Evaluation of serum IgG and IgM in covid-19 patients and their correlations with some clinic pathological parameters

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Abstract

Cross sectional study was carried out to evaluated of serum IgG and IgM in patients with COVID -19, conducted in Al-Yarmouk Hospital and Dar Al Salam field Hospital 1 in Baghdad city. The study started from February to July 2021 on Iraqi COVID-19 patients,age of the patients was above > (15) years old with confirmed infection documented by polymerase chain reaction (PCR). The total of subjects were 132 patients, 69 (52.3%) males and 63 (47.7 %) females. Patients divided into three pathological groups: mild patients (45), moderate patients (34) and severe patients (53), each group was divided into four weeks according to symptoms onset date. more comorbidities, included cardiovascular disease, diabetes, renal disease, liver disease and chronic lung disease. Sandwich-Enzyme-Linked Immunosorbent Assay kits were used to evaluate levels of IgG and IgM in patients. Our results for antibody responses in patients showed,IgG and IgM levels occurred simultaneously or sequentially across four weeks. In this study mild patients showed increased levels of IgM and IgG in the 2nd week after symptoms onset. Then, a relatively high level of IgG was still persistent after two weeks, while the level of IgM tended to decrease slightly. Serum IgG was gradually increased from week 1 to 4 with high significant differences (p-value= 0.0001). Serum IgM was increased in the early stage from symptoms onsets and gradually decreased significantly (p-value=0.0384). In moderate COVID-19 patients, IgG titers showed slightly increased in late stage with significant difference (p-value=0.0229), whereas no significant observed in IgM titers across four weeks (p-value=0.0239). For severe patients, our results showed no significant differences in IgG (p-value= 0.782), whereas showed high significant in IgM during disease progression in severe patients (p-value=0.0052). The present study showed,a positive correlation between IgG with LYM, but there was a negative correlation between IgG and (LDH,CRP,D-dimer, NEU) in COVID-19 patients.

Keywords: Covid-19, inflammatory parameters, antibodies,IgG, IgM

1. Introduction

The coronavirus disease 2019, (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been prevalent in almost all regions of the world, resulting in 276,436,619 confirmed cases and 5,374,744 deaths by December,23, 2021. On 11 March 2020, The World Health Organization declared COVID-19, as a pandemic caused by (SARS-CoV-2) [1]. The main effect of (SARS-CoV-2) is in human respiratory system cells. However, new studies have revealed the impact of the virus on the cells of the gastrointestinal tract, urinary system, liver, pancreas, eyes, and brain [2]. The SARS-CoV-2 virus is about 79% and 50%, similar to SARS and MERS viruses, respectively [3].

Many laboratory parameters make it possible to assess the severity of the disease and predict the risk of it evolving toward more serious afflictions such as acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC) and multiple organ failure (MOF) [4]. Some parameters for which an unfavorable course of the disease has been described are absolute neutrophilia, thrombocytopenia, hypoalbuminemia, the elevation of liver enzymes, creatinine and nonspecific inflammatory markers such as C-reactive protein (CRP) [5]. In addition to the that, the main progression predictors described are lymphopenia, elevated D-dimer and hyperferritinemia, although it is also necessary to consider LDH, CPK and troponin in the marker panel [6]. Clinical studies suggest that the dynamics of antibody response following acute infection with SARS-CoV-2 is similar to other HCoVs. Antibody responses are generally detected against the nucleocapsid (N) or spike (S) proteins, the S1 subunit of which contains the receptor-binding domain (RBD), antibodies against different antigens may have differential dynamics and neutralizing effect. The presence of neutralizing antibodies has been demonstrated in studies of vaccine research and therapeutic use of convalescent plasma [7, 8]. However, the neutralizing titers vary greatly and are correlated with Ab binding levels against RBD, spike, nucleocapsid, and with age, symptom duration, and symptom severity [9, 10]. Our study was aimed to evaluate several hematological, biochemistry and immunological parameters in COVID-19 patients are their relation with disease progression and severity.

2. Patients and methods

During the period from February– July 2020, a cross-sectional study was conducted to evaluate the IgG and IgM in covid -19 patients were hospitalized in Al-Yarmouk Hospital and Dar Al Salam field Hospital 1 in Baghdad city. One hundred thirty two Iraqi patients with COVID-19 infection documented by PCR participated in this study, these groups were age and gender matched, all participants are adults > 15 years old. The Ethics Committee (No.10223 in February 16/2020) at the Baghdad Al Karekh Health Directorate (Iraqi Ministry of Health and Environment) approved the study protocol. All patients were confirmed positive for SARS-CoV-2 using PCR retrieved from nasopharyngeal swab. Blood specimens were collected at the time of physician requests and the data of the patients was obtained from clinical records. Those patients were classified into 3 groups according to severity of disease: mild, moderate and severe, and each group classified into 4 weeks, according to date of symptoms onset. The severity of

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disease include:

- Mild disease: including patients without pneumonia or hypoxia, SpO2 ≥95 on room air
- Moderate disease: including patients with clinical signs pneumonia, SpO2 ≥90 on room air.
- Severe disease: including patients with severe respiratory distress, SpO2 <90 on room air,who hospitalized in respiratory care unit (ICU) thus requiring supplementation of oxygen.

Disease severity of COVID-19 was assigned into the following groups in accordance with guidance given by WHO (WHO,2021).

From each patient, 5 mL of venous blood was collected using 5mL disposable syringe. The obtained blood was distributed into two tubes; a serum separated tube (Gel) and ethylene-diamine-tetra-acetic acid (EDTA) tubes. Five milliliters of blood was dispensed, 2 ml in EDTA tube for CBC assay, and 3 ml in Gel tube for biochemical and immunological assays, gel tube was allowed to clot (for minimum of 30 minutes) at room temperature (20-25°C), then sera are separated by centrifugation (The samples were centrifuged at 2000-3000 rpm for 20 minutes.) and stored at -20°C until assayed. Serum levels of in the samples were measured by sandwich - Enzyme Linked Immunosorbent Assay (ELISA) at wavelength of 450 nm. A commercial ELISA kits for the quantitative determination of IgG and IgM (CUSABIO, Chine) were used. This test was performed following the procedure protocol included within the kit packing as issued from the manufacturer company. The detection ranges of the kits are given as follows:

IgG: The detection range of the kit is 0.59 µg/ml -150 µg/ml; Sensitivity= 0.487 µg/ml

IgM: The detection range of the kit is 4.68 ng/ml-300 ng/ml; Sensitivity= 1.17ng/ml

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01) probability. Estimate of correlation coefficient between variables in this study.

3. Results

4.1. Demographic and clinical characteristics of COVID-19 groups across the four weeks

The present study evaluated the levels of IgG and IgM of COVID-19 in adult Iraqi patients. The present study included 132 cases..Three pathological groups of the disease progression over time have been proposed for COVID -19; mild,moderate and severe groups, numbered, 45, 34,53 respectively. The disease progression in mild, moderate and severe patients with COVID-19 was divided into four weeks (1st week, 2nd week, 3rd week and 4th week).

The mean ± SD of age for mild patients across four weeks was (39.91±3.64, 40.62±5.72, 38.87±5.60, 34.53±2.09) respectively, while the mean age for moderate patients was (55.78±6.68, 51.00±5.99, 56.60±3.49, 47.42±5.50) respectively, for severe patients the mean age was (54.50±4.36, 55.94±3.92, 57.33±3.32, 65.22±3.23) respectively. In the present study no significant differences was observed among four weeks for mild and moderate (p-value=0.0912, 0.1552) respectively, whereas was significant differences in severe (p-value=0.0319). Patients were divided by sex into 69 (52.3%) males and 63 (47.7 %) females. Moderate and severe cases did not have any significance across four weeks (p-value= 0.1552, 0.0319) respectively, but there is a significant sign in mild cases (p-value 0.0368), as shown in table (4.1).

Table 4-1: Demographic of COVID -19 groups distributed according to symptoms onset.

COVID-19 groups	Variables	1st Week	2nd Week	3rd Week	4th Week	p-value
Mild N=45	Age Mean± SD	39.91±3.64	40.62 ±5.72	38.87±5.60	34.53±2.09	0.0912
	Gender (%) Male Female	7(21.21) 5(15.15)	4(12.12) 4(12.12)	7(21.21) 1 (3.03)	11(33.33) 6(18.18)	0.0368
Moderate N=34	Age Mean± SD	55.78 ±6.68	51.00 ±5.99	56.60±3.49	47.42±5.50	0.1552
	Gender (%) Male Female	5(15.15) 4(12.12)	5 (15.15) 3 (9.09)	4 (12.12) 6 (18.18)	2 (6.06) 5 (15.15)	0.0.084
Severe N=53	Age Mean± SD	54.50 ±4.36	55.94 ±3.92	57.33±3.32	65.22±3.23	0.0319
	Gender (%) Male Female	6 (18.18) 6 (18.8)	5 (15.15) 12(36.36)	7 (21.21) 8 (18.18)	6 (18.18) 3 (9.09)	0.073

4.2. Clinical characteristic

It was observed that most of COVID-19 cases have comorbidities were strongly related to COVID-19 hospitalization and severity. In this study COVID-19 cases with underlying medical problems like cardiovascular disease, diabetes, renal disease, liver disease and chronic lung disease were more at risk of developing a serious illness and required referral for intensive care due to their low immune

status. Cancer, Hyperthyroidism, Hypothyroidism, Lupus, G6PD deficiency and Psoriasis have also been observed, but it were uncommon, as shown in Table (4.2).

able (4.2):Clinical characteristics in COVID-19 patients across the four weeks			
Clinical Characteristic	Mild	Moderate	Severe

	Week1 N=12	Week2 N=8	Week3 N=8	Week4 N=17	Week1 N=9	Week2 N=8	Week3 N=10	Week4 N=7	Week1 N=12	Week2 N=17	Week3 N=15	Week4 N=9
Comorbidities(%)												
Diabetes	1(8.3)		2(25)		2(22.2)	3(37.5)	3(30)		4(33.3)	7(41.1)	7(46.7)	2(22.2)
Cardiovascular disease	3(25)			4(23.5)	6(66.6)	6(75)		1(14.3)	8(66.6)	9(52.9)	10(66.6)	4(44.4)
Liver disease					1(11.1)		2(20)	1(14.3)		1(5.8)		1(11.1)
Renal disease					2(22.2)	1(12.5)		2(28.6)	3(16.6)	4(23.5)	2(13.3)	1(11.1)
Chronic lung disease			1(12.5)	1(5.9)	1(11.1)					3(17.5)	1(6.6)	
Any health problems and others (%)												
Cancer					1(11.1)					2(11.7)		1(11.1)
Hyperthyroidism										1(5.8)	1(6.6)	
Hypothyroidism	1(8.3)				1(11.1)	1(12.5)						1(11.1)
Lupus				1(5.9)								
G6PD deficiency				1(5.9)			1(10)					
Psoriasis				1(5.9)						1(5.8)		
Current smoker	4(33.3)	5(62.5)	3(37.5)	7(41.2)								
Pregnancy	2(16.6)		1(12.5)	2(11.7)	1(11.1)							

4.3. Levels of inflammatory parameters in COVID-19 groups across four weeks.

The study, including C-reactive protein, D-dimer,, Lactate dehydrogenase, lymphocytes and neutrophils) were measured for each patients across four weeks as summarized in table (4.3).

Table (4.3):- Levels of inflammatory parameters in COVID-19 groups across for weeks

Parameters	1st Week	2nd Week	3rd Week	4th Week	p-value
Mild					
LDH U/L	186.58 ±14.37 a	178.62 ±27.41 ab	199.00 ±15.35 a	138.11 ±5.51 ab	0.0006
CRP32 mg/dL	1.088 ±0.23 b	2.15 ±0.96 a	0.546 ±0.18 c	0.354 ±0.06 c	0.0001
D-dimer ng/ml	112.67 ±30.70	133.00 ±22.15	157.75 ±39.63	143.23 ±19.32	0.249
NUT 10^3/uL	4.65 ±1.08 b	6.01 ±1.63 ab	6.52 ±1.52 a	4.50 ±0.43 b	0.0452
LYM 10^3/uL	2.12 ±0.23 b	2.89 ±0.21 b	3.75 ±0.24 a	3.80 ±0.25 a	0.0341
Moderate					
LDH U/L	344.33 ±48.92 ab	402.87 ±35.19 a	411.00 ±56.87 a	295.43 ±39.51 b	0.0315
CRP32 mg/dL	6.35 ±3.07 a	3.73 ±1.35 b	7.12 ±1.70 a	3.90 ±1.67 b	0.0298
D-dimer ng/ml	270.67 ±77.07 b	380.37 ±56.09 ab	526.50 ±112.67 a	375.00 ±104.7 ab	0.0478
NUT 10^3/uL	6.86 ±1.57 b	10.24 ±1.96 a	8.60 ±2.53 ab	8.03 ±2.23 ab	0.0425
LYM 10^3/uL	1.57 ±0.16	2.17 ±0.25	1.81 ±0.27	2.13 ±0.23	0.077
Severe					
LDH U/L	779.75 ±149.90	636.88 ±83.75	627.73 ±63.26	843.89 ±102.71	0.0662
CRP32 mg/dL	10.64 ±23.19	11.72 ±2.45	8.15 ±1.85	13.08 ±2.93	0.266
D-dimer ng/ml	1572.25 ±175.99 a	1185.41 ±174.43 b	1359.27 ±151.62 ab	1424.44 ±240.10 ab	0.0480
NUT 10^3/uL	10.82 ±2.01 b	13.85 ±1.80 ab	16.05 ±2.86 a	9.38 ±1.88 b	0.0073
LYM 10^3/uL	1.54 ±0.22 a	1.78 ±0.18 a	1.15 ±0.09 b	0.949 ±0.08 b	0.0378

Means having with the different letters in same row differed significantly.
Means having with the similar letters in same row did not differ significantly.
Means did not have letters in the same row did not differ significantly

The mean ± SD of CRP, D-dimer, LDH, lymphocytes and neutrophils in mild, moderate and severe patients as shown in table (4.3).

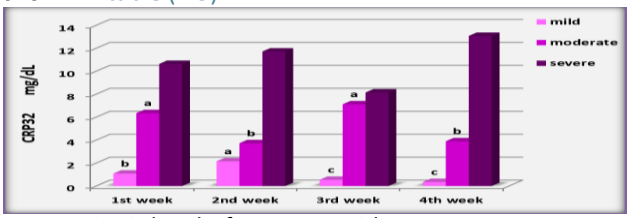

Figure (4.1): level of CRP32 mg/dL in COVID-19 groups across four weeks

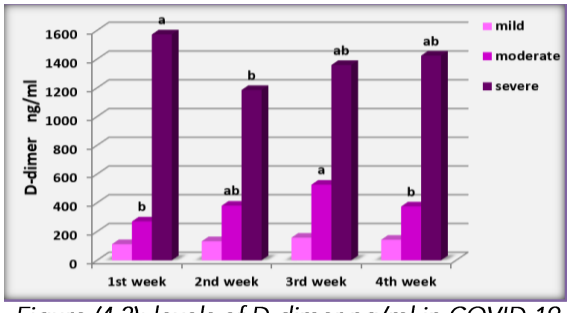

Figure (4.2): levels of D-dimer ng/ml in COVID-19 groups across four weeks

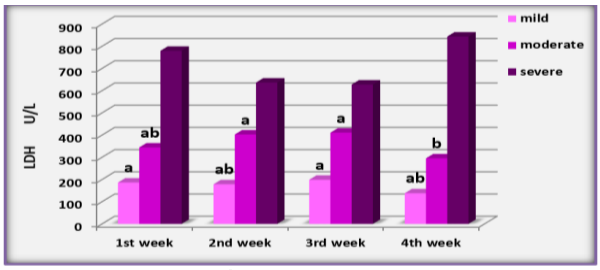

Figure (4.3): level of LDH in COVID-19 groups across four weeks

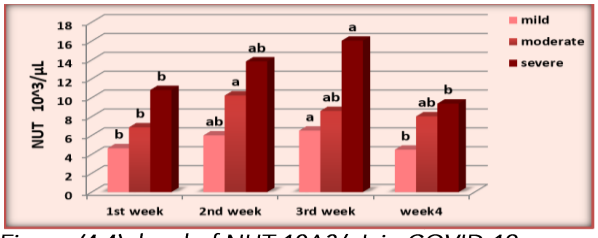

Figure (4.4): level of NUT 10^3/uL in COVID-19 groups across four weeks

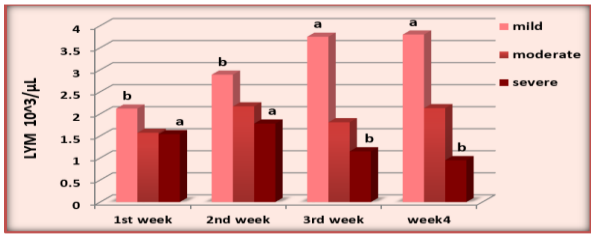

Figure (4.5): level of LYM 10^3/uL in COVID-19 groups across four weeks

In mild patients CRP levels increased rapidly after symptom onset, and reached peak levels on illness 2nd week, then gradually declined to low levels at the last weeks with significant differences(P-value=0.0001), The CRP in moderate patients were elevated in the 1st week and decreased in the 2nd week, then returned to rise at a level close to the 1st week, and continued to decrease gradually during the last week with significant differences (p-value=0.0298), in severe patients levels of CRP were elevated among the four weeks with no significance differences (p-value=0.266). In the present study no significance differences in D-dimer levels in mild patients (p-value=0.0672). Whereas increased with significant differences among the four weeks in moderate and severe patients (p-value=0.0478, 0.0480) respectively. LDH levels in mild patients increased after symptom onset in the 1st week, and reached peak levels on illness 3rd week, They declined to low levels in the last week with High significant (p-value=0.0006). moderate patients had an increase in LDH in the 1st week, 2nd week and 3rd week then gradually decreased in 4th week with significant differences (p-value=0.0315). In severe patients there were no significant differences were showed in elevated levels of LDH in severe cases (p-value=0.0662). Results of mild patients showed increased with significance differences for neutrophils and lymphocyte (p-value=0.0452, 0.0341) respectively. In moderate patients there was no significant difference in neutrophil (P-value= 0.077), and significant differences among the four weeks in Lymphocyte (p-value=0.0425) respectively. Severe patients showed increased with high significance differences in Neutrophil (p-value=0.0037), whereas showed decreased with significant differences in Lymphocyte (p-value =0.0378) respectively.

4.4. levels of antibodies in COVID-19 groups across four weeks.

Antibody responses to SARS-CoV-2 in patients were measured across four weeks, IgG and IgM levels occurred simultaneously or sequentially as shown in table (4.3).

Table (4.4): levels of IgG and IgM in COVID-19 groups across four weeks

Parameters	1st Week	2nd Week	3rd Week	4th Week	p-value
Mild					
IgG µg/ml	0.374 ±0.04 c	3.67 ±0.42 b	5.70 ±1.72 ab	7.20 ±0.92 a	0.0001
IgM µg/ml	65.39 ±5.12 ab	69.87 ±4.05 a	58.65 ±4.27 b	60.44 ±1.26 b	0.0384
Moderate					
IgG µg/ml	0.359 ±0.07 b	0.272 ±0.04 b	0.501 ±0.08 a	0.508 ±0.15 a	0.0229
IgM µg/ml	57.69 ±5.51	57.06 ±5.46	60.35 ±4.41	58.57 ±5.07	0.239
Severe					
IgG µg/ml	0.345 ±0.02	0.335 ±0.02	0.316 ±0.02	0.334 ±0.02	0.782
IgM µg/ml	51.22 ±5.92 b	60.86 ±4.57 ab	52.72 ±3.33 b	69.01 ±4.48 a	0.0052
Means having with the different letters in same row differed significantly. Means having with the similar letters in same row did not differ significantly. Means did not have letters in the same row did not differ significantly					

The mean ± SD of IgG and IgM levels for covid-19 patients, as shown in table(4.4).

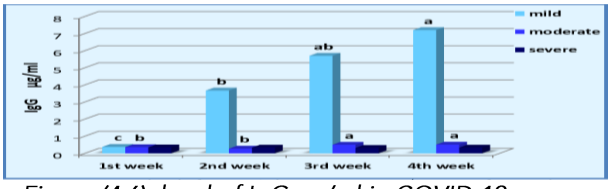

Figure (4.6): level of IgG µg/ml in COVID-19 groups across four weeks

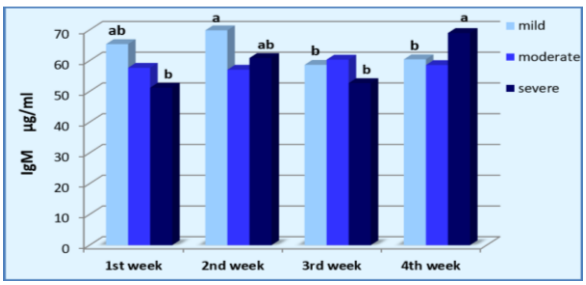

Figure (4.7): level of IgM µg/ml in COVID-19 groups across four weeks

In this study mild patients exhibit elevated levels of IgM

and IgG in the 2nd week after symptoms onset. Then, a relatively high level of IgG was still persistent after two weeks, while the level of IgM tended to decrease slightly. Serum IgG was detected around 1st week after illness onset, then gradually increased from week 1 to 4 and peaked in week 4 with high significant differences (p-value= 0.0001). Serum IgM was detected around 1st week after illness onset, peaked in 2nd weeks, and then gradually decreased significantly from 2nd week to 4th week (p-value=0.0384). levels of anti-SARS-CoV-2 IgG and IgM antibodies were measured in moderate COVID-19 patients, IgG titers showed significant difference (p-value=0.0229), whereas no significant observed in IgM titers across four weeks (p-value=0.0239). For severe patients, our results showed no significant differences in IgG (p-value= 0.782), whereas showed high significant in IgM during disease progression in severe patients (p-value=0.0052).

4.5. Correlation coefficient (r) between IgG with some parameters in covid-19 patients.

To assess the correlations between parameters IgG with some parameter (LDH, CRP32, D-dimer, NEU, LYM), Spearman's correlation coefficient (rs) was estimated in mild, moderate and severe patients, as shown in table (4.5).

Table (4.5):Correlation coefficient (r) between IgG with some parameters in covid-19 patients.

Variables		LDH	CRP32	D-dimer	NEU	LYM
Mild						
IgG	rs	-0.204	-0.175	0.051	-0.067	0.598
	p-value	0.179	0.251	0.740	0.660	0.000
Moderate						
IgG	rs	-0.156	-0.152	0.018	-0.141	0.422
	p-value	0.378	0.391	0.920	0.427	0.013
Severe						
IgG	rs	-0.120	0.026	-0.087	0.013	0.061
	p-value	0.394	0.855	0.538	0.929	0.663

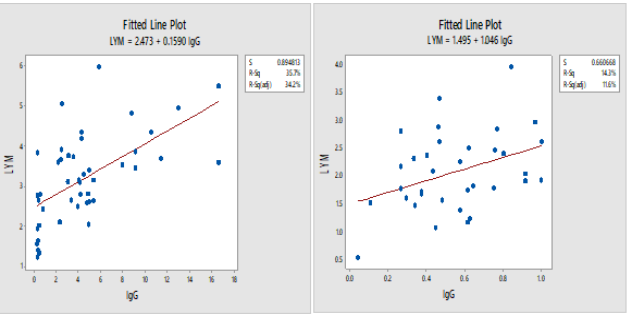

Figure (4.8): Correlation between IgG Figure (4.9): Correlation between IG with LYM in mild patients. With LUM in moderate patients.

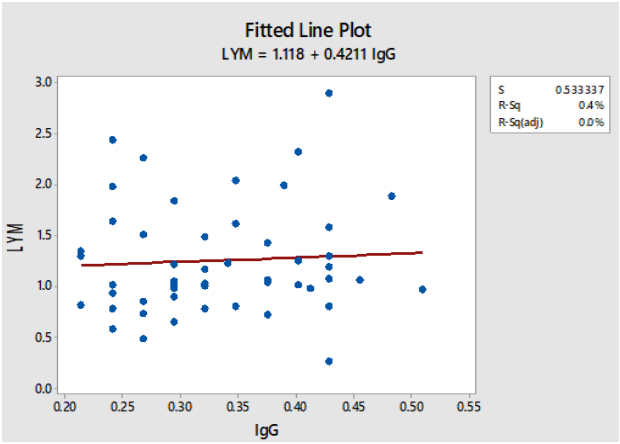

Figure (4.10): Correlation between IgG With LYM in severe patients.

4. Discussion

We found the patients with comorbid diseases was higher than the findings. In the current study, hypertension and diabetes mellitus were the most common comorbidities and these results supported the findings of the studies conducted in hospitalized COVID-19 patients [11-13]. This discrepancy could be attributed to variation in the prevalence of chronic diseases across age, gender distribution, and geographic region. Current smokers were more commonly observed only in among people who are diagnosed as a milder disease. This study is agreement with the study done by Hippisley-Cox et al. [14], they found that smoking was associated with lower risks of COVID-19 severity on adjustment for multiple prognostic factors, in contrast, smoking was associated with higher risks of COVID-related death, adjusted for age and sex, in another large population-based study conducted by Williamson et al. [15]. A history of smoking was not related to the severity of COVID-19, and the same results were also found in a study conducted with a total of 7162 patients, suggesting that smoking is not significantly associated with the severity and mortality of COVID-19. The reasons behind the discrepancy may be linked to the lack of high-quality data and studies about the relationship between smoking and the severity of COVID-19 [16], since some researchers found a positive relationship [17, 18]. In addition, there are currently no clinical or laboratory results about the influence of smoking on the disease. I think that smoking damage lungs and therefore causing damage to ACE2 receptors, or nicotine interferes with ACE2 receptors, which may prevent the virus from entering cells. Pregnant women do not appear more likely to contract the infection than the general population., pregnancy itself alters the body's immune system pregnancy itself. However,this study showed that cases of COVID-19 in pregnancy are milder and with good recovery. There are limited data on the impact of COVID-19 on pregnant women and their babies. Compared with SARS and MERS, COVID-19 appears to be less lethal, most of the pregnant women with COVID-19 were asymptomatic or only had mild symptoms, however

particular attention should be given to those with underlying diseases, since they are at a higher risk of developing severe disease as with the general population [19, 20]. Our results for mild which is consistent with prior reports in COVID-19 [21-23]. A study reported there was increased in day 3 in serum CRP levels, which then dramatically dropped down around Day 6 to 9 in both moderate and severe group of patients [24]. Previous research conducted by Tan *et al*., (2021), has shown that CRP decreased after 14 days from symptoms onsets. Another study showed there was a fluctuation around day 3 in serum CRP which then dramatically dropped down around Day 6 to 9 in severe group of patients, then it rose again [24]. CRP is key indication of immunological deterioration. COVID-19, on the other hand, is a highly contagious disease caused by SARS-CoV-2, and nothing is known about how the virus interacts with the immune system after it infects the human body, except from significant studies on the virus' genomic structure and sequence [25]. In severe COVID19 patients, increased CRP levels may be associated with an overproduction of inflammatory cytokines. Although cytokines attack microorganisms, when the immune system becomes overactive, it can cause lung tissue damage. In COVID19 patients, inflammatory cytokines and tissue damage both trigger CRP production [26].Increased C-reactive protein levels in COVID-19 patients may be correlated to inflammatory cytokine overproduction. Although cytokines target pathogens, an overactive immune system may harm lung tissue.

A study conducted by Zhou et al. [27], showed that differences between mild patients in D-dimer levels were significant from illness onsets to day 22, except for day 4 after illness onsets,while in severe patients continued to rise from illness onsets to 25 day. On contrary, another study observed in the 1st week after symptoms onset, mild patients showed slightly elevated in D-dimer levels, D-dimer were at the peak (bottom) in the 2nd week, then decreased at a significantly low during the 4th week to the 5th week after symptoms onset [28]. In moderate patients,our results showed,that the highest levels of D-dimer was noticed in the 3rd week after symptoms onsets,on contrast, a study conducted by Lin et al. [28], showed in the 2nd week after symptoms onsets the hematological and inflammatory parameters were elevated, indicating that a high immune response might exist during this time. D-dimer assays are commonly used in clinical practice to exclude a diagnosis of deep vein thrombosis or pulmonary embolism, and elevated D-dimer indicates increased risk of abnormal blood clotting. Elevated levels of D-dimer were also found to be related with higher mortality rate of community-acquired pneumonia [29]. Patients with severe community-acquired pneumonia had significantly higher D-dimer levels, and D-dimer within normal range indicated low risk for complications. The level of coagulation function parameters, including prothrombin time, fibrinogen, fibrinogen degradation products, and D-dimer, were found elevated in patients with severe COVID-19. Presumably, the severity of COVID-19 might also be associated with coagulation dysfunction [30].

LDH levels in mild patients agreement in one aspect and did not agree in another with a study done by Zhou et al. [21],They found lactate dehydrogenase increased, peaked on illness days 6-8 and 8-11 and gradually declined. This may be related to low sample size in the present study and family history of disease was defined as the presence of diabetes in patients was correlated with elevated LDH, the incidence of LDH was associated with presence of diabetes, this phenomenon might be due to reduced glycogen synthesis, change in glucose oxidative metabolism and elevated whole-body rate of non-oxidative glycolysis. These mechanisms cause elevated lactate in patients with diabetes compared with those without Wu et al. [31]. Our results of LDH in moderate which agreement with Lin et al. [28]. Whereas, Yuan et al. [24], found that LDH decreased in hospitalization patients after day 12. Levels of LDH were elevated without significance differences among four weeks in severe patients,its agreement with Zhou et al. [27], they observed that LDH continued to rise during all four weeks. Another study noticed serum LDH concentration elevated during the 1st week until day 12, which appeared more stable [24].

LDH elevation in patients with COVID-19 indicates lung and tissue injuries. Another possible cause of liver impairment in patients infected with SARS-CoV-2 is a systemic inflammatory response [32]. A “cytokine storm” is triggered by an uncontrolled overproduction of inflammatory cytokines in this scenario, resulting in acute lung damage and acute respiratory distress syndrome [33].

Mild patients showed that lymphocytes levels gradually increased over time and had a high level in the last week, this results in agreement with the previous study [28]. Neutrophilia in COVID-19 patients also observed, neutrophils gradually increased in the 2nd and 3rd weeks, then decreased significantly in the 4th week in survivors. another study mentioned, white blood cell counts and neutrophil counts were in normal range during week1, with neutrophilia as later findings was common in survivors and non survivors throughout the disease’s course [22]. In moderate, the neutrophils also elevated in the 2nd week then slightly decreased, while no change in the level of lymphocytes among four weeks. Previous study noticed, no significant difference on numbers of lymphocyte, when the increased of neutrophil cell numbers were significantly high on day 9 [24].

In the present study, we showed a gradual increase in neutrophils in severe patients with time during the first three weeks and then decreased the last week, whereas Lymphocytes were observed through the study to continue to decline overtime in severely symptomatic patients. Other study noticed Lymphocyte levels and blood lymphocyte percentages in severe and critical COVID-19 were consistently lower from 1 to 30 days post-illness onset, by contrast, the neutrophil levels and blood neutrophil percentages appeared normal at the early stage of severe and critical COVID-19, after that, gradually increased between 10- and 28-day post illness onsets. These results indicated that neutrophil activation slightly lag behind acute lymphocyte losses. After about 1-month

post-illness onset, when severe and critical patients were in convalescence, the blood counts of lymphocytes and neutrophils gradually regressed to normal ranges. Our results in agreement with previous study, WBCs showed a gradual increase with time, the same applies to neutrophils, the rate was gradually high. Patients in this category had lymphopenia as lymphocytes continued to decline gradually).

It is uncommon for white blood cells to rise in viral infections. Neutropenia is observed in patients infected with Herpes viruses (EBV, CMV, HHV6, and HSV), tract respiratory viruses (RSV, influenza A and B, and parainfluenza), and hepatitis viruses, as well as other viral exanthematous diseases, including chickenpox, Measles (rubeola), and German measles (Rubella). Neutropenia often happens during the first few days of the viral disease and lasts for 3–8 days. Munshi et al. [34] illustrated that viral infections are common causes of neutropenia, due to either bone marrow suppression or peripheral destruction. But through this study, a significant increase in the number of neutrophil cells or neutrophilia was seen. A previous study highlighted that increased neutrophil counts in the blood of severely affected people were discovered to be a prominent clinical feature of SARS COV 2 disease.

Our results of mild patients which in agreement with previous studies [28, 35], one milestone of the host immune response was that the SARS-Cov-2 specific IgG antibody was produced stably in the majority of COVID-19 patients since 14 days after symptoms onset and serum IgM was detected around week 1 after illness onset, peaked in weeks 2 and 3 and then gradually decreased from week 4 to 6. The details such as the peak timing and the duration of the antibodies differed from those in SARS and MERS Lee et al. [36], found that serum SARS-CoV IgG was first detected on day 4 after illness onset, seroconversion occurred at a median of 16 days, and IgG peaked in week 4. In moderate IgG titers changed during disease progression and slightly increased in the 3rd week to 4th week in our results, a similar pattern of IgM dynamics was observed in all moderately ill Jing et al. [37], reported that Anti-SARS-CoV-2 IgG titers were increased in the first month after symptoms onset in moderately ill patients followed by decline in the 7th week, but such difference was not observed for IgM.

Jing et al. [37], showed a steady increase in anti-SARS-CoV-2 IgG levels in the first month after symptom onset, and severe patients preserved relatively high levels of IgG even at 10th week. In contrast, IgM peaked at nearly 5 weeks after symptom onset and almost disappeared by week 10 another study observed IgM antibodies, accounting for approximately 10% of human immunoglobulins, are often produced during the early phase of acute infection, IgG antibodies, accounting for approximately 75% of human immunoglobulins, provide long-term immunity after viral infection [38]. In an Italian study of asymptomatic to critical patients, IgM seroconversion disappeared at 4 months and 47% of IgG seroconversion was observed at 10 months after symptom onset. Importantly, the timing of IgM and IgG antibody occurrence in patients varies greatly, and this variation in timing may be associated with age as well as comorbidity.

Many studies revealed detectable levels of IgM and IgG antibodies could provide information regarding serological convention over the disease course, as the detection of IgM antibody indicates a recent exposure to SARS-CoV-2 and the detection of IgG antibody in the absence of detectable IgM antibody indicates prior virus exposure. The positive rates of IgM and/or IgG detection were not significantly different between the mild, severe and critical groups. However, quantitative analyses of antibody levels over the disease course revealed that SARS-CoV-2-specific IgM levels were higher and neutralizing IgG levels were lower in patients in the critical group, as compared with the other groups, which might be because of high disease activity and/or a compromised immune response in these patients. In contrast, in the mild group patients, IgG was maintained at a high level, whereas IgM levels gradually decreased when most of the patients were in the recovery state of infection. Furthermore, the level of IgM antibody was higher in the group of deceased patients than that in the group of recovered patients, whereas the IgG level was not significantly different between these groups. The IgM level showed heterogeneity within the group of deceased patients, and some patients had very high IgM levels which might be in the active status of disease or very low IgM levels due to the long disease course. The elevated IgM level in the deceased patients group might be related to the higher disease severity in these patients and indicate a poor prognosis. Alternately, cytokine storm, severe immune dysfunction and other comorbidities might be the important risk factors in these cases [39-41].

5. Conclusion

1. Considering the laboratory results, D-dimer, LDH, CRP, neutrophils, lymphocytes, and respiratory rate were potential factors that could be used to predict the severity of COVID-19.

2. IgG and IgM levels differ significantly among COVID-19 patients with different illness severities and outcomes. Active immune responses drive production of high IgG titers in the late stage of disease in mild patients.

3. Quantitative levels of IgM and IgG levels in covid-19 patients are helpful for the diagnosis, severity classification, and management of COVID-19 patients, and these levels should be monitored in each stage of this disease.

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