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Article

Association between Liver Function Indicators, Immunoglobulins, and Toll-like Receptors and Neuropilin-1 Expression in Patients with COVID-19

Salma A. El Kazafy[1,*], Amr E. Ahmed[1], Yasser M. Fouad[2], Azza F. Said[3], Tarek M. Ali[4] Ahmad El Askary[5], Hebatallah H. Assal[6] and Osama M. Ahmed[7]

[1] Biotechnology Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt; amreahmed@psas.bsu.edu.eg
[2] Internal Medicine, Faculty of Medicine, Minia University, Minia Egypt; Yasserfouad10@yahoo.com
[3] Pulmonary Medicine, Faculty of Medicine, Minia University, Minia Egypt; azza20022@yahoo.com
[4] Department of Physiology, College of Medicine, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; tarek70ali@gmail.com
[5] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; ahmedelaskary3@gmail.com
[6] Department of Chest Medicine, Faculty of Medicine, Cairo University, Cairo 11562, Egypt; hebatallah.assal@kasralainy.edu.eg
[7] Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, P.O. Box 62521, Beni-Suef, Egypt; osamamoha@yahoo.com; osama.ahmed@science.bsu.edu.eg
*Corresponding author: salma.kazafy2010@gmail.com

Abstract: Purpose: The study aimed to investigate if there were any links between liver function biomarkers and immunoglobulins levels in serum, and Toll-like receptors (TLRs) and neuropilin-1 (NRP1) in COVID-19 patients. The study also aimed to assess the accuracy—sensitivity, specificity, and area under the curve (AUC) by the receiver operator curve (ROC) analysis for immunoglobulins levels and TLRs expressions. **Patients and Methods:** This study included 150 patients (100 patients with confirmed COVID-19 and 50 healthy volunteers as a control group). Patients with COVID-19 were subdivided into two groups according to the severity of symptoms (moderate and severe, with 50 patients each). Serum C-reactive protein (CRP), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), albumin, lactate dehydrogenase (LDH), immunoglobulin (Ig) G, and IgM levels were estimated. TLRs (TLR2 and TLR4) and NRP1 gene expression in blood samples were investigated using quantitative real-time polymerase chain reaction (qRT-PCR). ROC analysis was also applied to determine the accuracy of various detected parameters in predicting the possibility of COVID-19 infection. **Results:** In COVID-19 patients, serum parameters related to liver function, except serum albumin, CRP, IgG, IgM, and TLR2, TLR4, and NRP1 mRNA expression levels, significantly elevated compared to controls. Severe COVID-19 patients exhibited significantly higher liver enzymes (ALT, AST and LDH), CRP, and TLR2 mRNA expression levels and lower albumin levels than the moderate group. In the moderate and severe groups, ALT, CRP, TLR2, and TLR4 had a significant positive correlation with IgM levels. ALT, AST, LDH, CRP, TLR2, and TLR4 showed a significant positive correlation with IgG levels in both groups. In both the moderate and severe groups, NRP1 expression was found to be significantly correlated with CRP, IgG, IgM, TLR2, and TLR4. In contrast, serum albumin levels exhibited a significant negative correlation with IgG and IgM levels only in the severe group, but they showed a significant negative correlation with TLR2, TLR4, and NRP1 expression in both moderate and severe groups. Serum ALT and AST activities were positively correlated with NRP1 expression in the moderate group but not in the severe group and as well as TLR2 and TLR4 expression in both the moderate and severe groups. ROC analysis indicated that AUC was higher than 0.800 for serum IgM level and TLR4 gene expression in moderate COVID-19 group. **Conclusions:** The increased liver function biomarkers in serum and NRP1 expression are closely correlated with sustained activations in humoral and innate immune responses during COVID-19 infection. As a result, TLR2, TLR4, and NRP1 could be targets for limiting COVID-19 infection and impairment effects on liver function. Moreover, detection of IgM level in

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serum and TLR4 expression in blood have a good accuracy in predicting the possibility of infection with COVID-19 in moderate cases.

Keywords: liver; Neuropilin-1; Toll-like receptors; COVID-19

1. Introduction

SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) is highly isomorphic to another human coronavirus, SARS-CoV-1 [1], which is transmitted by droplets. Dry cough, fever, bodily ache, weakness, and diarrhea are all symptoms of COVID-19 (Coronavirus Disease 2019). Patients with a severe condition have a high fever, breathlessness, and oxygen deprivation, and the majority of critically ill patients die from respiratory and multiorgan failure as a result of cytokine storms [2,3]. According to the World Health Organization(WHO), there were 156,496,592 confirmed cases of COVID-19 worldwide as of March 8, 2021, with 3,264,143 deaths [4].

A cohort research in China [5] included 1099 COVID-19 patients, 21 (2.1%) of whom had hepatitis B and 22.2 and 21.3 % of whom had higher aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) levels, respectively. In hospitalized COVID-19 patients, the incidence of abnormal liver function ranged from 10.5 to 69 % [6]. Increased AST and ALT values are the most prevalent causes of abnormal liver function tests, according to most research, with AST being more common than ALT.

An IgM test can indicate whether a patient has been infected lately, while an IgG test can identify whether a patient has been infected before [7,8]. Using serum IgG antibody testing, a comprehensive assessment of COVID-19 epidemiology and a retrospective estimation of population prevalence in target populations can be performed [9]. The majority of recent studies reported serological diagnostics focused on detecting SARS-CoV-2-specific IgM and IgG [10,11].

Toll-like receptors (TLRs) are innate immune receptors involved in innate immunity activation, cytokine regulation, adaptive immune system indirect activation, and pathogen-associated molecular pattern recognition [12-14]. Toll-like receptor (TLR) pathways, which are part of innate immunity, could have a role in the pathogenesis of SARS-CoV-2, as previous research has shown that TLRs are important in the pathogenesis of SARS-CoV and the Middle East respiratory illness (MERS) [13]. TLRs are located on immune cells such dendritic cells (DCs), macrophages, natural killer cells, and T and B cells, among others [15]. TLRs may play a critical role in COVID-19, according to research on other coronaviruses (CoVs).

Neuropilin-1 (NRP1) is a pleiotropic transmembrane polypeptide that serves as a growth factor coreceptor [16]. NRP1 has been recognized as a SARS-CoV-2 host receptor that enhances cellular entry and infectivity [16, 17]. SARS-CoV-2 has a polybasic cleavage site (RRAR) between the S1 and S2 spike protein subunits. This location allows furin and other proteases to cleave the virus, affects viral infectivity in cells, and increases the pathogenicity of SARS-CoV-2. After RRAR cleavage by furin, NRP1 on the cell surface is activated [17]. Within the S1/S2 junction cleavage site, the SARS-CoV-2 spike protein has a furin cleavage motif (RXXROH) [18]. Regrettably, the specific etiology of COVID-19 remains unknown. As a result, the majority of information about COVID-19 pathogenesis comes from MERS-CoV and SARS-CoV data [15].

Therefore, this study aims to determine whether there were links between liver function biomarkers, IgG and IgM in serum and TLRs and NRP1 expression in COVID-19 patients.

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2. Patients and Methods

2.1. Patients and exclusion criteria

This study includes a group of 100 COVID-19 patients with a mean age of 61.05 years. From March to July 2021, all participants were patients at the Misr International Hospital in Cairo, Egypt. The research followed the Declaration of Helsinki and best practices guidelines. A formal consent form was signed by all participants. The study was approved by the ethics committee of Misr International Hospital, Cairo, Egypt, and the institutional review board of the Ministry of Health, Cairo, Egypt (No. 3-2021/19).

Fifty healthy controls were included in the study. Thyroid dysfunction, autoimmune disorders, eczema, chronic respiratory disorders, malignancy, kidney failure, liver hepatitis, liver cirrhosis, cerebrovascular diseases, ischemic heart disease, pregnancy and lactation, and patients taking immunomodulatory drugs were among the key exclusion criteria for all enrolled patients (healthy and COVID-19 patients).

2.2. Laboratory assay

Participants' blood samples were obtained in plain tubes (4 mL each). After a 30-minute incubation period at room temperature, blood in plain tubes was centrifuged for serum isolation. Rapidly serum samples were separated, aliquoted, and frozen at -40°C until biochemical tests [ALT, AST, albumin, and lactate dehydrogenase (LDH)] could be performed. Gella et al. [19] used reagent kits obtained from Biosystem S.A. (Spain) to evaluate ALT and AST activity. Using a reagent kit acquired from Human Diagnostics (Germany), the concentration of serum albumin was measured according to Doumas et al. [20]. Using reagent kits bought from Stanbio Laboratories (Boerne, TX, USA), according to Buhl and Jackson [21], serum LDH activity was measured.

2.3. RNA isolation and quantitative real-time polymerase chain reaction (RT-PCR)

Individual blood samples from patients in each group were used to extract total RNA using the TRIzol reagent (MBI Fermentas, Germany). The High-Capacity cDNA Reverse Transcription Kit (Invitrogen, Germany) was used to make cDNA according to the manufacturer's instructions. In a 20 μL system, RT-PCR was carried out using 10 μL of 1x SsoFast EvaGreen Supermix (Bio-Rad, Hercules, CA, USA), 2 μL cDNA, 6 μL RNase/DNase-free water, and 500 nM of the primer pair sequences [TLR2: Forward, 5′ATCCTCCAATCAGGCTTCTCT-3′ and Reverse, 5′ACACCTCTGTAGGTCACTGTTG3′ (NM_001318789.2); TLR4: Forward, 5′ATATTGACAGGAAACCCCATCCA-3′ and Reverse, 5′AGAGAGATTGAGTAGGGGCATTT3′ (NM_138554.5); NRP1: Forward, 5′AACAACGGCTCGGACTGGAAGA3′ and Reverse, 5′GGTAGATCCTGATGAATCGCGTG3′ (NM001024628); and β-actin: Forward, 5′GGAACGGTGAAGGTGACAGCAG3′ and Reverse, 5′TGTGGACTTGGGAGAGGACTGG3′ (XM004268956.3)]. Then, 30 seconds at 95 °C, followed by 40 cycles of 5 seconds at 95 °C and 10 seconds at 60 °C were used in the thermal cycler. With a melting curve analysis, each reaction was carried out at 65°C–95°C. The relative amount of mRNA was measured for each process when the fluorescent signal exceeded an arbitrarily chosen threshold close to the middle of the log-linear amplification step. The manufacturer's program was used to examine the amplification data, which was then normalized to β-actin using Livak and Schmittgen techniques [22].

2.5. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 20 for Windows was used to analyze the data (2011; IBM Corp., Armonk, NY, USA). The data is presented as a mean with standard error of mean (SEM). Duncan's procedure for post hoc analysis was applied to compare groups with each other. The Pearson correlation coefficient approach was used to estimate correlation between the various examined factors. A statistically significant P value of 0.05 was used. Using the area under the receiver operator characteristic curve, we calculated the diagnostic accuracy of cytokines and other biomarkers to identify

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COVID-19 patients (AUROC). We choose the AU-ROC as a general indicator because we know that a model is a perfect classifier when the AUC is 1.

3. Results

Figure 1 shows the baseline demographics of all patients. Age was nonsignificantly changed in the moderate and severe patient groups when compared to controls. The moderate group had similar percentages of male and female patients, whereas there were 66% male and 34% female patients in the severe group ($P = 0.263$).

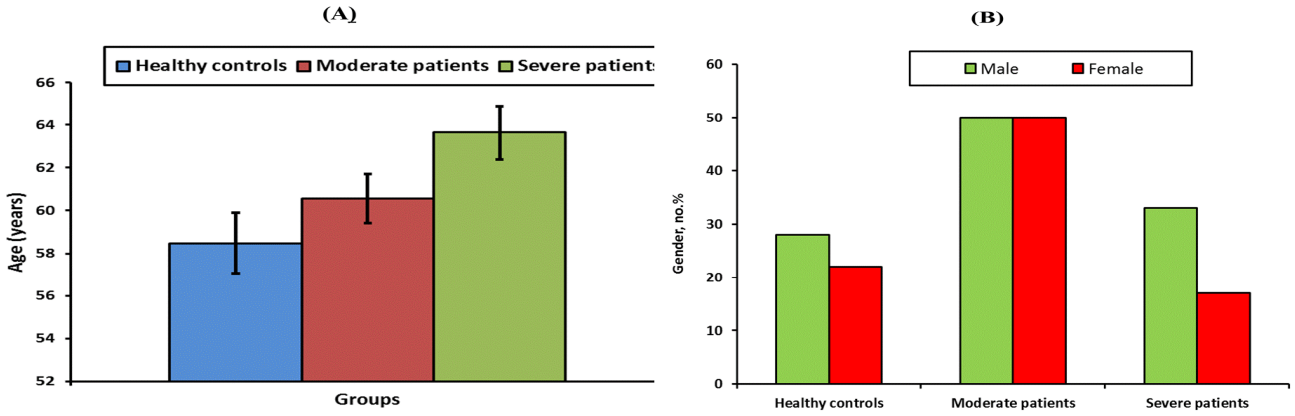

Figure 1. (A) Age (years) of healthy controls, moderate and severe groups. (B) Gender of healthy controls, moderate and severe groups (Results were presented as No. and % and compared using Chi-square test).

Serum ALT, AST and LDH activities were detectably higher in the moderate and severe groups than in the controls. While the effect on ALT and AST activities was significant in both moderate and severe COVID-19 patients, the effect on LDH activity was significant only in severe group only. Compared to healthy controls, the change in serum albumin level was significant ($P<0.001$) only in severe group **(Figure 2).**

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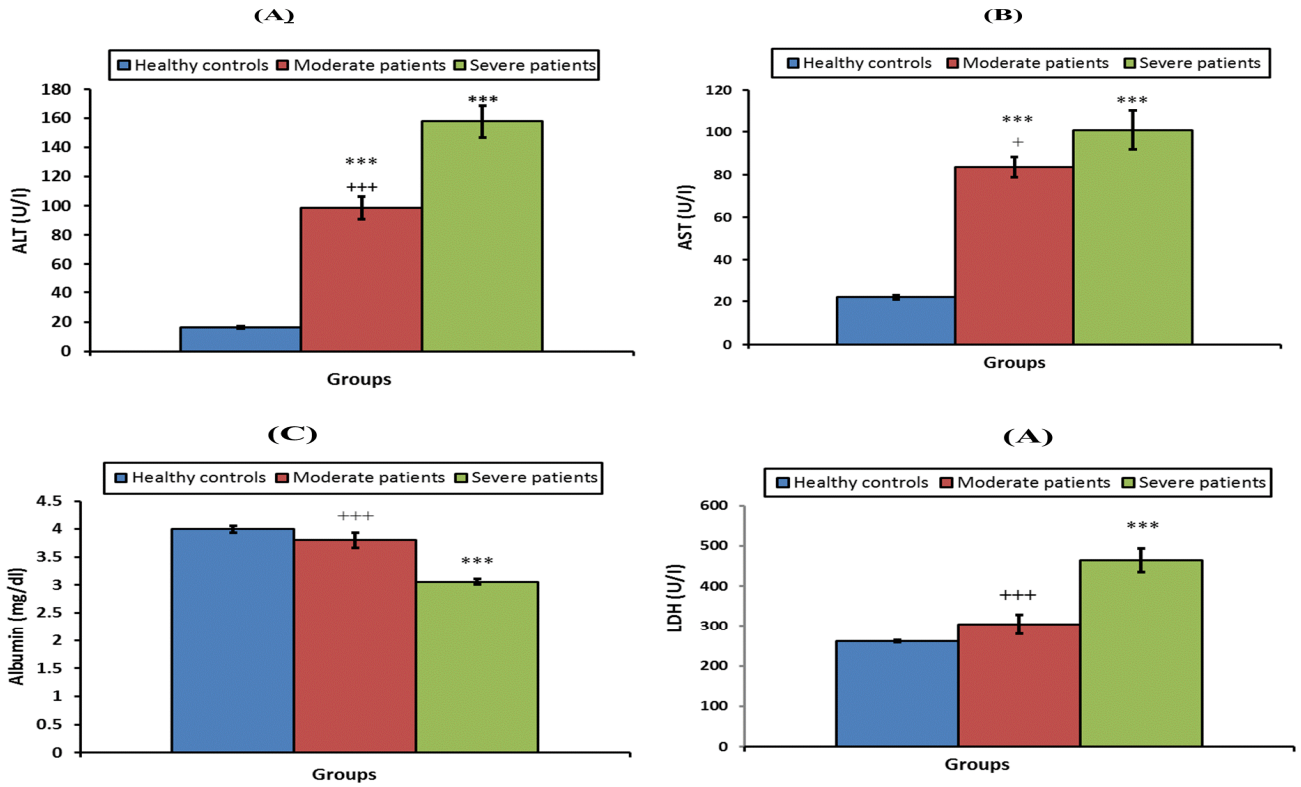

Figure 2. (A) ALT (U/I); (B) AST (U/I); (C) Albumin (mg/dl); (D) LDH(U/I) of healthy controls, moderate and severe groups. Data are expressed as mean ± standard error (SE). Values were considered significantly different at $^{***}P< 0.001$ versus healthy controls and $^{+}P< 0.05$ and $^{+++}P < 0.001$ versus severe group.

CRP, IgG and IgM serum levels and TLR2, TLR4 and NRP1 mRNA expression levels in the moderate and severe groups significantly elevated (P<0.001) compared to controls. Moreover, CRP levels in severe patients significantly increased (P<0.001) compared to the moderate group. Additionally, TLR2 mRNA expression level were significantly higher (P<0.01) in severe group of patients in comparison with moderate patients (**Table 1**).

Table 1. C-reactive protein, Immunoglobulins, and Toll-like receptors and NRP1 mRNA expression of all studied groups.

	Healthy controls (n=50)	Moderate patients (n=50)	Severe patients (n=50)
CRP (mg/dl)	2.01 ±0.19	51.70 ±6.00^{***+++}	77.84 ±2.99***
IgG (mg/dl)	238.99 ±3.12	717.42 ±19.02***	724.57 ±18.55***
IgM (mg/dl)	49.24 ±1.43	74.28 ±2.70***	78.67 ±1.62***
TLR2 relative expression	1.02 ±0.003	3.09 ±0.16^{***++}	3.69 ±0.22***
TLR4 relative expression	1.03 ±0.003	2.64 ±0.18***	3.04 ±0.20***
NRP1 relative expression	1.01 ±0.002	4.28 ±0.16***	3.98 ±0.19***

Data are expressed as mean ± standard error (SE). Values were considered significantly different at $^{***}P< 0.001$ versus healthy controls and $^{++}P< 0.01$ and $^{+++}P < 0.001$ versus severe group.

Table 2 demonstrates the correlation between IgG and IgM levels and TLR2, TLR4, and NRP1 mRNA expression with all other tested parameters in the moderate group. IgG and IgM levels had a significant positive correlation with age, ALT, AST, CRP, TLR2, TLR4 and NRP1. While IgG had significant (P<0.05) positive correlation with LDH activity

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in moderate group, IgM had not (P>0.05). Both IgG and IgM showed an insignificant negative correlation with albumin (P>0.05) in the moderate group. Also, TLR2 and TLR4 had a significant positive correlation with age, ALT, AST, CRP, and NRP1 expression, and a significant negative correlation with albumin in the moderate group. NRP1 expression showed a significant (P<0.001) positive correlation with age, ALT, AST, CRP, IgG, IgM, TLR2, and TLR4 while it non-significant (P>0.05) negative correlation with albumin level.

Table 2. Correlation between TLR2, TLR4, IgG, IgM and NRP1 with all parameters in moderate group.

	IgG		IgM		TLR2		TLR4		NRP1	
	r	p	r	p	r	p	r	p	r	p
Age	.725***	.000	.545***	.000	.667***	.000	.595***	.000	.728***	.000
ALT	.217*	.030	.293**	.003	.296**	.003	.288**	.004	.417***	.000
AST	.202*	.044	.264***	.008	.258**	.009	.375***	.000	.350***	.000
Alb.	-.146	.148	-.138	.170	-.241*	.016	-.269**	.007	-.161	.110
LDH	.200*	.046	.150	.136	.254**	.011	.266**	.007	.158	.116
CRP	.581***	.000	.508***	.000	.657***	.000	.656***	.000	.627***	.000
IgG	1		.634***	.000	.807***	.000	.607***	.000	.791***	.000
IgM	.634***	.000	1		.523***	.000	.358***	.000	.561***	.000
TLR2	.807***	.000	.523***	.000	1		.750***	.000	.774***	.000
TLR4	.607***	.000	.358***	.000	.750***	.000	1		.640***	.000
NRP1	.791***	.000	.561***	.000	.774***	.000	.640***	.000	1	

* Correlation is significant at the 0.05 level, ** at the 0.01 level, *** at the 0.001 level.

Table 3 demonstrates the correlation between IgG and IgM serum levels and TLR2, TLR4, and NRP1 mRNA expressions with all other tested parameters in the severe group. IgG and IgM levels had a significant (P<0.001) positive correlation with all other tested parameters, except for AST which showed an insignificant (P>0.05) association with IgM level, and albumin level which had negative correlation with both IgG and IgM levels. TLR2 and TLR4 expressions had a significant positive correlation with all other tested parameters at P<0.001, except for ALT, which showed an insignificant (P>0.05) with TLR2, and AST, which had a significant positive correlation with TLR2 at P<0.01. TLR2 and TLR4 expressions showed a significant (P<0.001) negative correlation with albumin. NRP1 showed a significant (P<0.001) positive correlation with all other tested parameters except for its correlation with ALT and AST activities which was insignificant (P>0.05). On the other hand, NRP1 showed a significant (P<0.001) negative correlation with albumin.

Table 3. Correlation between TLR2, TLR4, IgG, IgM, and NRP1 with all parameters in severe group.

	IgG		IgM		TLR2		TLR4		NRP1	
	r	p	r	p	r	p	r	p	r	p
Age	.744***	.000	.651***	.000	.592***	.000	.610***	.000	.667***	.000
ALT	.337***	.001	.282**	.004	.185	.065	.331***	.001	.103	.309
AST	.291**	.003	.174	.083	.266**	.008	.434***	.000	.092	.361
Alb.	-.749***	.000	-.606***	.000	-.615***	.000	-.586***	.000	-.715***	.000
LDH	.570***	.000	.421***	.000	.542***	.000	.521***	.000	.435***	.000
CRP	.858***	.000	.808***	.000	.706***	.000	.670***	.000	.745***	.000
IgG	1		.761***	.000	.722***	.000	.669***	.000	.774***	.000
IgM	.761***	.000	1		.571***	.000	.579***	.000	.673***	.000
TLR2	.722***	.000	.571***	.000	1		.668***	.000	.614***	.000
TLR4	.669***	.000	.579***	.000	.668***	.000	1		.615***	.000
NRP1	.774***	.000	.673***	.000	.614***	.000	.615***	.000	1	

* Correlation is significant at the 0.05 level, ** at the 0.01 level, *** at the 0.001 level.

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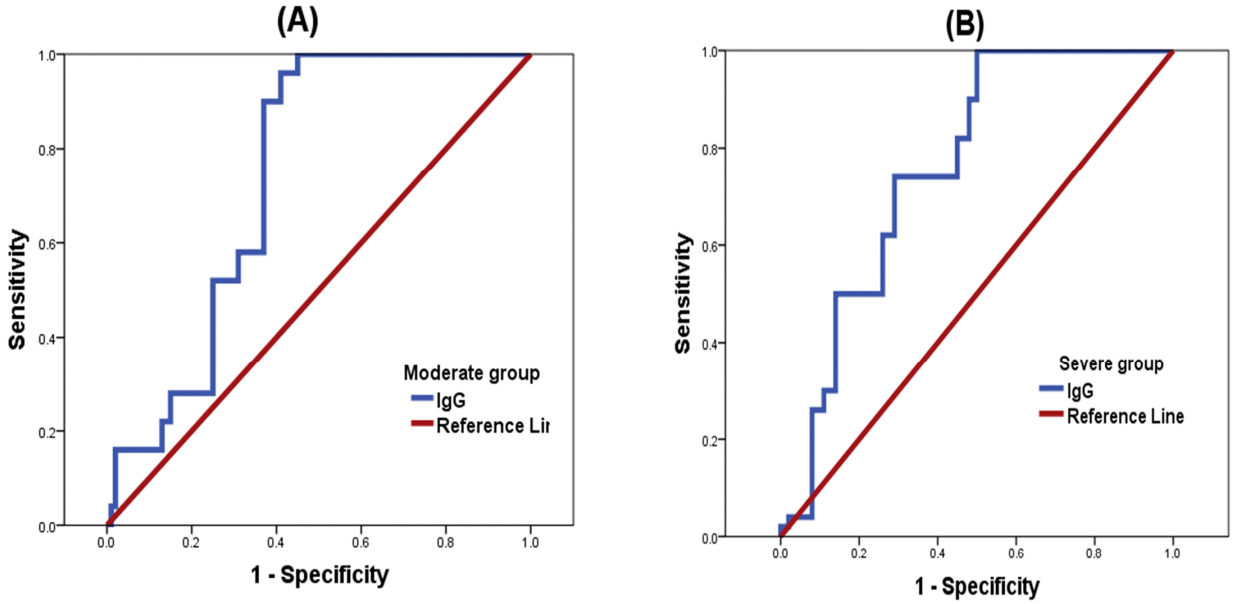
ROC analyses in moderate and severe COVID-19 patients was applied to determine the cut-off values of IgG (**Figure 3**). The AUC of IgG were 0.759 and 0.741 in moderate and severe, respectively with *P<0.001*. The cut-off values of IgG in moderate and severe, that's 511.35 and 712.0 pg/mL, respectively. When the sensitivity and specificity were calculated at these cut-off values, the sensitivity and specificity were 100% and 55%, respectively in moderate group; those of severe patients were 74% and 71%, respectively.



(C)

	AUC	CI 95%	*P*	Cut-off value	sensitivity	specificity
IgG (in moderate)	0.759	0.684 - 0.834	< 0.001	511.35 pg/ml	100%	55%
IgG (in severe)	0.741	0.663 - 0.818	< 0.001	712.0 pg/ml	74%	71%

Figure 3. IgG . (**A**) Area under the ROC curve (AUC) analysis for evaluating the accuracy of IgG in moderate group. (**B**) Area under the ROC curve (AUC) analysis for evaluating the accuracy of IgG in severe group. (**C**) Sensitivity and specificity calculation for TLR2 cut-off point.

Similarly, the ROC analyses in moderate and severe COVID-19 patients were calculated to determine the cut-off values of IgM (**Figure 4**). The AUC of IgM were 0.807 and 0.614 with *P<0.001* and *P<0.05* in moderate and severe, respectively. The cut-off values of IgM in moderate and severe, that's 57.25 and 63.7 pg/mL, respectively. When the sensitivity and specificity at these cut-off values were calculated, the sensitivity and specificity were 76% and 40%, respectively in moderate patients; those in severe patients were 100% and 73%, respectively.

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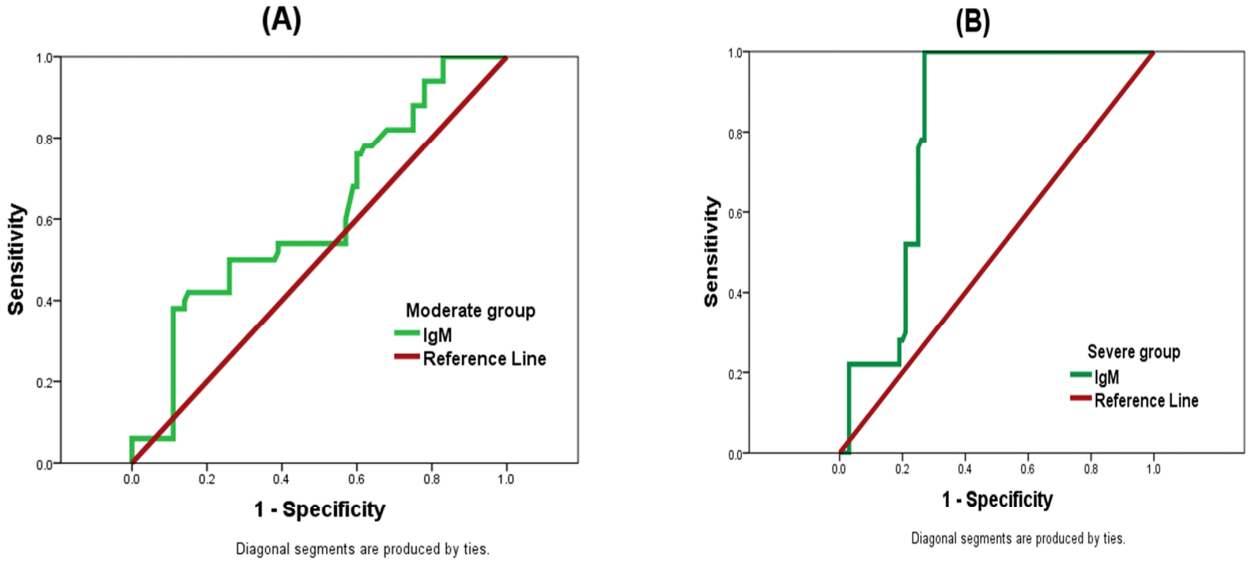

(C)

	AUC	CI 95%	*P*	Cut-off value	sensitivity	specificity
IgM (in moderate)	0.807	0.737 - 0.877	<0.001	57.25 pg/ml	76%	40%
IgM (in severe)	0.614	0.518 - 0.710	< 0.05	63.7 pg/ml	100%	73%

Figure 4. IgM. (**A**) Area under the ROC curve (AUC) analysis for evaluating the accuracy of IgM in moderate group. (**B**) Area under the ROC curve (AUC) analysis for evaluating the accuracy of IgM in severe group. (**C**) Sensitivity and specificity calculation for TLR2 cut-off point.

In the same line, the ROC analyses in moderate and severe COVID-19 patients, was performed to determine the cut-off values of TLR2 (**Figure 5**). The AUC of TLR2 were 0.798 and 0.702 in moderate and severe, respectively, with p<0.001. The cut-off values of TLR2 in moderate and severe, that's 1.55 and 2.65 pg/mL, respectively. When the sensitivity and specificity at these cut-off values were calculated, the sensitivity and specificity of moderate were 100% and 50%, respectively. Those of severe were 78% and 68%, respectively.

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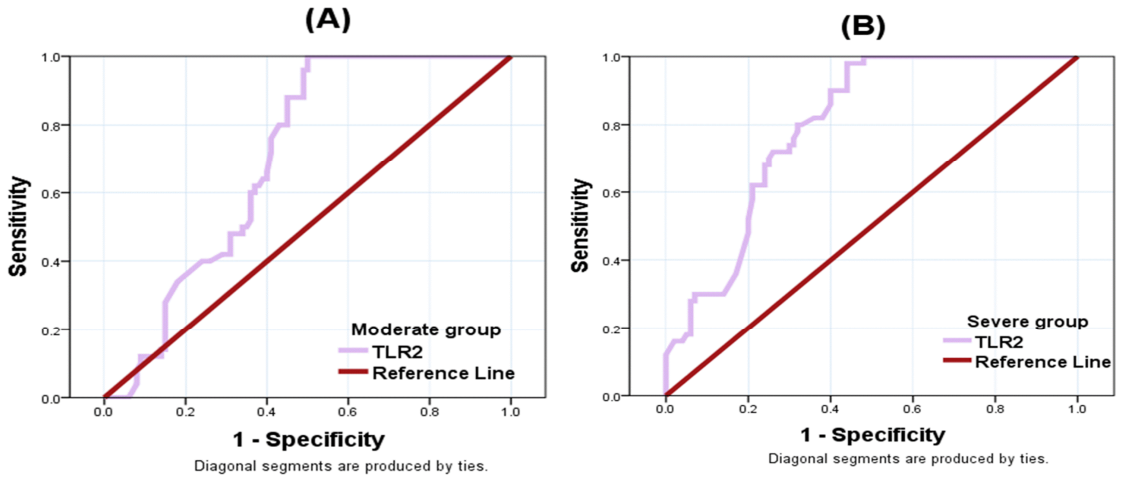

(C)

	AUC	CI 95%	*P*	Cut-off value	Sensitivity	Specificity
TLR2 (in moderate)	**0.798**	**0.730 - 0.867**	**<0.001**	**1.55 (relative to control)**	**100%**	**50%**
TLR2 (in severe)	**0.702**	**0.621 - 0.782**	**<0.001**	**2.65 (relative to control)**	**78%**	**68%**

Figure 5. TLR2 . (**A**) Area under the ROC curve (AUC) analysis for evaluating the accuracy of TLR2 in moderate group. (**B**) Area under the ROC curve (AUC) analysis for evaluating the accuracy of TLR2 in severe group. (**C**) Sensitivity and specificity calculation for TLR2 cut-off point.

Similar to TLR2, the ROC analyses in moderate and severe COVID-19 patients was also applied to determine the cut-off values of TLR4 (**Figure 6**). The AUC of TLR4 were 0.802 and 0.698 with *P<0.001* in moderate and severe, respectively. The cut-off values of TLR4 in moderate and severe, that's 1.145 and 2.06 pg/mL, respectively. When the sensitivity and specificity at these cut-off values were calculated, the sensitivity and specificity of moderate were 100% and 50% respectively. Those of severe were 76% and 76%, respectively.

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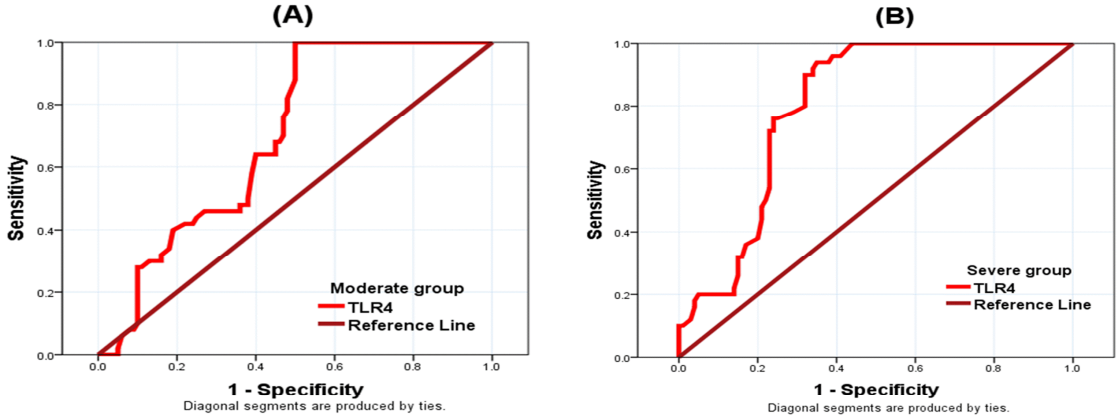

(C)

	AUC	CI 95%	*P*	Cut-off value	sensitivity	specificity
TLR4 (in moderate)	**0.802**	**0.733-0.870**	**<0.001**	**1.15** (relative to control)	**100%**	**50%**
TLR4 (in severe)	**0.698**	**0.616-0.780**	**<0.001**	**2.06** (relative to control)	**%76**	**76%**

Figure 6. TLR4 . (**A**) Area under the ROC curve (AUC) analysis for evaluating the accuracy of TLR4 in moderate group. (**B**) Area under the ROC curve (AUC) analysis for evaluating the accuracy of TLR4 in severe group. (**C**) Sensitivity and specificity calculation for TLR4 cut-off point.

4. Discussion

According to a previous study on SARS-CoV and MERS-CoV, around 60% of patients exhibited hepatopathy, mainly shown as increased liver enzymes in blood [23]. This study aimed to focus on immunoglobulins (IgG and IgM), TLRs (TLR2 and TLR4), and NRP1 expression as possible related factors to liver injury in COVID-19 patients. In the current study, serum IgG and IgM levels were significantly increased in COVID-19 patients than in controls. Interestingly, as markers of virus-specific antibodies to SARS-CoV-2, IgG and IgM revealed a significant positive correlation with age, ALT, TLR2, TLR4, and NRP1 in both moderate and severe COVID-19 patients. According to Bogarapu and Aruna [24], most COVID-19 patients had elevated IgM and IgG levels. Furthermore, Ma et al. [25] discovered that serum IgM and IgG levels in moderate and severe patients are much greater, although no significant difference between severe and moderate patients, which is consistent with current data. On the other hand, Liu *et al.* [26] reported no significant antibody response in 7.1% of the 32 COVID-19 patients they studied. Furthermore, Marklund *et al*. [27] discovered that 9.4% of the 47 individuals studied did not have a predicted antibody response. These patients exhibited mild symptoms in both studies. This occurrence could be explained by the fact that these relatively mild infections were limited to mucosal cells of the respiratory system and now elicit a major secretory immune response in the form of IgA and little or no IgG.

In the current study, in all COVID-19 patients, there was a significant elevation in both TLR2 and TLR4 expression levels relative to controls. Furthermore, both had a positive correlation with ALT, AST, LDH, CRP, IgG, IgM, and NRP1, and a negative correlation with albumin. In viral infections, TLRs have a dual role. They cause the Janus kinase transducers (JAK/STAT) to become activated, resulting in macrophage activation syndrome [28, 29]. TLRs also stimulate the expression of the major histocompatibility complex on DCs, which helps to activate the adaptive immune system [30]. In COVID19 patients, oxidized phospholipids, which are generated by neutrophil myeloperoxidase, are higher, leading to TLR4 pathway activation in the pulmonary phase of the infection, which causes oxidative stress. As a result, TLR4 could be involved in the etiology of COVID19 [31]. Several investigations have shown that the SARSCoV2 spike protein links to TLR1, TLR4, and

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TLR6, with TLR4 having the highest affinity. As a result, TLR4 antagonists may be used to treat COVID19 patients in the future [32]. Surprisingly, TLR2 expression in the current data increased in severe patients compared to moderate patients.

COVID-19 patients have abnormal liver function. SARS-COV-2 has been linked to liver dysfunction or injury in a prior investigation [33]. In the present study, all patients with COVID-19 showed significantly increased ALT, AST, and LDH levels. Fang et al. [34] showed that 39.1% of 305 Wuhan patients with COVID-19 had increased ALT and AST, which is compatible with the current findings. Patients with abnormal liver function also demonstrated increased LDH activity than those with normal liver function [35]. According to Cai et al. [36], liver injury occurs in 14.8% of SARS-CoV-2 infected patients overall and 36.2% of severe patients. In most individuals with COVID-19, elevated liver function tests are usually transitory and are usually linked with higher muscular and cardiac enzymes; without hepatic morbidity or death these liver enzyme abnormalities may begin to resolve [37]. Stress-induced liver injury in COVID-19 patients induced by sympathetic nervous and adrenocortical system stimulation, hypoxia–reoxygenation, overstimulation of Kupffer cells, intestinal endotoxemia, and oxidative stress, on the other hand, has been linked to hepatic morbidity [37]. In agreement with Hao et al. [38], who identified twenty-five fatal cases in patients infected with COVID-19, and practically all patients had decreased albumin (28.6–36.0 μmol/L), all patients in this study had lower albumin. Remarkably, in agreement with the current results, Jiao *et al.* [39] established a prediction model showing that in elderly patients, low albumin, high CRP, serum LDH, and direct bilirubin are linked with severe COVID-19 infection. In addition, Huang et al. [40] observed that hypoalbuminemia can predict COVID-19 outcome without regard to age or comorbidities. Huang *et al.* [40] showed that hypoalbuminemia is a self-determining risk factor for death. This study showed that liver function biomarkers were significantly higher in severe patients than in non-severe patients. This was in line with the findings of Henry [41], Sultan et al. [42, 43], who found that biomarkers of liver and renal function, inflammation, cardiac injury, and coagulation profile were higher in severe and critical patients compared to non-severe patients. According to Wu et al. [44], liver injury can be caused by viral infection of bile duct cells or antiviral medication-induced functional impairment. It is necessary to determine the causes of liver injury as well as the pathophysiological shifts induced by COVID-19. This study showed that significantly increased NRP1 mRNA expression levels positively correlate with ALT, CRP, IgG, IgM, TRL2, and TRL4. NRP1 is a newly identified SARS-CoV-2 virus coreceptor that is overexpressed in the injured and diseased liver. Increased NRP1 expression in the pathologic liver of COVID-19 patients could be an amplifying route that worsens the prognosis and severity of the disease. NRP1 expression in the liver and SARS-CoV-2 infection have been associated [45]. NRP1 RNA expression was shown to be higher in the lungs of severe patients, according to Mayi et al. [46]. The fact that the NRP1 protein is upregulated in diabetic kidney cells suggests that it is important as a risk factor for severe COVID-19.

In this study, the ROC analyses in moderate and severe COVID-19 patients were calculated to determine the accuracy of serum IgG and IgM levels and TLR2 and TLR4 expression in identifying COVID-19 infection and severity. The study revealed that the AUC for serum IgG and IgM levels and TLR2 and TLR4 were higher than 0.500 in severe and moderate COVID-19 patients reflecting these parameters may be useful to various degrees in predicting the infection. However, IgM serum level and TLR4 expression have AUC that was higher than 0.800 in moderate group. So, in moderate COVID-19 patients, IgM and TLR4 tests could be more effective than others in predicting the possibility of infection with COVID-19.

5. Conclusion

Higher liver enzymes and CRP and lower albumin levels in serum of patients with COVID-19 are parallel with the severity of infection. In non-severe patients, IgM, CRP, and ALT are associated with TLR2 and TLR4 expression. Additionally, NRP1 expression

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is associated with CRP, IgG, IgM, and TLR2 and TLR4 expression. Hypoalbuminemia in severe patients may be negatively linked to IgM levels. Increased ALT and CRP levels in these patients are positively associated with TLR2 and TLR4 expression levels. Increased levels of CRP, IgM, IgG, TLR2, and TLR4 are linked to NRP1 expression. TLR2, TLR4, and NRP1 could thus be possible targets for managing infection in the early and advanced stages of the disease, as well as vaccine development against COVID-19 and avoiding liver function deficits. The ROC analysis indicated that serum IgM and IgG levels and TLR2 and TLR4 expressions may be useful in expecting the possibility of infection with COVID-19, but IgM and TLR4 could be more effective because the AUC for each was higher than 0.800 in moderate COVID-19 patients.

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