Original Research Article

Biochemical and Serological Tests for People Recovering from COVID-19

Abstract

Aims: The purpose of this study was to detect some biological and serological factors in COVID-19 recovery patients, as well as their relationships with antibody levels 8 months after infection. ***Materials and methods***: 92 blood samples were obtained; 67 of them had COVID-19 infections 4 months prior, and 25 blood samples served as control samples. The participants' ages ranged from 17-75 year. Determination levels of immunoglobulin IgG and IgM antibodies was carried out on (188) samples of infection over for ages ranging from 15-75 years by using enzyme linked immunosorbant assay (ELISA). ***Results:*** The people under research have 94.03% IgG and 55.22% IgM. Furthermore, the maximum concentration of IgG was seen after 6 months of infection. Aberrant lactate dehydrogenase (LDH) levels were found in 20 people at 41.6% after 4 months, in 25 people at 52.08% after 6 months, and in 8 people at 8.33% after 8 months. In contrast, aberrant C- reactive protein (CRP) levels were found in 10.4% of the participants after 4 to 8 months of infection and 12.5% after 6 months, with no significant association between them. ***Conclusion:*** The maximum IgG level was reported 6 months after infection, and the LDH and CRP tests were abnormal 6 months later. On the other hand, 4 months after the infection, both urea and creatinine levels were abnormal, as was the IgG concentration.

Keywords: COVID-19, IgG, IgM, LDH, ELISA, SARS-CoV-2

Introduction

SARS-CoV-2 is a novel virus that first surfaced in the end of 2019 in Wuhan, China, and has since spread around the world. By June 2020, more than 7 million people had been infected with COVID-19, which had killed more than 400,000 people [1]. The severity of this sickness ranges from asymptomatic to deadly multi-organ failure to mild illness [2].

Coagulopathy, which manifests as venous and arterial thromboembolic thrombosis, is one of the most significant consequences of the condition and has been associated to poorer outcomes. Despite preventative and therapeutic anticoagulation, instances of thrombosis raise questions about the pathogenesis of COVID-19 [3]. In addition to coagulopathy markers like D-dimer, other hematologic parameters have been studied. In contrast, in order to develop successful interventional approaches for this lethal disease, a deeper understanding of pathophysiology and the identification of biomarkers predictive of clinical outcomes are required [2].

One of the notions proposed is a substantially heightened inflammatory response that generates thrombi inflammation via mechanisms such as cytokine storm, immune complex, and endothelins. Retrospective research has so created clinical signs that suggest a poor prognosis. Similarly, the severity of the disease is linked to neutrophil count, lymphocyte qualification, neutrophil/lymphocyte ratio, and platelet count. As a result, it is now clear that people infected with COVID-19 have a significantly higher risk of thrombosis, which persists despite anticoagulation. Furthermore, it has been claimed that a virus may be capable of initiating the coagulation cascade on its own. Although several institutions have developed policies and processes for delivering preventative and treatment anticoagulation, the optimum course of action is rapidly evolving as we learn more about the pathophysiology of this disorder. Including. Furthermore, thrombosis has been connected to prior coronavirus epidemics such as SARS-CoV-1 and MERS-CoV.

As evidenced by an upsurge in reports of arterial, venous, and catheter-related thrombosis around the world [3, 4 and 5], the novel SARS-CoV-2 appears to produce a highly prothrombotic environment. Individuals with COVID-19 disease have a significantly increased risk of thrombosis, which persists despite anticoagulation. Concurrently, a better understanding of pathophysiology was gained.

The goals of this study are to discover the serological and biochemical characteristics of coronavirus-cured patients.

Materials and Methods

During the eight months following infection, 188 blood samples were collected. 67 were from patients who had COVID-19 infections 4 months before, 48 after 6 months, and 48 after 8 months, with 25 blood samples serving as controls. The ages of the participants ranged from 17 to 75 years. The study lasted from October 2020 to March 2021. Age and gender of the patient, date of injury and recovery, symptoms experienced during infection, comorbidities, treatments,

and residency were all recorded. Blood serum was obtained and frozen in preparation for the next phase. COVID-19 antibodies were detected using two different serological techniques.

ELISA

The IgG and IgM kits were utilised (Vircell Spain S.L.U., Granada, Spain). SARS- COV-2 (S) and (N) Protein, as well as when diluted samples were added and during the incubation period COVID- 19 antibodies bind to their antigens. After washing, the antibodies labelled with enzyme were added, with which the complex in the pits associate to induce colour change when the enzyme's base material was introduced. The intensity of the colour is directly proportional to the concentration of antibodies in the sample, the reaction is halted with an acid solution, and the light intensity is measured at (450) nm.

Biochemical tests

1. **C-reactive protein quantification using nephelometry**: Nephelometry is a technique used in immunology to quantify the quantities of various proteins in blood plasma. This approach measures the level of C-reactive protein (CRP) using latex-enhanced immuno-nephelometry. Latex is dependent on the interaction of the soluble protein to be evaluated with the appropriate antigen or antibody attached to the latex particles. A machine Specific protein analyser is used to determine the concentration of this immunological complex, and the reagents employed in this device are pre-calibrated. The curve calibration is recorded inside the Magcard assay kit [7].

2. Determination of lactate dehydrogenase (LDH): This test relies on the conversion of pyruvate LDH to lactate in the presence of NADH. The oxidation of NADH detected at 340 nm, which is directly proportional to the concentration of LDH present in the sample, as illustrated in the attached figure [8].

3. Quantification of urea:

The test is based on the hydrolysis of urea in the sample by urease to ammonium ion and carbon dioxide. In the presence of glutamate dehydrogenase (GLDH), the generated ammonium ions react with -ketoglutarate and NADH to create glutamate and NAD +. The concentration of urea is measured at 340 nm [9].

4. Quantification of creatinine:

In an alkaline environment, creatinine in the sample interacts with picric acid to generate a yellow-orange salt detected at 510 nm. The intensity of a colour that emerges over time is proportional to the amount of creatinine in the sample [10].

Statistical analysis

The data were statistically examined using (SPSS version 25) software to determine the percentage of concordance between different parameters [11].

Results and Discussion

The determination of COVID-19 antibodies (IgG and IgM) after 4 months of infection and their percentages were depicted in the table (1). This table displays the total number of diagnosed samples, which was 67. 94.03% of them tested positive for IgG. As control samples, 25 healthy adults with no indications of illness were collected. Also, IgG antibodies were found in 68% of participants, whereas IgM antibodies were found in 55.22% of 37 people and 88% of 22 people in control samples.

Table 1: IgG and IgM in people after 4 months of infection and their percentages.

Antibodies	ELISA					
	Total No. Sample	**+ Samples**	**%**	**Total No. Control**	**+ Control**	**%**
IgG	67	63	94.03	25	17	68
IgM	67	37	55.22	25	22	88

The average concentration of IgG appeared in units/ml during the three periods and began to drop in the third period to 37.54 U/ml. The IgM appeared at a rate of 14.46U/ml in the first period, grew to 18.52U/ml in the second period, and remained at a close rate of 19.18U/ml in the third period, Table (2). This suggests that the level of IgG has begun to decline after 6 months of infection, whereas the concentration of IgM is determined by the extent to which the person is exposed to the virus again during the recovery period [12].

Table 2: The average concentration of IgG and IgM in the three periods. **Ave..: Average** Std.E**:** Standard error.

Ig	**Total No.**	**+ After 4 Months**	**Ave. & Std.E**	**+ After 6 Months**	**Ave. & Std.E**	**+ After 8 Months**	**Ave. & Std.E**
IgG	48	48	33.40 ±1.55 (10.76)	48	43.22 ±0.65 (4.50)	48	37.54 ±1.27 (8.82)
IgM	48	28	14.46 ±0.62 (3.29)	30	18.52± 0.7 (3.86)	36	19.18±0.6 (3.61)

The LDH test was carried out on 48 persons. 20 samples (41.67%) were found to be abnormal in the first period, 25 samples (52.08%) in the second period, and 4 samples (8.33%) in the third period. The CRP test was abnormal in just 5 samples out of 48 total patients (10.42%) in the first and third periods, Table (3).

Table 3: The average concentration of IgG for the three periods according to serological tests in the subjects under study.

Parameters		4 M After infection			6 M After infection		8 M After infection			
	Total No.	Positive sample	%	Ave ± Std.E	Positive sample	%	Ave ± Std.E	Positive sample	%	Ave ± Std.E
LDH	48	20	41.67	638.1±36.68 164.1-	25	52.08	642.92±35.97 179.86-	4	8.33	55.52 ±633 111.03-
CRP	48	5	10.42	16.71 ±1.61 3.59-	6	12.5	12.81±1.14 2.79-	5	10.42	13.41±0.68 1.52-

The investigation involved the determination of several biochemical and serological tests in the patients under study, as well as their correlation with the rate of IgG. LDH and CRP levels were determined. As kidney functions, urea and creatinine were examined in Table (4).

When assessing LDH after 4 months of infection, the concentration of IgG in patients reached its peak. The level of IgG began to drop in the first period with LDH, with a clear substantial association between IgG and LDH at a significant level ($P< 0.05$). After 6 months, the IgG concentration reached its peak with LDH. After 4 and 8 months, the concentration of IgG converged at LDH. There was no significant association between IgG levels and LDH 6 months after infection.

When CRP was measured in the body, the maximum level of IgG was seen in the first 8 months following infection. When assessing CRP 8 months after infection, the level of IgG was at its maximum in the second period. When CRP was evaluated 6 months after infection, the maximum level of IgG was seen in the third period, Table (4).

Table 4: The average concentration of IgG for the three periods according to serological tests in the subjects under study

Parameters	IgG Concentration Rate (IU/ml)			Correlation coefficient	P-value
	Standard error ± IgG 4 M (standard deviation)	Standard error ± IgG 6 M (standard deviation)	Standard error ± IgG 8 M (standard deviation)		
CRP 4 M	**36.25 ± 3.11 6.21-**	**42.42 ± 2.11 4.22-**	**39.21 ± 3.40 6.8-**	**0.107**	**P > 0.05**
CRP 6 M	**38.54 ± 4.27 10.46-**	**42.37 ± 1.21 2.96-**	**46.19 ± 2.90 7.11-**	**0.142**	

CRP 8 M	**39.37 ± 5.7** **12.74-**	**46.41 ± 1.82** **4.08-**	**44.03 ± 5** **11.17-**	**0.266**	

For the first time, kidney functioning appeared normal. When creatinine serum and urea levels were measured, IgG levels appeared to be normal (4 months after infection). During the second and third periods of the trial, no significant changes in kidney function were seen. A significant link was found between the average IgG level after 4 months of infection and the LDH level after 4 months of infection, with a correlation coefficient of ($P< 0.05$). A significant association between the average level of LDH and urea ($P< 0.05$). There is also a significant association between and level of urea after 4 months with age at ($P< 0.01$) and between LDH and CRP after 8 months at ($P< 0.05$), Table (5).

Table 5: Biochemical and serological tests for people under study and their percentages.

Parameters	**IgG (U/ml)**			**Significant**	**P-value**
	Std.E± IgG4 M	**Std.E± IgG 6 M**	**Std.E± IgG 8 M**		
LDH 4 M	34.71 ± 2.72 (12.16)	42.99 ± 0.92 (4.11)	36.56 ±2.1 (9.39)	0.260*	P < 0.05
LDH 6 M	33.41 ± 2.15 (10.96)	44.34 ± 0.94 (4.78)	39.81 ± 1.75 (8.91)	0.057	P > 0.05
LDH 8 M	25.89 ±25.71 (11.41)	40.19 ± 1.8 (3.60)	33.16 ± 2.91 (5.82)	0.082	
Creatinine for 92 samples	42.876	-	-	0.047	
Urea for 92 samples	26.58 ± 15.87 (22.45)	-	-	0.188	

The level of IgM was assessed for each period, as well as its association to biochemical and serological assays such as LDH, creatinine, urea, and CRP. After 4 months of infection, the second period had the greatest level of LDH, 15.75U/ml, and it also appeared in the third period with the highest level of LDH, 16.58U/ml, Table (6).

Table 6: The average IgM concentration in the three periods and its relationship to the biochemical tests of the people under study.

Parameters	IgM (U/ml)			Significant	P-value
	IgM±Std.E 4 M	IgM±Std.E 6 M	IgM±Std.E 8 M		
LDH 4 M	12.6±1.05 -4.71	15.75±1.42 -6.35	16.58±1.43 -6.39	0.082	P > 0.05
LDH 6 M	79.0±11.2 -4.03	13.93±1.33 -6.76	15.23±1.17 -5.94	0.169	
LDH 8 M	11.97±2.83 -5.67	12.37±2.47 -2.94	18.15±3.25 -6.5	0.252	
Creatinine for 92 samples	8.35	-	-	0.047	
Urea for 92 samples	12.1±1.97 -2.78	-	-	0.071	

The highest levels of IgM were seen at CRP 8 months after infection in the first and second periods. This finding suggests that in cases of SARS-CoV-2 infection, adverse effects may remain beyond recovery, depending on the severity of the injury. There was a significant connection between IgM levels 6 and 8 months after infection and CRP levels 6 and 8 months after infection (P< 0.05), Table (7).

Table 7: The average concentration of IgM in the three periods and its relationship to the serological tests.

Parameters	IgM Concentration Rate (U/ml)			Correlation coefficient	P-value
	Standard error ± IgM 4 M (standard deviation)	Standard error ± IgM 6 M (standard deviation)	Standard error ± IgM 8 M (standard deviation)		
CRP 4 M	**1.24±13.3** **-2.48**	**4.48±9.57** **-8.97**	**2,45±14.22** **-4.91**	**0.074**	**P > 0.05**
CRP 6 M	**9.76 ± 1.42** **-3.48**	**8.61 ± 2.35** **-5.76**	**19.37 ± 1.43** **-3.49**	**0.302***	**P < 0.05**
CRP 8 M	**13.71 ± 2.36** **-5.27**	**17.78 ± 2.99** **-6.7**	**12.3 ± 2.80** **-6.27**	**0.302***	**P < 0.05**

A study involved 114 patients, 36 of whom experienced a severe injury and 78 of whom did not. Patients with severe clinical symptoms had older ages, lower lymphocyte counts, higher levels of inflammatory biomarkers like CRP and IL-6, and greater organ damage indices like LDH and D-dimer, which was consistent with earlier research [13, 14].

From week 3 to week 8, the amount of IgM was positively connected with CRP, LDH, and alkaline phosphatase (ALP) and negatively correlated with albumin, showing that IgM is implicated in uncontrolled inflammatory reactions and, in severe cases, organ damage [15, 16].

A Chinese study comprised 49 individuals who have recovered from COVID-19. It was discovered that their infection was severe due to high levels of both LDH and CRP and major lung abnormalities, as well as a significant (p0.05) connection with high levels of antibody to the disease and a low lymphocyte count [17].

A research on 40 COVID-19 patients in Greece discovered (31 males and 9 females). 30% of the 40 patients were diagnosed as having mild to moderate cases, while 70% were classed as having severe to critical cases. This study discovered a definite relationship between infection and the quantity of white blood cells (WBC), lymphocytes, and platelets that suggest lung damage, particularly in individuals with severe disease, with a positive relationship between IgG and D-dimer [18].

Total antibody levels correlate positively with ALT, LDH, and ferritin; however, different results were observed when antibodies were associated with creatinine, CRP, and urea, depending on illness severity [19]. CRP, LDH, and D-dimer levels have also been linked to an increased risk of death in individuals with comorbidities [20]. The D-dimer and urea, in combination with troponin, were found to be predictive of ICU admission [21]. Severe cases have also been reported to have greater levels of antibody response, inflammatory cell counts, and CRP [22].

According to one study, SARS-CoV-2 antigen was inversely linked with total immunoglobulin, PLT, ferritin, and ALT in a group of patients with mild to moderate illness. Total Ig levels were found to be related to WBC, LYMP, ferritin, and ALT. Total Ig and D-dime levels were found to have a substantial negative association. SARS-CoV-2 antigen was found to be inversely related to total Ig, WBC, LYMP, PLT, and ALT levels in the severe-critical group. Antigens were linked to higher levels of CRP and urea. Total Ig levels were found to be favorably associated to WBC, neutrophils, and D-dimer. Total Ig and creatinine levels also had a strong negative connection [23]. This is consistent with our study.

Because viral load and particular immune response to antibodies are inversely related. Several laboratory indicators of tissue infection, acute phase response, and innate immune activation were found in patients with severe COVID-19 infection. At the time of diagnosis and admission to the hospital for therapy, IL-6, CRP, and D-dimer levels are all very high. Average levels of LDH, IL-6, and CRP remained elevated for the first 10 days following hospitalization, and D-dimer increased slightly before decreasing around day 20 [24].

The findings revealed that an increasing proportion of patients had biomarker levels that were below the threshold limit. On day 20, 30% of individuals had LDH levels of less than 400 U/L,

23% had IL-6 levels of less than 500 ng/L, 32% had CRP levels of less than 50 ng/L, and 40% had D-dimer levels of less than 2 ng/L, and antibody titers rose as infection progressed [25]. It agrees with our results here. Antibody levels were significantly ($P< 0.01$) inversely associated to CRP levels. However, the relationship between IL-6 levels was weaker.

This is consistent with our findings, as there was no significant association between CRP and antibody levels. As a result, a transitory relationship was discovered between the immune response and levels of IL-6 and CRP, which eventually decrease with recovery [26-28]. During the second week of infection, there was an increase in antibody levels. High levels of IL-6 and CRP were also found to remain in certain patients over extended periods of time [29]. The reason could be the existence of secondary problems such as bacterial and fungal infections [30]. This is consistent with our CRP findings.

A study involving 622 patients in Switzerland discovered that higher levels of SARS-CoV-2 antibodies were substantially related with lower levels of viral loads, LDH, IL-6, and CRP [31].

The number of antibody-producing cells (CD19+, CD27+, CD38+) was also connected to CRP, IL-6, LDH, and the quantity of atypical memory B cells in the serum (CD27-, CD21-). Inflammation indices (IL-6, LDH, and ferritin) and the number of antibody-producing cells (plasmablasts) (CD19+, CD27+, CD38+) and atypical memory B cells (CD27-, CD21-) were higher in individuals with the most severe COVID-19 pathways. Following COVID-19 recovery, a quick return to normal levels of atypical memory B cell count was also observed [25].

Another study with 84 patients discovered statistically significant differences between antibody titers and CRP and LDH levels (P 0.001), as their values coincided with the high level of antibodies. After recovery, the antibody titer reduced [32].

This is consistent with the data we observed in terms of lowering antibody titers and CRP and LDH readings as recovery time progressed. The researchers discovered that patients with severe COVID-19 had higher levels of IL-6 than non-critical instances. In addition to IL-6, CRP was shown to be linked with illness severity [33, 34]. Although disease severity and CRP may overlap, it is thought that CRP is more likely to be a factor related with higher antibody titers [32].

Conclusions

The maximum level of IgG antibodies was detected 6 months after infection, and the LDH and CRP tests looked abnormal 6 months after infection, whereas both urea and creatinine appeared abnormal 4 months after infection. In general, the concentration of IgG was higher in the second period with LDH and CRP than in the other periods. Our work continue to detect other immunological parameters of COVID-19 recovering people.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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