make a long-term memory and fight against the virus in the future (Racine and Winslow, 2009). Therefore, in our data both SARS-COV-2 IgG and IgM antibody detection in 20 participants (4.6%) are normal as any other viral infection. The presence of both antibodies at the same time represents the overlap production of both antibodies during microbial infection throughout the course of the disease. A study conducted by Nakano et al. in 2021 showed that anti- SARS-COV-2 IgM titer would increase until day 18 after the symptoms appeared, after that it will decline and, IgG remains stable above 400AU/ml after day 13, it increases afterwards (Nakano, et al., 2021).

The seropositive IgM was significantly higher among participants with chronic diseases such as hypertension and diabetes (3.1%) (George, et al., 2019) and (42.7%) of participants were IgG positive had family members infected with COVID-19, which agrees with other research done in India (George, et al., 2021) one of the first protective strategies against SARS-COV-2 was wearing the mask, the governments were asking, or in some order to wear masks, since then many researches and public reports showed the significance

Table III
Comparison of Blood Cell Parameters in Seroprevalence of Anti-SARS-COV-2

Blood parameters	IgG (Mean±SD)		*P* value	IgM (Mean±SD)		*P* value
	Positive *n* (159)	Negative *n* (277)		Positive *n* (66)	Negative *n* (369)	
WBC (10^3/µl)	6.29±1.44	6.67±1.61	0.014*	7.01±1.74	6.45±1.51	0.007*
LYM (10^3/µl)	1.9±0.47	1.89±0.55	0.865	1.95±0.43	1.88±0.54	0.368
MON (10^3/µl)	0.397±0.18	0.4±0.18	0.66	0.38±0.15	0.4±0.19	0.465
GRA (10^3/µl)	3.99±1.21	4.37±1.41	0.004*	4.67±1.59	4.15±1.29	0.004*
LYM%	30.98±7.31	29.05±7.5	0.009*	28.77±6.96	29.93±7.56	0.264
MON%	6.38±2.76	6.2±2.79	0.517	5.64±2.24	6.38±2.85	0.021*
GRA%	62.81±7.31	64.77±8.05	0.012*	65.58±7.37	63.79±7.89	0.087
RBC (10^6/µl)	5.33±2.89	5.18±0.612	0.434	5.3±0.736	5.22±1.94	0.744
HGB (g/dl)	14.8±1.99	15±1.94	0.175	15.08±1.8	14.95±1.99	0.618
HCT (%)	44.4±4.57	44.95±5.21	0.268	45.48±4.2	44.61±5.11	0.195
MCV	87.47±7.47	87.08±8.16	0.628	86.68±8.73	87.32±7.76	0.542
HCH (pg)	29.27±3.36	29.32±3.53	0.897	28.79±4.0	29.39±3.36	0.195
MCHC (g/dl)	33.47±2.4	33.52±2.63	0.847	33.15±2.46	33.57±2.56	0.225
RDW	12.81±1.25	12.86±2.32	0.810	12.7±1.34	12.85±2.1	0.765
PLT	245.13±74.4	245.87±54.4	0.904	246.61±54.54	245.42±63.76	0.887
PDW	15.05±1.32	15.13±1.414	0.544	14.92±1.182	15.13±1.41	0.259

* indicate significant levels ($P \leq 0.05$)

Table IV
Frequency anti-SARS-CoV-2 Immunoglobulins among participants

Anti-SARSCoV-2	Positive	Negative
IgM	66 (15.1%)	370 (84.9%)
IgG	159 (36.5%)	277 (63.5%)
IgM and IgG	20 (4.6%)	416 (95.4%)

of wearing the mask in decreasing the transmission of the virus as this may help in reducing the direct contact with the droplets of an infected person (Howard, et al., 2020). In our finding, the seropositive IgG of SARS-COV-2 was significantly lower among participants wearing mask daily (30%) ($P < 0.01$) in comparison to those not wearing a mask on a daily basis (43.1%), which mean good protection against virus transmission, this result is compatible with many other researches done regarding the efficiency of wearing a mask in reducing virus transmission. A study by Leffler et al. (2020) comparing the effect of wearing a mask in different countries, they found that the transmission of the virus was 7.5% higher in countries wearing a mask was not compulsory.

The relationship between blood group and microbial infection has been documented previously (Leffler, et al., 2020). This relation was connected to the difference in antigens found in erythrocytes that react differently with different bacteria, parasites and viruses (Acik and Bankir, 2021); this can be true for SARS-COV-2 infection as well. The results of our study showed that the highest seropositive IgG was found among B+ blood group participants (50.4%) who disagree with (Pourali, et al., 2020). Our result, similar to other researches, showed that the blood group O+ was more resistant to SARS-COV-2 infection (30%) (Wu, et al., 2020; Zhao, et al., 2020; Acik and Bankir, 2021).

Since the onset of COVID-19, many factors were connected to the severity and mortality rate among infected people, for example, chronic disease cardiovascular diseases and diabetes, age and gender (Guzik, et al., 2020; Teng, et al., 2020). Moreover, WBC count is one of the factors that have been linked to COVID-19 infection severity and mortality. Possibility of death will be higher in COVID-19 patients with elevated WBC count (Zhu, et al., 2021). Increase total WBC count and neutrophils, decreased lymphocyte count has reported in many studies about COVID-19. However, other studies in the same field reported different and variable results regarding the WBC profile of COVID-19 patients (Sun, Zhou and Ye, 2021). These inconsistence in the results of WBC in Coronavirus infected people make it difficult to connect it to the severity of the disease or use it as a prognosis tool, since this difference or changes in the WBC counts are strongly related to the immune system and variability in immune response within people (Siedlinski, et al., 2020; Sun, Zhou and Ye, 2021).

As a result of the importance of the WBCs in establishing the immune response and inflammation, it is used as a parameter in determining the severity of COVID-19 infection and increased risk of mortality (Wang, et al., 2021). Coronavirus infection, as any other infection, is companied by immune response and inflammation, which indicated by many inflammatory markers in the body, including inflammatory cytokines and WBCs. The results of our study showed increased total WBC in IgM positive or recent infection ($P > 0.01$), which agree with the meta-analysis review study done by Feng, et al., 2020. However, there was no significant difference between infected and non-infected participants regarding total lymphocytes and monocytes.

On the other hand, our data showed significant reduction in total WBC among seropositive IgG participants compared with seronegative individuals ($P > 0.05$) which agree with a study contacted by Liu et al. (2020), demonstrating that approximately 80% of the patients suffered from COVID-19 had a normal or reduced WBC counts. Lymphocytes levels were low in 72.3 percent (Liu, et al., 2020). By studying the relation between coronaviruses infection and the innate immune response, the possibility of persisting and developing of chronic inflammation among these population will be more clear (Zhu, et al., 2021).

In most COVID-19 seroprevalence assessments up to date, the cumulative rate of infection was identified primarily by age group, race, ethnicity, and nationality. Antiviral IgG screening of people in high-risk jobs to COVs due to repeated or high social activities could be needed for public health decisions on immediate lockdowns or return to work policies (WHO, 2020a; Bendavid, et al., 2021; Rosenberg, et al., 2020).

V. Conclusion

Many people in the community are infected with coronavirus without any symptoms, which has a bigger threat on spreading the virus to a non-infected person. The previous infected asymptomatic individuals can be detected just by antibody testing, giving a clear picture of the rate of COVID-19 infection among the population. This figure could be used to assist the health sectors in confirming better identify infection, death rates and make policy decisions. Based on the studied samples from people at Koya University, we recommend that the analyses with a vast sample size yield more reliable estimation of the proportion that has historically been infected with SARS-COV-2 and, as a result, provide more detailed information on herd immunity.

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