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Determination of IgG and IgM against COVID- 19 for Recovered Patients at Different Intervals and by Different Techniques

Enas Wa'ad Ubaid Al- Hadidi*, Mahmood Abdaljabbar Al- Tobje

Department of Biology, College of Sciences, Mosul University, Mosul-Iraq
(Enass.scp23@student.uomosul.edu.iq, mahtsbio@uomosul.edu.iq)
**Correspondence: Enass.scp23@student.uomosul.edu.iq*

Abstract

The numbers of infected cases and deaths associated with the novel coronavirus disea-19 (COVID-19), are still increasing daily. Although antibodies have been detected in serum of COVID-19 patients, their dynamics and association with outcomes have not been fully characterized. This study aimed to determine the concentrations of IgG and IgM antibodies for different intervals after recovery from COVID-19, namely 4, 6 and 8 months. Also, to identify the accordance between two types of immunity techniques used to identify the existence of antibodies. The presence of antibodies IgM and IgG to SARS-COV-2 was evaluated in serum samples from recovered patients from COVID-19 over 8 months after infection using ELISA technique and rapid test cassette. The study was conducted in Nineveh Governorate in Iraq on 92 recovering from COVID-19 disease at least two months ago. The results showed the existence of IgG antibodies with a percentage of 94.03%, and IgM antibodies with a percentage of 55.22% in recruited individuals using ELISA technique. The percentages of these antibodies were (86.56%) for IgG and (16.42%) for IgM when using a rapid test cassette for diagnosis; the matching was 67% between the two methods. Samples of the control group also showed the presence of IgG and IgM with percentages of 68% and 88% respectively. The average concentrations ±SD of IgG antibodies were 33.05 ± 10.76, 43.21± 4.5 and 37.53 ± 8.82 at P ≥ 0.01 after 4, 6 and 8 months after infection respectively; the peak was at the 6th month after infection. The averages of IgM ± SD were 14.45 ± 3.3, 18.52 ± 3.86 and 19.18 ± 3.61 at P< 0.05 after 4, 6 and 8 months of being infected respectively; the peak was at the 8th month after infection. It was concluded that the presence of antibodies against SARS-CoV-2 continued for six months after infection and its level began to decrease gradually after eight months of infection whereas the concentration of IgM depends on the patient's exposure again to the virus during recovery period.

Keywords: *ELISA, IgG, IgM, Rapid test cassette*

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I. INTRODUCTION

In the year 2019, virus SARS- COV- 2 and acute respiratory infection was diagnosed for the first time in China, and it was called COVID-19 (Figueiredo-Campos *et al*., 2020). It is of great importance to deal with COVID-19 epidemic in case of future spread or in regard to developing new strategies of sustainable vaccination to determine the activity and the pattern of immunity. Therefore, it is necessary to acquire further accepting of the immunity duration and its relatedness to disease harshness and clinical management (Sasisekharan *et al*., 2021).

After 7- 14 days from infection by SARS-COV-2, seroconversion takes place (Krajewski *et al*., 2020; Long *et al*., 2020a; To *et al*., 2020) with the presence of peak in the level of antibodies which was seen in 30- 35 days after the manifestation of symptoms (Crawford *et al*., 2020; Wang *et al*., 2020). Rapid decline was noticed in the concentration of (anti- SARS- COV- 2 IgG antibodies) for a period of about 3 months after being infected (Beaudoin-Bussiere *et al*., 2020; Röltgen *et al*., 2020), while there was stability in the titer of antibodies for several months, the thing that indicates the presence of long-term immunity (Gudbjartsson *et al*., 2020; Wang *et al*., 2020; Liu *et al*., 2021). COVID- 19 cases are confirmed through the detection of virus RNA sequences using (NAAT) test, such as (RT-PCR) in phlegm and throat swabs of lower respiratory system (WHO, 2020; Vengesai *et al*., 2021), and the standard reference method rRt- PCR diagnoses the infection of SARS- COV-2 accurately and with great sensitivity in the acute stage of COVID- 19 (Lee *et al*., 2020).

Serological checks can be completed more quickly and with higher productivity and less cost and work burden (Lee *et al*, 2020; Wolff *et al*., 2020) and when the virus is present in patients less than the detection border of RT- PCR criteria, then diagnosis becomes of high value and can be used in

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complementation to NAATs (Wolff *et al*., 2020; To *et al*., 2020), however, the first diagnosis of COVID- 19 using serological check only might not be probable since seroconversion takes pace usually within 3- 14 days after the manifestation of the disease. The specificity and sensitivity of the tests are certainly affected by the period of infection (Wang *et al*., 2020). In general, serological checks can be appropriate in the following cases: 1- Diagnosis through 7 days of symptoms appearance, 2- Diagnosis with RT- PCR negative tests and having epidemical and clinical evidence that indicate being infected with COVID- 19, 3- Tracing patient contacts, 4- Determining probable immunity and protection against reinfection, 5-Serological epidemiological studies to recognize the spread of COVID- 19 in the society (Lassaunière *et al*., 2020; Lou *et al*., 2020; Rastawicki *et al*., 2020).

Serological tests for diagnosis include (ELISAs) assays, (CLIAs) assay and (LFIAs) assay, in addition to that neutralization assays (NT) (Ravi *et al*., 2020). The current study aimed to iidentify the most sensitive and accurate technique to diagnose the infection and the degree of matching between techniques. Also, to determine the period of retention for IgG and IgM antibodies after infection and recovery from the disease.

II. Materials and Methods

A. *Materials*

The research was conducted in Nineveh Governorate in Iraq on 92 individuals, 67 were infected with COVID-19 disease recovered at least two months ago and 25 control samples.
92 blood samples were collected of which 67 from recovered patients from COVID- 19 for a period of recovery not less than two months, and 25 blood samples from individuals that showed no symptoms of ages (17- 75) years and during the period from October/2020 to March/ 2021. Patient information were recorded in an information form which included: sample number, age, gender, date of infection, date of recovery, symptoms that the patient suffered during infection, comorbidities, treatments used and place of residency. Samples collection included three different time intervals (4 months, 6 months and 8 months) after infection with COVID- 19.

B. *Preparation of blood serum*

5 ml of venous blood was drawn via sterile medical syringe, put in gel tube and left for half an hour till blood coagulation took place, and serum separated using centrifuge at (3000) rpm for (15) minutes, and then distributed on a number of eppendorf tubes. The samples were then preserved at (-20) C_o until the required tests were conducted.

C. *Serological methods*

Two different Serological methods were used for detection of antibodies of COVID-19.

1. ELISA

COVID- 19 IgG- IgM detection kit was used from (Vircell Spain S.L.U., Granada, Spain), Vircell COVID- 19 ELISA IgG. These tests use SARS- COV- 2 antigen of (S) protein and (N) Protein, and when the diluted samples were added and during the incubation period, COVID- 19 antibodies link with their antigens, and after washing, the antibodies marked with enzyme were added with which the complex in the pits associate to cause color change when adding the base material of the enzyme. The intensity of color is directly proportional with the concentration of the antibodies in the sample, the reaction is stopped using an acid solution, and then light intensity is measured at (450) nm and results are calculated allowing:
Ab- index= (sample O.D. /cut off serum mean O.D.) x 10
Results: Positive values ≥ 6.0, negative values <4.0, and questionable values :4.0 to 6.0, (Wölfel *et al*., 2020)

2. Rapid test cassette

Using cassette test to detect the existence of COVID-19 virus antibodies; IgG and IgM supplied by (Biozek Medical,). Immunochromatographic test as used for the fast lateral flow qualitative discovery of IgG , IgM –Ab of SARS- COV-2 in blood according to the instructions of the manufacturer; the result was read within 10- 15 minutes (Cui *et al*. 2019).

D. *Statistical analysis*

Statistical analysis of the data was conducted via Kappa program to find the percentage of matching between different methods (McHugh, 2012) and the Statistical Package for Social Science (SPSS) version 25 (SPSS Inc., Chicago, IL) program was used in the statistical analysis for the purpose of finding the significant relationship and extracting a p-value (Kirkwood and Sterne, 1988).

III. Results

Two serological methods were followed to identify the presence of Diseases IgG, IgM -Ab in the serum, namely: ELISA and Rapid test Cassette and table (1) shows that the total number of the diagnosed samples was (67) of which (63) gave positive result for IgG antibodies according to ELISA with a percentage of (94.03%). Tests were also conducted on (25) samples of healthy people who showed no symptoms of infection, as control samples, of which (17)

Table 1. Methods used in identifying positive samples of IgG and IgM in individuals and their percentages after 4 months of infection

Antibodies	EISA						Rapid test cassette				Percentage of match
	total	Positive samples %	Percentage %	Total control sample	Positive control samples	percentage %	Positive samples	percentage %	Positive control samples	percentage	67%
IgG	67	63	94.03	25	17	68	58	86.56	13	52	
IgM	67	37	55.22	25	22	88	11	16.42	10	40	

had IgG antibodies with a percentage of (68.56%). IgM antibodies were found in (37) persons with a percentage of (55.22%) and in (22) persons of the control sample with a percentage of (88%). In the other hand, when Rapid test cassette was used, there were (58) persons out of the total number that showed positive results for IgG with a percentage of (86.56%) and (13) of the control samples showed positive IgG with a percentage of (52%), whereas IgM appeared in (11) persons with a percentage of (16.42%) and in the control samples in (10) persons with a percentage of (40%).

Through the performance of statistical analysis of results, the match ratio between the two methods was found to be (67%) and this result indicates medium matching between the two techniques.

Sensitivity and specificity are two quantitative standards to evaluate methods of diagnosis (Mandrekar, 2010), where sensitivity pointing to the ability of the test to determine the least possible amount of antibodies in the sample, and the higher the sensitivity of the test was, the less false- negative results obtained.

As for specificity, it is the ability of the test to qualitative determination of antibodies under investigation, and the more specific the test was, the more accurate the results we get (Li *et al*., 2020: Goudouris, 2021). Sufficient specificity and sensitivity eventually lead to optimal diagnosis.

Among several studies that addressed the determination of Rapid test cassette sensitivity, only few of them proved it to be of good sensitivity which varied between 90% - 100% (Zhao *et al*., 2020; Zhong *et al*., 2020; Yang *et al*., 2020). Many other studies have proved that it was of low sensitivity (Yang *et al*., 2020; Pan *et al*., 2020; To *et al*., 220; Xu *et al*., 2020) and this is in line with the results we came to. The sensitivity values of test kits vary according to the type of test and the manufacture. A comparative study was conducted among the different methods for diagnosing infection with COVID- 19 and found that ELISA was the most sensitive among other methods used to diagnose infection with COVID-19 (Zhong *et al*., 2020).

In general, ELISA has superior diagnostic accuracy in determining the concentration of IgG and IgM with sensitivity and specificity that reached (85%) and (99%) respectively (Vengesai *et al*., 2021) and this is in congruency with the results we obtained. The sensitivity results in this statistic study is compatible with the statistical analysis which showed that Rapid test cassette has less sensitivity than CLIA and ELISA in each class of antibodies (Deeks *et al*., 2020; Bastos *et al*., 2020; Wang *et al*., 220). This contradicted the statistical analysis by Deeks *et al*. who found that regarding IgG and IgG- IgM, the Rapid test cassette had higher sensitivity than ELISA (Deeks *et al*., 2020).

According to another statistical analysis, serological tests to determine the level of IgM had the lowest sensitivities compared to serological test to determine the level of IgG in each method (Deeks *et al*., 2020; Bastos *et al*., Wang *et al*., 2020) and this is in line with our study. Low concentrations of IgM may be due to its late formation after the infection or due to the late performance of test till their disappearance (Kontou *et al*., 2020).

Table 2. The mean concentration of IgG and IgM ± SD antibodies in the three intervals.

Ig	Total Number	Number of positive samples after 4 months	Mean ±SD IU/ml	No. of $+^{ve}$ samples after 6 months	Mean ± SD IU/ml	No. of $+^{ve}$ samples after 8 months	Mean ± SD IU/ml	P- value
IgG	48	48	33.05 ± 10.76	48	43.21± 4.5	48	37.53 ± 8.82	$P \geq 0.01$
IgM	48	28	14.45 ± 3.3	30	18.52 ± 3.86	36	19.18 ± 3.61	$P< 0.05$

Table (2) demonstrates the mean concentrations ± standard deviation (SD) of IgG and IgM antibodies during the three intervals; the average concentration ±SD of IgG was (33.05± 10.76) IU/ml in the first interval, increased to (43.21± 4.5) IU/ml in the second interval, and started to decline as it was (37.53± 8.82) IU in the third at ($P \geq 0.01$). The average ±SD of IgM was (14.45 ± 3.3) IU/ml in the first interval, increased to (18.52 ± 3.86) IU/ml and maintained a close average in the third interval with (19.15 ± 3.61) IU/ml at ($P < 0.05$). We may conclude that we have a reduced IgG average at 8 months after infection whereas the concentration of IgM depends on the patient's exposure again to the virus during recovery period.

Evidence indicate that adults infected confirmed with RT-PCR develop IgM antibodies with a percentage up to (80%) after 20 days of symptoms manifestation (Dave *et al*., 2020; Liu *et al*., 2020; Shu *et al*., 2020) and the results of these studies indicate also that IgM antibodies are first noticed in an average of 7 days and begin to decline in 27 days and this contradicts our study.

Many other studies also showed that the heights of IgG against SARS- COV-2 did not decline after 4 months of infection and it continued to 6 months after the appearance of symptoms (Zhao *et al*., 2020; Isho *et al*., 2020; Dan *et al*., 2020; Lee *et al*., 2020; Figueiredo *et al*., 2020; Liu *et al*., 2020; Wang *et al*., 2020; Cervia *et al*., 2021; Marklund *et al*., 2021; Wajnberg *et al*., 2020).

Another study which lasted for about 8 months found that (95%) of individuals who developed IgG had a slight decline in its concentration after 3 months, and IgG bodies still present with a percentage of 90% after 8 months. IgA and IgM antibodies were less common and there levels declined faster in 8 months at which IgM antibodies were not seen. Age and severity of infection are independently associated with the high levels of IgG antibodies (Glück *et al*., 2021).

Some reports indicate that IR to SARS- COV- 2 could decrease rapidly (Ibarrondo *et al*., 2020; Brochot *et al*., 2020) and a big percentage of patients go back to seronegative (Self *et al*., 2020), the study results, with other studies, proved that anti- SARS- COV- 2 IgG may keep a stable level respectively

or show a slow reduction for 6 months at least (Isho *et al*., 2020; Dan *et al*., 2020; Wajnberg *et al*., 2020; Iyer *et al*., 2020; Choe *et al*., 2021) and this coincides with the results we obtained; IgG antibodies were seen with the highest level at 6 months and started to decine at 8 months. This also coincides with another study that followed COVID-19 cases for 6 months and found that antibodies persist in all cases after 6-7 months from COVID- 19 infection in addition to T- cell memory (Tan *et al*., 2020).

Many recent studies have showed that most patients have responses (Ab) to SARS- COV- 2 that can be seen after 6-8 months from infection (Crawford *et al*., 2020; Iyer *et al*., 2020, Wajnberg *et al*., 2020; Grandjean *et al*., 2020; Seow *et al*., 2020; Gudbjartsson *et al*., 2020).

IV. CONCLUSION

The highest IgG antibodies level was seen after 6 months of infection whereas the highest level of IgM antibodies was seen in the eighth month after infection. ELISA was the more sensitive and accurate technique in diagnosis of antibodies than Rapid test cassette and the percentage of accordance between them was 67%.

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