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Prognostic impact of toll-like receptors gene polymorphism on outcome of COVID-19 pneumonia: A case-control study

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A B S T R A C T

Toll-like receptor 3 (TLR3) and TLR7 genes are involved in the host immune response against viral infections including SARS-COV-2. This study aimed to investigate the association between the TLR3(rs3775290) and TLR7 (rs179008) polymorphisms with the prognosis and susceptibility to COVID-19 pneumonia accompanying SARS-COV-2 infection. This case-control study included 236 individuals: 136 COVID-19 pneumonia patients and 100 age and sex-matched controls. Two polymorphisms (TLR3 rs3775290 and TLR7 rs179008) were genotyped by allelic discrimination through TaqMan real-time PCR. This study also investigated predictors of mortality in COVID-19 pneumonia through logistic regression. The mutant 'T/T' genotypes and the 'T' alleles of TLR3 (rs3775290) and TLR7(rs179008) polymorphisms were significantly associated with increased risk of COVID-19 pneumonia. This study did not report association between the mutant 'T/T' genotypes of TLR3(rs3775290) and TLR7(rs179008) and the disease outcome. In multivariate analysis, the independent predictors of mortality in COVID-19 pneumonia were male sex, $SPO_2 \leq 82\%$, $INR > 1$, $LDH \geq 1000$ U/l, and lymphocyte count $< 900/mm3$ ($P < 0.05$).

1. Introduction

In December 2019, a novel infectious disease, called COVID-19 pneumonia, emerged in Wuhan City, China, and is spreading globally. Unfortunately, the ultimate fate of the COVID-19 pneumonia outbreak is unknown, as the situation is rapidly evolving [1].

The innate immune system provides the first-line protection against invading organisms and it is potentially important in SARS-CoV-2 infection [2]. The innate immunity includes a family of receptor proteins, named pattern recognition receptors (PRRs); of which, toll-like receptors (TLRs) are important members. TLRs can identify pathogen-associated molecular patterns (PAMP) and respond by eliciting inflammatory responses to eliminate the invading organisms [3].

TLR signaling has a pivotal role in the regulation of cytokine expression; hence TLR signaling could be crucially implicated in cytokine storm of SARS-CoV-2 infection [4]. Furthermore, an interaction of SARS-CoV-2 spike glycoprotein with the cell surface TLRs was found

which could modify the existing knowledge of COVID-19 immunobiology [5].

TLR3 showed implication in the development of a protective response against coronaviruses [6]. TLR3 is a crucial component in the innate immune responses, causing the release of interferon-regulatory factor-3 and -7 and the production of pro-inflammatory cytokines (TNF-α, IL-1 beta, and IL-6), that are responsible for innate antiviral responses and COVID19 immunopathogenesis [7,8]. Moreover, there is a design of multi-epitope (B-cell and T-cell epitopes) peptide vaccines that can strongly bind to human TLR3 and can produce an adequate level of immune response to circumvent the SARS-CoV-2 infection [9].

Interestingly, other TLRs, such as TLR7, provide anti-viral immunity via the identification of viral single-stranded RNA (ss-RNA) including SARS-CoV-2[10]. Moreover, the activation of TLR7 results in the release of pro-inflammatory cytokines and chemokines, like IFN-alpha, IFN-beta and IFN-lambda, which have been shown to aid in viral clearance and reduced replication [7].

There is a growing recognition that genes, especially those regulating the host immune response might confer differential vulnerability and influence the outcomes of SARS-COV-2 infection [4,11,12]. It is important to identify contributing genes that can help the accurate prediction of clinical outcome and fatality from COVID-19 pneumonia and therefore allow for preventive strategies in patients with a higher risk of death.

TLR genes display genetic variations and allelic polymorphisms resulting in numerous immunopathological consequences in viral infections [13]. Thus, we hypothesized that the study of TLRs immuno-polymorphisms may provide important clues on the susceptibility and clinical outcomes of SARS-COV-2 infection. Our secondary endpoint will be to find other predictors of mortality among patients with COVID-19 pneumonia.

2. Subjects and methods

2.1. Study design

This is a case-control analysis performed in accordance with the Declaration of Helsinki. Written informed consents were obtained from all participants. The study protocol was approved by the institutional research board of the Faculty of Medicine, Mansoura University, Egypt (approval: R.20.06.900). This study was conducted between October 2020 and April 2021 in Medical Biochemistry Department, Clinical Pathology Department, Mansoura research Center for cord blood stem cells (MARC), and Mansoura University Hospitals, Faculty of Medicine, Mansoura University.

2.2. Patients

The present study included 236 individuals: 136 patients diagnosed with COVID-19 pneumonia and 100 age and sex-matched healthy controls. The patients with COVID-19 pneumonia were admitted to the quarantine department in Mansoura University Hospital with a positive result on real-time PCR for SARS-COV2 RNA and diagnosed with COVID-19 pneumonia by a respiratory physician according to the guidance of MOHP November 2020 (Ministry of Health and Population, Egypt: Management protocol for COVID-19 patients, 2020)[14].

Patients with COVID-19 pneumonia included moderate cases [cases who show CT features of COVID-19 and peripheral capillary oxygen saturation (SPO$_2$) $\geq$ 92%]; and severe cases [cases who displayed any of the following: respiratory rate > 30breaths/min, SPO$_2$ < 92% at room air, PaO$_2$ (arterial oxygen partial pressure)/FiO$_2$ (fraction of inspired oxygen) < 300, chest radiology showing>50% lesion].

We excluded COVID-19 patients without signs of pneumonia, COVID-19 patients with missing data, patients aged $\leq$18 years and pregnant females with COVID-19.

For all patients, the following data were recorded upon hospital admission: age, sex, comorbidities (hypertension, diabetes, ischaemic heart disease, and chronic liver disease), CT chest, laboratory investigations and the clinical outcome (either required invasive mechanical ventilation or not; either survived or not).

3. Methods

3.1. Genetic studies

1 ml of the study participants' peripheral blood was collected into an EDTA-containing test tube. A QIAamp Blood Mini Kit was used to extract genomic DNA from whole blood *(Qiagen, Hilden, Germany)* according to the manufacturer's instructions and stored at −20 °C until genotyping assay.

Two SNPs (rs3775290 in TLR3 and rs179008 in TLR7) were successfully genotyped using allelic discrimination TaqMan real-time PCR. The SNP probes and primers were designed and synthesized by Applied Biosystems *(Applied Biosystems, Foster City, USA)*. The allele-specific probes for wild-type and varian'T' alleles were labeled with reporter fluorescent dyes FAM and VIC, respectively.

For rs3775290, probes sequence was AATGGAGAGGTCTAGAAAA-TATTTT[C/T]GAAATCTATCTTTCCTACAACAAGT. For rs179008, probes sequence was TTTCCCAATGTGGACACTGAAGAGAC[A/T] AATTCTTATCCTTTTTAACATAATC. The PCR mixture contained 20× SNP Genotyping Assay 1.25 μl, 2× genotyping Master Mix 12.5 μl, 10 ng template DNA and DNase-free water up to a total volume of 25 μl. Reaction conditions were 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The discrimination of genotypes was performed based on the relative fluorescence intensity of VIC and FAM using 7500 Real-Time PCR apparatus *(Applied Biosystems, Foster City, USA)*.

3.2. Statistical analysis

Statistical analysis was done using SPSS software (version 25.0) and SNPStats software. The categorical data were compared using the Chi-square test. Quantitative data were presented as median (25th – 75th percentiles) being non-normally distributed as examined by Shapiro-Wilk test with $P > 0.05$. Quantitative non-normally distributed data was compared by Mann–Whitney U test, and Kruskal-Wallis H-test between two, and three groups, respectively. Z-test used for column proportions (with adjusted P value by Bonferroni method) was presented by letters; similar letters = insignificant difference while different letters = significant difference. TLR3 and TLR7 SNPs were tested for Hardy–Weinberg equilibrium (HWE) then the genotypic and allelic disease association analyses were performed. Multiple inheritance models were tested to select the best inheritance model.

Univariate logistic regression was used to predict the likelihood of mortality in patients with COVID-19 pneumonia using only one predictor. The optimal cutoff value that discriminates survivors from non-survivors was estimated by receiver operator characteristics (ROC) curves. Then the multivariate logistic regression model was applied to the variables significant at the univariate analysis to create a prediction model. The data were presented as odds ratio (OR), P-value and 95% confidence interval (CI). For any used tests, results were considered statistically significant if P-value <0.050.

4. Results

4.1. Genotype and allele frequencies of TLR3 rs3775290 and TLR7 rs179008 polymorphisms in patients with COVID-19 pneumonia and controls

Table 1 showed that the mutant homozygous 'T/T' genotypes, as well as the 'T' alleles of TLR3 rs3775290 and TLR7 rs179008 were found to be more significantly associated with COVID-19 pneumonia. Patients with 'T/T' genotype of TLR3 had 3.6 times higher odds to exhibit COVID-19 pneumonia vs. those with the wild homozygous 'C/C' genotype, whereas, patients with 'T/T' genotype of TLR7 had 4.76 times higher odds to exhibit COVID-19 pneumonia vs. those with the wild homozygous 'A/A' genotype.

The exact test for Hardy-Weinberg equilibrium showed that TLR3 rs3775290 and TLR7 rs179008 of the 'control' group were in HWE ($P = 0.80$, and 0.17, respectively). When we study the association of different models of inheritance for TLR3 rs3775290 and the risk for COVID-19 pneumonia, the recessive model was the best inheritance in which participants with mutant 'T/T' genotype of TLR3 had 1.8 times higher odds (95% CI = 0.69–4.92) to exhibit COVID-19 pneumonia vs. the combined (C/C + C/T) genotypes. However, the significant association between TLR7 rs179008 and the COVID-19 pneumonia was observed using the log-additive mode ($P = 0.079$).

Male participants with 'T/T' genotype of TLR3 and 'A/A' genotype of TLR7 had 49.2- and 2.6-times higher odds than female participants to

Table 1

Genotype and allele frequencies of TLR3 rs3775290 and TLR7 rs179008 polymorphisms in patients with COVID-19 pneumonia and controls.

	Case (n = 136)	Control (n = 100)	P1	COR (95% CI)	P2
Genotype frequency					
TLR3					
'C/C'	60 (44%) a	54 (54%) a		R	
'C/T'	52 (38%) a	40 (40%) a		1.17 (0.67–2.03)	0.577
'T/T'	24 (18%) a	6 (6%) b	**0.025**	3.6 (1.37–9.47)	**0.009**
TLR7					
'A/A'	106 (78%) a	84 (84%) a		R	
'A/T'	18 (13%) a	14 (14%) a		1.02 (0.48–2.17)	0.961
'T/T'	12 (9%) a	2 (2%) b	0.090	4.76 (1.04–21.83)	**0.045**
Allele frequency					
TLR3					
'C' allele	172 (63%)	148 (74%)		R	**0.014**
'T' allele	100 (37%)	52 (26%)	**0.013**	1.66 (1.11–2.47)	
TLR7					
'A' allele	230 (85%)	182 (91%)		R	**0.040**
'T' allele	42 (15%)	18 (9%)	**0.038**	1.85 (1.03–3.32)	

P1 value by Chi-square test (data are presented as count and percentage). Z-test for column proportions (with adjusted P value by Bonferroni method) is presented by letters; similar letters = insignificant difference while different letters = significant difference. P2 value by simple logistic regression. Abbreviations: COR: crude odds ratio; CI: confidence interval; R: reference; TLR: toll-like receptor.

exhibit COVID-19 pneumonia, respectively. *P* values (test for interaction in the trend) were 0.037 for TLR3, and 0.0056 for TLR7 (Table 2).

4.2. Association between the different TLR3 rs3775290 and TLR7 rs179008 genotypes and patients characteristics in COVID-19 pneumonia

Table 3 showed a statistically significant association between the different TLR3 genotypes among patients of COVID-19 pneumonia and sex (*P* = 0.002), mechanical ventilation (*P* = 0.02), haemoglobin level (*P* = 0.001), platelet count (*P* = 0.02) and CRP level (*P* = 0.03). As regards TLR7 rs179008, there was a statistically significant association

Table 2

TLR3 rs3775290 and TLR7 rs179008 polymorphisms and sex cross-classification interaction (adjusted for Age).

Polymorphism	Genotype	Control	Case	OR (95% CI)
TLR3 rs3775290	'C/C'			
	Female	34	30	R
	Male	20	30	2.19 (0.96–4.98)
	'C/T'			
	Female	22	20	R
	Male	18	32	2.29 (0.93–5.67)
	'T/T'			
	Female	4	2	R
	Male	2	22	**49.15 (5–483.43)**
TLR7 rs179008	'A/A'			
	Female	44	36	R
	Male	40	70	**2.55 (1.35–4.83)**
	'A/T'			
	Female	14	14	R
	Male	0	4	–
	'T/T'			
	Female	2	2	R
	Male	0	10	–

Abbreviations: 95%CI: 95% confidence interval; OR: Odds ratio; R: reference; TLR: toll-like receptor.

between the three TLR7 genotypes among patients of COVID-19 pneumonia and sex (*P* = 0.001), presence of comorbidities (*P* = 0.002), INR (*P* = 0.02) and CRP level (P = 0.002).

4.3. Patients' clinic-demographic and laboratory characteristics

Of 136 patients with COVID-19 pneumonia, there were 80 survivors (58.8%) and 56 non-survivors (40.2%). Compared to survivors, non-survivors were older in age (*P* = 0.012) and had statistically significantly higher proportion of chronic liver disease (*P* = 0.03), higher respiratory rate (*P* < 0.001) and lower SPO$_2$ (*P* = 0.001). All 56 non-survivors required mechanical ventilation vs. 2 (2.5%) only in survivors (Table 4).

As regard laboratory findings, the non-survivors showed a statistically significantly higher proportion of 'C/T' genotype of TLR3, increased INR (*P* < 0.001) and LDH (*P* = 0.001) than did the survivors. There was a significant decrease in lymphocyte count (*P* = 0.017) and serum albumin level (P < 0.001) in non-survivors compared to survivors (Table 4).

4.4. Predictive factors of mortality in patients with COVID-19 pneumonia

The univariate and multivariate analysis for predictive indicators of mortality in patients with COVID-19 pneumonia were summarized in Table 5. In order to find a cutoff value for quantitative predictors that discriminate survivors from non-survivors, ROC curve analysis was conducted (Fig. 1).

In the univariate analysis, the following eleven risk factors, including older age $\geq$ 61 years, chronic liver disease, respiratory rate $\geq$ 26 breaths/min, SPO$_2$ $\leq$ 82%, severe COVID-19, C/T TLR3 genotype, serum albumin$\leq$3 g/dl, INR > 1, LDH $\geq$1000 U/l, lymphocyte count$\leq$900/ mm^3, were found to be associated with mortality in patients with COVID-19 pneumonia (*P* < 0.05).

Then, the multivariate stepwise logistic regression model was run including all the 11 predictor variables. The model was statistically significant [χ^2 (11) = 106.696, *P* < 0.001]. The model correctly classified 88.2% of participants with sensitivity, and specificity of 90%, and 86%, respectively. Of the 11 predictor variables, 5 were considered statistically significant independent predictors of mortality; male sex, SPO$_2$ $\leq$ 82%, high INR > 1, high LDH $\geq$ 1000 U/l, and low lymphocyte count <900/mm^3 (*P* < 0.05).

5. Discussion

Accumulating evidence supports the role of dysregulated TLR signaling in the pathogenesis of infectious diseases. TLR7 and TLR3 expression were involved in Respiratory syncytial virus-induced lung inflammation [15]. The expression of TLR3 was positively regulated by the influenza A virus [16], and rhinovirus infection [17]. Also, TLR7 gene variations were associated with respiratory diseases. For instance, TLR7 rs179008 polymorphism showed a strong association with the development of bronchial asthma [18].

TLRs have a dual role in in confronting COVID-19 infection. TLRs play an important role in recognition of viral particles and initiation of the innate immune system with secretion of pro-inflammatory cytokines, although it can also harm the host due to persistent inflammation and tissue destruction via activation of inflammasome and production of IL-1β, which induces IL-6 leading to hyperactivation of the immune system which can contribute to acute lung injury [19]. In addition, activation of Janus kinase transducers (JAK/STAT), which is induced by TLRs, could lead to macrophage activation syndrome. Besides this, TLRs also contribute to the activation of the adaptive immune system via the upregulation of major histocompatibility complex on dendritic cells [20].

Different TLRs, like TLR2, TLR3, TLR4, TLR6, TLR7, TLR8, and TLR9 are potentially important in eliminating SARS-COV-2 infection [8],

Table 3

Association between the different TLR3 rs3775290 and TLR7 rs179008 genotypes and patients characteristics in COVID-19 pneumonia.

Parameters	TLR3 (rs3775290) genotypes				TLR7 (rs179008) genotypes			
	C/C ($n = 60$)	C/T ($n = 52$)	T/T ($n = 24$)	P	A/A ($n = 106$)	A/T ($n = 18$)	T/T ($n = 12$)	P
Demographic characteristics								
Age (years)	60 (51–67)	61 (54–70)	60 (53.5–66.8)	**0.56	60 (52.8–67)	61 (54–67)	63 (59–71)	**0.35
Sex				*0.002				
Male	30 (50%) a	32 (61.5%) a	22(91.7%) b		70 (66%) a	4 (22.2%) b	10 (83.3%) a	*0.001
Female	30 (50%) a	20 (38.5%) a	2 (8.3%) b		36 (34%) a	14 (77.8%) b	2 (16.7%) a	
Outcome				*0.06				
Survivor	40 (66.7%)	24 (46.2%)	16 (66.7%)		64 (60.4%)	8 (44.4%)	8 (667%)	*0.36
Non-survivor	20 (33.3%)	28 (53.8%)	8 (33.3%)		42 (39.6%)	10 (55.6%)	4 (33.3%)	
COVID-19 severity				*0.84				
Moderate	16) 26.7%(	16 (30.8%)	6 (25%)		32 (30.2%)	4 (22.2%)	2 (16.7%)	*0.62
Severe	44 (73.3%)	36 (69.2%)	18 (75%)		74 (69.8%)	14 (77.8%)	10 (83.3%)	
Presence of comorbidities				*0.662				
No	14 (23.3%)	16 (30.8%)	6 (25%)		30 (28.3%) a	0 (0%) b	6 (50%) a	*0.002
Yes	46 (76.7%)	36 (69.2%)	18 (75%)		76 (71.7%) a	18 (100%) b	6 (50%) a	
Mechanical ventilation				*0.02				
Not required	40 (66.7%) a	22 (42.3%) b	16(66.7%)a,b		62 (58.5%)	8 (44.4%)	8 (66.7%)	*0.43
Required	20 (33.3%) a	30 (57.7%) b	8 (33.3%) a,b		44 (41.5%)	10 (55.6%)	"4 (33.3%)	
Laboratory characteristics								
Haemoglobin (g/dl)	12.5 (10.7–13.1)	11.5 (10–12.9)	13.5 (12.6–14)	0.001	12.7 (10.8–13.4)	11.5 (10–13.3)	12.3 (10.3–14)	0.53
WBC ($\times 10^3/mm^3$)	8.1 (6.3–12.7)	8.1 (6–12.2)	8.2 (7.1–13.9)	0.57	8.5 (6.6–12.4)	5.6 (5.3–14.5)	7.5 (5.4–14.1)	0.7
Lymphocyte count ($\times 10^3/mm^3$)	1.3 (0.9–1.9)	1.2 (0.8–1.7)	1.5 (1.2–1.6)	0.5	1.3 (0.8–1.8)	1.2 (0.8–1.4)	1.4 (0.9–1.6)	0.24
Platelets ($\times 10^3/mm^3$)	199 (148–235)	204 (146–268)	151 (94–204)	0.02	193 (145–236)	227 (142–263)	165 (156–261)	0.61
INR	1.0 (1.0–1.2)	1.1 (1.0–1.2)	1.1 (1.0–1.1)	0.42	1.1 (1.0–1.2)	1.2 (1.1–1.2)	1.0 (1.0–1.1)	0.02
D-dimer (ng/ml)	250 (170–900)	360 (200–438)	270 (100–400)	0.69	270 (193–438)	210 (170–2300)	300 (200–1100)	0.82
CRP (mg/l)	26.3 (12–48)	48 (24–92.2)	26 (10.1–57)	0.03	37 (23–66.7)	55 (9.9–96)	9.5 (7.4–14.5)	0.002
LDH (U/l)	752 (585–966)	885 (665–1110)	646 (589–1200)	0.59	829 (656–1110)	795 (375–1461)	621 (443–787)	0.09

P value by *Chi-square test (data are presented as count and percentage). Z-test for column proportions (with adjusted P value by Bonferroni method) is presented by letters; similar letters = insignificant difference. *P* value by ** Kruskal-Wallis H-test [data are expressed as Median (25th percentile – 75th percentile)]. Abbreviations: CRP: C-reactive protein; INR: international normalized ratio; LDH: lactate dehydrogenase; TLR: toll-like receptor; WBC: white blood cells.

however TLR7 and TLR3 are considered the most clinically relevant TLRs that have been shown to respond to coronaviruses. After endocytosis of SRS-COV-2 to the type II pneumocytes of the lungs, viral nucleotides are transported into endosomes where TLR3 and TLR7 are present. Through slightly different pathways, TLR3 and TLR7 activate NF-κB signaling, which results in the production of type I IFNs and proinflammatory cytokines IL-6, TNF-α, and IFN-γ [21].

The genetic background of human populations can influence the susceptibility and outcome of infectious diseases, especially COVID-19 [4]. Many studies reported the fundamental role of TLRs signaling pathways in COVID-19[5,9]. To our knowledge, none of the in vivo studies has examined the association of TLR3 rs3775290 and TLR7 rs179008 polymorphisms with the susceptibility to COVID-19 pneumonia and disease outcome, even though they have been associated with various infectious diseases.

Our study illustrated that the mutant 'T/T' genotype and the 'T' allele of TLR3 rs3775290 were significantly associated with an increased risk of COVID-19 pneumonia.

The TLR3 rs3775290 polymorphism is present in exon 4 of the TLR3 gene. The substitution of C to T at this position in the TLR3 gene (rs3775290) leads to an amino acid change from phenylalanine to leucine at position 459 of the protein- altering the TLR3 ectodomain and thereby affecting the ligand-receptor interaction [22] and the efficacy of signal transduction in TLR3 pathway and thus cause an altered immune response [23], which may explain the association of 'T' allele and risk of COVID-19 pneumonia in our study.

This finding partially agreed with the findings of another Egyptian study that showed a significantly higher 'T' allele of TLR3 rs3775290 in hepatitis C virus (HCV) patients than healthy controls; however, the heterozygous genotype 'C/T' was higher than both homozygous genotypes in HCV patients than controls [24]. On the other hand, our finding was in contrary with the Turkish study of Goktas et al. [25], which revealed that 'C/C' genotype was a risk factor for chronic HBV infection. These controversial findings could be due to the variations in ethnic background of the studied populations, the studied infectious diseases

and the number of patients and controls.

Furthermore, our study illustrated that the males harboring the 'T/T' genotype of TLR3 rs3775290 polymorphism could be more susceptible to COVID-19 pneumonia than females having the same genotype. This sex-dependent difference may be owing to gender-specific behaviors, genetic and hormonal factors, and sex differences related to SARS-COV-2 infection [26].

Regarding TLR7 rs179008, a significant association was found between the mutant 'T/T' genotype as well as the 'T' allele and the susceptibility to COVID-19 pneumonia.

These data could be explained as the mutant 'T' allele of TLR7 rs179008 was associated with a significant decrease in gene expression of TLR7 compared to the 'A' allele in HCV patients [27] and in HIV patients [28]. Additionally, The substitution of A to T at the position of TLR7 rs179008 leads to an amino acid change from glutamine to leucine at position 11 of the protein-altering the TLR7 processing and cause an altered immune response [18].

These results were in accordance with a German study done by Schott et al. [29] which showed that the 'T' allele was associated with enhanced susceptibility to chronic HCV infection. However, our results were dissimilar to those of Mosaad et al. [24] which showed no significant association between TLR7 rs179008 polymorphism and chronic HCV infection. In the genetic association studies of infectious diseases, these conflicting findings are frequent. These discrepancies could be due to microbe genetic heterogeneity, differences in the exposure rate to infectious agents, and various pathogen-induced immune responses, in addition to the interaction with environmental factors.

When comparing different genotypes of TLR7 rs179008 in a sex-dependent manner, the present study illustrated that males carrying the 'A/A' genotype might be more risky to exhibit COVID-19 pneumonia than females carrying the same genotype.

In the current analysis, the dissimilar results of TLR7 rs179008 genotyping in male and female patients are not surprising and could be explained by the situation of the *TLR7* gene in the X chromosome which is an immune regulatory gene whose biallelic expression leading to a

Table 4

Clinic-demographic and laboratory characteristics of patients with COVID-19 pneumonia.

Parameter	Survivor ($n = 80$)	Non-survivor ($n = 56$)	P
Demographic characteristics			
Age (years)	58.5 (52.3–65.8)	62.5 (54.3–73)	****0.012**
Sex			
Male	44 (55%)	40 (71.4%)	*0.052
Female	36 (45%)	16 (28.6%)	
Presence of comorbidities			
Diabetes	40 (50%)	26 (46.4%)	*0.682
Hypertension	36 (45%)	28 (50%)	*0.565
IHD	10 (12.5%)	14 (25%)	*0.060
CLD	4 (5%)	12 (21.4%)	***0.003**
Clinical characteristics			
COVID-19 severity			
Moderate	28 (35%)	10 (17.9%)	***0.028**
Severe	52 (65%)	46 (82.1%)	
SPO_2	90 (84.3–93)	83 (75.3–89.8)	****0.001**
Respiratory rate	24 (22–27.5)	30 (25–35)	**** < 0.001**
Laboratory characteristics			
TLR3 genotypes			
C/C	40 (50%)	20 (35.7%)	*0.061
C/T	24 (30%)	28 (50%)	
T/T	16 (20%)	8 (14.3%)	
TLR7 genotypes			
A/A	64 (80%)	42 (75%)	
A/T	8 (10%)	10 (17.9%)	*0.356
T/T	8 (10%)	4 (7.1%)	
Haemoglobin(g/dl)	12.6 (10.8–13.7)	11.9 (10–13.1)	**0.124
WBC($\times 10^3$/mm^3)	7.45 (5.45–12.6)	8.85 (7–13.6)	**0.077
Lymphocyte count($\times 10^3$/ mm^3)	1.4 (1.1–1800)	1 (0.7–1.7)	****0.017**
Platelet($\times 10^3$/mm^3)	191 (147.3–239.5)	206 (131–242.8)	**0.646
CRP(mg/l)	28 (17.9–74.8)	31 (12–63)	**0.974
D-Dimer(ng/ml)	250 (200–400)	370 (160–1400)	**0.451
LDH(U/l)	731 (589–935)	1200 (994–1718)	****0.001**
Serum creatinine(mg/dl)	1.2 (1.0–1.5)	1.2 (0.8–1.6)	**0.581
ALT(U/l)	38 (24.5–49.8)	44 (21.8–58.8)	**0.958
AST(U/l)	39.5 (30–57.5)	36.5 (27–78)	**0.906
Serum total bilirubin(mg/ dl)	0.7 (0.6–0.8)	0.7 (0.53–0.98)	**0.285
Serum albumin(g/dl)	3.5 (3.1–3.9)	3.1 (2.9–3.5)	**** < 0.001**
INR	1.0 (1.0–1.1)	1.2 (1.1–1.3)	**** < 0.001**

P value by *Chi-square test, whereas the Fisher's exact test was used for TLR7 genotypes (data are presented as count and percentage). *P* value by** Mann-Whitney U test [data are presented as median and (25th percentile – 75th percentile)]. Abbreviations: ALT: alanine transaminase; AST: aspartate transaminase; CLD: chronic liver disease; CRP: C-reactive protein; IHD: ischaemic heart diseases; INR: International normalized ratio; LDH: lactate dehydrogenase; SPO$_2$: peripheral capillary oxygen saturation; TLR: toll like receptors; WBC: white blood cells.

stronger immune response decreasing viral load levels and inflammation in women than in men [30].

Conflicting results were reported by different studies: Oh et al. [31] found that female carriers of the mutant 'T' allele are at an increased risk of HIV-1 infection; Buschow et al. [32] demonstrated that heterozygous genotype 'A/T' may reduce the risk of Caucasian females to develop Chronic hepatitis B infection; Mosaad et al. [24] reported that the risk of HCV infection was related to the 'T' allele in Egyptian females.

In the present study, COVID-19 pneumonia patients were classified into survivors (58.8%) and non-survivors (41.2%). Comparing the two groups as regard TLR3 rs3775290, the heterozygous 'C/T' genotype showed a significant increase in COVID-19 non-survivors. On the other hand, TLR7 rs179008 genotype frequencies did not reveal any significant difference between COVID-19 survivor and non-survivor.

Table 5

Predictors of the likelihood of mortality in patients with COVID-19 pneumonia.

Predictor	Univariable		Multivariable	
	COR (95% CI)	P	OR (95% CI)	P
Age (years)		**0.002**		0.253
<61	R		R	
≥61	3 (1.48–6.1)		2.19 (0.57–8.35)	
Sex		0.054		**0.006**
Female	R		R	
Male	2.05 (0.99–4.24)		7.21 (1.78–29.34)	
Chronic liver disease		**0.007**		0.204
Absent	R		R	
Present	5.18 (1.58–17.05)		0.22 (0.02–2.29)	
Respiratory rate		**<0.001**		0.054
<26 breaths / min	R		R	
≥26 breaths / min	4.64 (2.2–9.73)		3.56 (0.98–12.89)	
SPO$_2$		**<0.001**		**0.033**
>82%	R		R	
≤82%	4 (1.87–8.54)		5.23 (1.14–23.96)	
COVID-19 severity		**0.031**		0.352
Moderate	R		R	
Severe	2.48 (1.09–5.65)		2.04 (0.45–9.16)	
TLR3 genotype		**0.019**		0.158
C/C-'T/T'	R		R	
C/T	2.33 (1.15–4.74)		2.32 (0.72–7.43)	
Serum albumin		**<0.001**		0.108
>3 g/dl	R		R	
≤3 g/dl	5.28 (2.35–11.83)		2.98 (0.79–11.29)	
INR		**<0.001**		**0.024**
≤1	R		R	
>1	5.5 (2.52–11.99)		4.46 (1.22–16.31)	
LDH		**0.001**		**<0.001**
<1000 U/l	R		R	
≥1000 U/l	15.89 (6.79–37.18)		24.44 (6.05–98.75)	
Lymphocyte count		**<0.001**		**0.009**
>900/mm^3	R		R	
≤900/mm^3	5.54 (2.51–12.22)		12.67 (1.88–85.24)	

Abbreviations: 95% CI: 95% confidence interval; COR: crudes odds ratio; INR: International normalized ratio; LDH: lactate dehydrogenase; SPO$_2$: peripheral capillary oxygen saturation; TLR: toll like receptors.
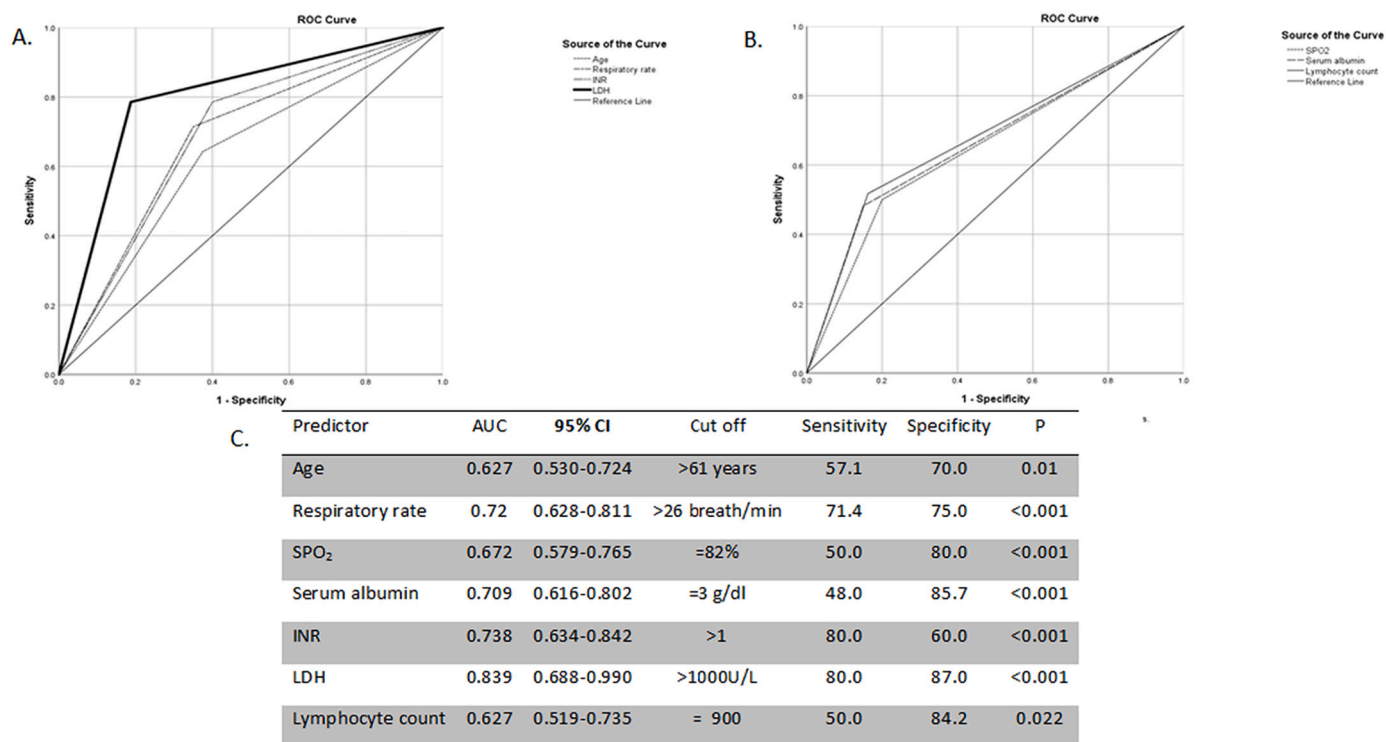
Obtaining these promising results about the TLR3 rs3775290 and TLR7 rs179008 polymorphisms, would allow for a better understanding of their possible roles in COVID-19 pneumonia development, outcome and prevention and could be very important in the field of genetically-based personalized medicine.

Early prediction of death among patients with COVID-19 pneumonia may help guide the clinical management and improve the clinical care of those patients. Therefore, this study aimed to investigate the predictors of mortality of COVID-19 pneumonia.

In the univariate analysis, the 'C/T' genotype of TLR3 rs3775290 was found to be associated with mortality in patients with COVID-19 pneumonia; however it was dropped out during the multivariate regression.

Male sex and SPO$_2$ ≤ 82% were important independent predictors of mortality. Many studies had confirmed that male sex and hypoxaemia were associated with death in patients with COVID-19 pneumonia [33,34].

Additionally, we also found that high INR > 1 was a high-risk factor for mortality in patients with COVID-19 pneumonia. This finding was in accordance with another study [35]. Obviously, severe COVID-19

Predictor	AUC	95% CI	Cut off	Sensitivity	Specificity	P
Age	0.627	0.530–0.724	>61 years	57.1	70.0	0.01
Respiratory rate	0.72	0.628–0.811	>26 breath/min	71.4	75.0	<0.001
SPO₂	0.672	0.579–0.765	=82%	50.0	80.0	<0.001
Serum albumin	0.709	0.616–0.802	=3 g/dl	48.0	85.7	<0.001
INR	0.738	0.634–0.842	>1	80.0	60.0	<0.001
LDH	0.839	0.688–0.990	>1000U/L	80.0	87.0	<0.001
Lymphocyte count	0.627	0.519–0.735	= 900	50.0	84.2	0.022

Fig. 1. ROC curves for quantitative predictors to find a cutoff value that discriminate survivors from non-survivors. A. ROC curves to find cutoff value for age, respiratory rate, LDH and INR; B. ROC curves to find cutoff value for SPO_2, serum albumin, lymphocyte count. Abbreviations: AUC: area under the curve; CI: confidence interval; INR: International normalized ratio; LDH: lactate dehydrogenase; ROC curve: receiver operating characteristics.

pneumonia-induced immune responses can cause inflammatory storms, damage microcirculation and activation of the blood coagulation system increasing INR levels [36].

Further, high LDH level $\geq$ 1000 U/l was an effective predictor of mortality in our study. This result was in harmony with other studies [37,38], which revealed that elevated LDH level was an independent predictor of mortality in COVID-19. The increase of LDH reflects tissue destruction suggesting viral infection or lung damage, such as the pneumonia induced by SARS-COV-2 [39].

Moreover, our study illustrated that low lymphocyte count<900/ mm3 was associated with an increased risk of death in COVID-19 pneumonia. This finding was in parallel to other studies [40,41]. Lymphopenia could be explained by targeting of SARS-COV-2 virus to ACE2 lymphocyte surface receptor combined with a deranged cytokine milieu [42].

In view of our findings, male sex, $SPO_2 \leq 82\%$, high INR > 1, high LDH $\geq$ 1000 U/l, and low lymphocyte count<900/mm3 could be used as strong early predictors for death from COVID-19 pneumonia in an attempt to reduce fatality and improve the clinical outcome.

6. Conclusions

This study concluded that the TLR3 rs3775290 and TLR7 rs179008 polymorphisms may be possible risk factors for vulnerability to COVID-19 pneumonia. However, there was no significant association between neither the TLR7 rs179008 nor TLR3 rs3775290 polymorphisms and the outcome of COVID-19 pneumonia cases. Moreover, it was found that male sex, lower SPO2%, high INR, high LDH, and lymphopenia were independently predictive of mortality among patients with COVID-19 pneumonia, which helps start personalized treatment for better disease outcome.

Declaration of Competing Interest

No external funding and no competing interests declared.

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