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ORIGINAL RESEARCH

Impact of Toll-Like Receptor 2 and 9 Gene Polymorphisms on COVID-19: Susceptibility, Severity, and Thrombosis

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Background: Toll-like receptors (TLRs) play an important role in activation of innate and adaptive immune responses.

Aim: We aimed to detect the association between TLR2 rs5743708 G>A and TLR9 rs5743836 C>T variants and COVID-19 disease susceptibility, severity, and thrombosis by using neutrophil extracellular traps (NETs).

Subjects and Methods: We included 100 adult COVID-19 patients as well as 100 age- and gender-matched normal controls. Participants were genotyped for TLR2 rs5743708 and TLR9 rs5743836. Citrullinated Histone (H3) was detected as an indicator of NETs.

Results: The mutant (G/A and C/C) genotypes and (A and C) alleles of TLR2 rs5743708 and TLR9 rs5743836, respectively, have been significantly related to a higher risk of COVID-19 infection, representing a significant risk factor for the severity of COVID-19. There was no significant association between the two variants and citrullinated histone (H3).

Conclusion: TLR2 rs5743708 and TLR9 rs5743836 variants have been significantly related to a higher risk and severity of COVID-19 infection but had no effect on thrombus formation.

Keywords: coronavirus disease 2019, single-nucleotide polymorphism, toll-like receptor, high resolution-CT, susceptibility, severity

Introduction

The WHO Director General proclaimed the COVID-19 (coronavirus disease 2019) pandemic, caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a global health emergency of concern worldwide in January 2020. SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA (ssRNA) virus.[1]

The immune system protects the host against foreign pathogens. Adaptive immune responses are primed by innate immunological reactions, which are the body's first line of defence versus invaders. Toll-like receptors (TLRs) as well as retinoic acid-inducible gene-I-like receptors, which recognise pathogen-associated molecular patterns (PAMPs), are various pattern-recognition receptors expressed by innate immune cells.[2]

TLR2 is a surface receptor that identifies various ligands generated by bacteria, viruses, fungi, and parasites and is implicated in sensing PAMPs via SARS-CoV-2.[3] Likewise, TLR9 identifies CpG-rich fragments of DNA produced by viruses, bacteria, and mitochondrial DNA.[4]

According to Zheng et al,[5] TLR2 expression was found to be increased with COVID-19 severity, and El Kebir et al[6] suggested number of mechanisms explaining the link between stimulation of TLR2 and the coagulation system in

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COVID-19. Similarly, TLR9 was proposed as an important factor explaining hyper-inflammation as well as multi-organ and thrombotic complications in COVID-19 patients.[7]

Infection-related neutrophil extracellular traps (NETs), which form a scaffold and stimulate adhesion of platelets and formation of thrombus, represent a component of the innate immunity response.[8] Interestingly, in COVID-19, NETs were suggested to lead to organ damage and death.[9] Mitochondrial DNA, which activates polymorphonuclear leukocytes via TLR9, is a strong inducer of NETs. A TLR9 antagonist was found to completely block the formation of mitochondrial DNA-induced NETs.[10] Additionally, previous studies have demonstrated that various types of viruses are involved in NETosis through the activation of platelet receptors (C-type lectin), enhancing NETosis, and production of pro-inflammatory cytokines via TLR2.[11–13]

Even in the absence of apparent hazard variables like elderly age, high BMI, and associated morbidities, disease development may happen quickly in some patients. As a result, it seems likely that additional variables, including the genetic makeup heterogeneity and the host's various immune responses, may contribute to disease severity.[14] Interestingly, recent studies found that there is upregulation of some genes (as ACE2, CD147, FURIN and TMPRSS1) and down regulation of others (as BMAL1) in SARS-CoV-2 infection and periodontitis conditions.[15]

Individuals exhibit a wide variety of susceptibilities to infections due to genetic changes like single nucleotide polymorphisms (SNPs), which strongly affect innate immune responses to pathogen challenges and illness outcomes. As a result, some people are susceptible to specific diseases, whereas others are protected.[16]

Thus, it is crucial to pinpoint the genes involved in the incidence and severity of COVID-19 cases to develop preventive measures for individuals who are predisposed to disease and complications.[17]

We therefore aimed to detect the connection between these variants of TLR2 and TLR9 and COVID-19 disease susceptibility, severity, and thrombosis. To our knowledge, no study has investigated these variants in COVID-19 disease in the population of Egypt.

Subjects and Methods

Selection of Participants

This case–control research enrolled 200 participants selected from Al-Zahraa University Hospital and categorized them into 2 groups: Group I (n = 100), consisting of individuals diagnosed with COVID-19 infection; and Group II (n = 100), consisting of healthy individuals with similar ages and sexes who served as controls. Group I was subdivided into two groups according to disease severity: group IA (n = 50), diagnosed with moderate to severe COVID-19 infection, and group IB (n = 50), diagnosed with mild COVID-19.

Calculation of Sample Size

The sample size was calculated by G*Power (version 3.1.9.2; Germany). Up to our knowledge, there are no previously published studies about association between TLR2 rs5743708, TLR9 rs5743836 SNP and COVID-19 disease, thus we conducted a pilot study on 20 COVID-19 patients and 20 healthy controls to calculate the sample size. Pilot study data revealed that the probability of exposure to A allele of rs5743708 TLR2 and C allele of rs5743836 TLR9 among controls was 7.5% and 10% respectively, probability of exposure among cases was 25% and 30% respectively, thus the minimum required sample size was 80 patients and 80 controls for TLR2 rs5743708 and 72 patients and 72 controls for TLR9 rs5743836 with a power of 80% and a level of significance of 5%. We add 20 individuals in each group for better statistical analysis.

Inclusion Criteria

This research included adult patients aged 20–60 years, diagnosed using SARS-COV-2 -reverse-transcriptase polymerase chain reaction (RT-PCR). Patients have been classified according to disease severity based on the interim guidance of the WHO[18] and the CO-RADS classifications based on CT findings.[19]

Exclusion Criteria

Patients with known chronic lung disease, chronic liver or kidney disease, malignancies, autoimmune diseases, diabetes, hypertension, hypo/hyperthyroidism, and those receiving any medical treatment were excluded from this research. Children and pregnant females were also excluded from this study.

Ethical Consideration

The research has been carried out in accordance with the World Medical Association's Helsinki Declaration for Human Subjects Studies. This study has been accepted by Al-Azhar University's Institutional Review Board (IRB) (study code 1203, 12th. January 2022), and each patient gave their written informed consent.

Assessment and Procedures

All patients underwent a detailed history-assessment, thorough clinical and radiological evaluation, and the following investigations:

(A) Routine laboratory investigations:

1. The automated haematology analyser Sysmex KX 21N (Kobe, Japan) was used to perform a complete blood count (CBC), coagulation profile using Stago STA (Diagnostica Stago, Paris, France), D-dimer using Immulite 1000 (Siemens Healthcare Diagnostics, New York, USA), and biochemical analyses using the Cobas c311 system (Roche Diagnostics, Mannheim, Germany) for kidney and liver function parameters; for ferritin, use the Cobas e411 system; and for C-reactive protein (CRP), use the Cobas Integra 400 plus.
2. Human citrullinated histone H3 was measured by ELISA using an ELISA kit supplied by the Bioassay Technology Laboratory (lot No 202203013, Zhejiang, China) in accordance with the manufacturer 's directions. The assay had a sensitivity of 1.352 ng/mL with a range of 32–105 ng/mL.
3. Molecular analyses of TLR2 rs5743708 and TLR9 rs5743836 variants:

Genomic DNA Extraction

A Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit (Lot. No.01176830) was used to extract DNA from blood specimens. DNA samples were tested for quality and concentration using a QIAxpert (QIAGEN, Hilden, Germany) and preserved at −20°C.

Genotyping of TLR2 rs5743708 and TLR9 rs5743836 by TaqMan Genotyping Assay

The PCR process consists of 25–50 cycles with the following steps: initial denaturation at 95°C to separate the nucleic acid double chain, annealing at 58°C for binding of primers, and extension by DNA polymerase at 72°C. The genotyping PCR reaction has been conducted in a 20 µL reaction volume having 10 uL of TaqMan Genotyping Master Mix (Lot. No. 01187540), 0.5 uL of TaqMan Genotyping Assay Mix, and 9.5 uL of diluted DNA template (5.5 µL nuclease free water, Lot No. 01069419, and 4 µL template). After amplification, data was collected and read based on fluorescence signals using a Rotor Gene real-time system (QIAGEN, Hilden, Germany). Every TaqMan® SNP Genotyping Assay contains sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest and two TaqMan® minor groove binder (MGB) probes with non-fluorescent quenchers (NFQ): one VIC™ labelled probe to identify the allele 1 sequence and one FAM™ labelled probe to identify the allele 2 sequence. The TaqMan® SNP Genotyping Assay of TLR2 rs5743708 and TLR9 rs5743836 were obtained from Applied Biosystems by Thermo Scientific, USA (Lot No. P211222-005A10 and P211222-005A11respectively).

The allelic discrimination data were plotted as a comparison of allele 1 (VIC™ dye) and allele 2 (FAM™ dye) using real-time PCR instrument software (QIAGEN, Hilden, Germany). Every specimen is represented as a separate point on the allelic discrimination (AD) plot, sometimes referred to as a cluster or scatter plot (Supplementary Data Figures 1–6).

(B) Radiological examination by high resolution – CT (HRCT) scan (Toshiba 160 multidetector CT scan) (Supplementary Data Figure 7).

Statistics

The Statistical Package for Social Sciences, Windows, version 21.0 (SPSS Inc., Armonk, NY, USA) has been employed to analyse the data. Normally distributed quantitative data has been represented as the mean and standard deviation. The Student's *t*-test was used for comparison between two groups, whereas the Mann–Whitney test was for non-parametric data. Qualitative variables were presented as frequencies and percentages, and the Fisher's exact test or the χ^2 test was employed to compare qualitative variables. TLR2 and TLR9 genetic variants were assessed for Hardy-Weinberg equilibrium (HWE) with a χ^2 test before genotypic and allelic disease correlation analysis. The odds ratio (OR) of genotypes between groups was assessed using logistic regression. The level of statistical significance was fixed at $P < 0.05$.

Results

Genotype and Allelic Frequencies of TLR2 rs5743708and TLR9 rs5743836 Variants in Patients with COVID-19 and the Control Group

In the control group, we found that TLR2 rs5743708 and TLR9 rs5743836 were in Hardy-Weinberg equilibrium (HWE) ($p = 0.74$ and 0.49, respectively), as indicated by the respective test.

Table 1 illustrates the association between TLR2 rs5743708 and TLR9 rs5743836 genotypes as well as the risk of COVID-19 infection. We observed that wild (G/G and T/T) genotypes have been the most prevalent genotypes in the studied population and have been related to a significantly lower risk of COVID-19 infection. We detected the mutant homozygous (A/A) genotype in 3% of patients with COVID-19, whereas the mutant homozygous (C/C) genotype was detected in 16% of COVID-19 patients and 6% of the control group, representing a significant risk factor for COVID-19 infection ($p = 0.02$). We found that patients with the (G/A) genotype of TLR2 rs5743708 were 3.7 times more at an increased risk of being infected with COVID-19 than those with wild homozygous genotypes. Moreover, we detected

Table 1 The Association Between TLR2 rs5743708 and TLR9 rs5743836 Genotypes and the Risk for COVID-19 Infection

TLR2 rs5743708	Group I (n=100)		Group II (n=100)		Group I versus Group II
	No.	%	No.	%	OR (CI 95%)
Genotypes					
GG	71	71.0%	91	91.0%	Reference
GA	26	26.0%	9	9.0%	3.7 (1.6–8.4), p=0.002*
AA	3	3.0%	0	0.0%	p= 0.5
Allele frequency					
G	168	84.0%	191	95.5%	Reference
A	32	16.0%	9	4.5%	4.04 (1.9–8.7) p<0.001**
Dominant model					
GG	71	71.0%	91	91.0%	Reference
GA+AA	29	29.0%	9	9.0%	4.1 (1.8–9.3), p<0.001**
Recessive model					
GG+GA	97	97.0%	100	100.0%	Reference
AA	3	3.0%	0	0.0%	p= 0.2

(*Continued*)

Table 1 (Continued).

TLR2 rs5743708		Group I (n=100)		Group II (n=100)		Group I versus Group II
		No.	%	No.	%	OR (CI 95%)
TLR 9 rs5743836		**Group I (n=100)**		**Group II (n=100)**		**Group I versus Group II**
		No.	**%**	**No.**	**%**	**OR (CI 95%)**
Genotypes						
TT		63	63.0%	74	74.0%	Reference
TC		21	21.0%	20	20.0%	1.2 (0.6–2.5) p=0.5
CC		16	16.0%	6	6.0%	3.1 (1.2–8.5) p=0.02*
Allele frequency						
T		147	73.5%	168	84.0%	Reference
C		53	26.5%	32	16.0%	1.9 (1.2–3.1) p=0.01*
Dominant model						
TT		63	63.0%	74	74.0%	Reference
TC+CC		37	37.0%	26	26.0%	1.7 (0.9–3.1) p=0.1
Recessive model						
TT+TC		84	84.0%	94	94.0%	Reference
CC		16	16.0%	6	6.0%	2.9 (1.1–7.9) p=0.03*
TLR2 rs5743708	**TLR9 rs5743836**	**Group I (n=100)**		**Group II (n=100)**		**Group I vs Group II**
		No.	**%**	**No.**	**%**	**OR (CI 95%)**
A	C	7	3.5%	1	0.5%	7.2 (0.9–59.2), p=0.06
A	T	25	12.5%	8	4.0%	3.4 (1.5–7.8), p=0.003*
G	C	46	23.0%	31	15.5%	1.6 (0.9–2.7), p=0.06
G	T	122	61.0%	160	80.0%	0.4 (0.2–0.6), p<0.001**

Notes: *p*-value >0.05 non-significant; **p*-value <0.05 significant; ***p*-value <0.001 highly significant.
Abbreviations: TLR 2, Toll-like receptor 2; TLR 9, Toll-like receptor 9; COVID-19, coronavirus disease 2019; No., number; OR, odds ratio; CI, confidence interval.

that the (A and C) alleles of TLR2 rs5743708 and TLR9 rs5743836, respectively, have been related to a significantly increased risk of COVID-19 infection.

The association of different models of inheritance for TLR9 rs5743836 revealed that the recessive model in which patients with the mutant (C/C) genotype of TLR9 were linked to a significantly increased likelihood of COVID-19 infection was the best inheritance model.

Regarding haplotype analysis, we observed that the frequency of the (A-T) haplotype was linked to an increased risk of COVID-19 infection. In contrast, the frequency of the (G-T) haplotype has been linked to a reduced risk of COVID-19 infection.

Genotype and Allelic Frequencies of TLR2 rs5743708 and TLR9 rs5743836 Variants and Disease Severity

Table 2 shows the association between TLR2 rs5743708 and TLR9 rs5743836 genotypes as well as the likelihood of severe COVID-19 infection. We detected the mutant homozygous (A/A) genotype in 6% of Group IA patients (moderate

Table 2 The Association Between TLR2 rs5743708 and TLR9 rs5743836 Genotypes and the Risk for Severity of COVID-19 Infection

		Group IA (n=50)		Group IB (n=50)		Group IA versus Group IB
		No.	%	No.	%	OR (CI 95%)
TLR2 rs5743708 Genotypes						
GG		30	60.0%	41	82.0%	Reference
GA		17	34.0%	9	18.0%	2.6 (1.01–6.6), p=0.047
AA		3	6.0%	0	0.0%	p= 0.141
TLR2 rs5743708 Allele frequency						
G		77	77.0%	91	91.0%	Reference
A		23	23.0%	9	9.0%	3 (1.3–6.9) p=0.009*
TLR2 rs5743708 Dominant model						
GG		30	60.0%	41	82.0%	Reference
GA+AA		20	40.0%	9	18.0%	3 (1.2–7.6), p=0.02*
TLR2 rs5743708 Recessive model						
GG+GA		47	94.0%	50	100.0%	Reference
AA		3	6.0%	0	0.0%	p= 0.02*
TLR 9 rs5743836 Genotypes						
TT		24	48.0%	39	78.0%	Reference
TC		13	26.0%	8	16.0%	2.6 (0.9–7.3) p=0.06
CC		13	26.0%	3	6.0%	7 (1.8–27.3) p=0.005*
TLR 9 rs5743836 Allele frequency						
T		61	61.0%	86	86.0%	Reference
C		39	39.0%	14	14.0%	3.9 (1.9–7.8) p<0.001**
TLR 9 rs5743836 Dominant model						
TT		24	48.0%	39	78.0%	Reference
TC+CC		26	52.0%	11	22.0%	0.3 (0.1–0.6) p=0.002*
TLR 9 rs5743836 Recessive model						
TT+TC		37	74.0%	47	94.0%	Reference
CC		13	26.0%	3	6.0%	5.5 (1.5–20.7) p=0.01*
Haplotype		**Group IA (n=50)**		**Group IB (n=50)**		**Group IA versus Group IB**
TLR2 rs5743708	***TLR9 rs5743836***	**No.**	**%**	**No.**	**%**	**OR (CI 95%)**
A	C	6	6.0%	1	1.0%	6.3 (0.7–53.5), p=0.09
A	T	17	17.0%	8	8.0%	2.4 (0.9–5.7), p=0.06
G	C	33	33.0%	13	13.0%	3.3 (1.6–6.7), p<0.001**
G	T	44	44.0%	78	78.0%	0.2 (0.1–0.4), p<0.001**

Notes: *p*-value >0.05 non-significant; **p*-value <0.05 significant; ***p*-value <0.001 highly significant.
Abbreviations: TLR 2, Toll-like receptor 2; TLR 9, Toll-like receptor 9; COVID-19, coronavirus disease 2019; No., number; OR, odds ratio; CI, confidence interval.

to severe COVID-19), whereas the mutant homozygous (C/C) genotype was detected in 26% of Group IA patients with COVID-19 and 6% of Group IB patients (mild COVID-19), representing a 7-times elevated risk of severe COVID-19 infection ($p < 0.05$). We further observed that the presence of (A and C) alleles of TLR2 rs5743708 and TLR9 rs5743836, respectively, were risk factors for severe infection of COVID-19. In particular, we found that the (G/A) genotype of TLR2 was at a 2.6-times elevated risk of moderate to severe infection of COVID-19 than the wild homozygous genotypes.

The association of various inheritance models for TLR2 rs5743708 and TLR9 rs5743836 revealed that the recessive model, in which patients with mutant (A/A) and (C/C) TLR2 and TLR9 genotypes, respectively, were linked to a significantly increased risk of moderate to severe COVID-19 infection, was the best inheritance model.

Regarding haplotype analysis, we noticed that the frequency of the (G-C) haplotype was strongly linked to an increased chance of developing a severe COVID-19 infection. In contrast, the frequency of the (G-T) haplotype was linked to a lower chance of severe COVID-19 infection.

Association Between TLR2 rs5743708 and TLR9 rs5743836 Genotypes and Laboratory Characteristics

We discovered a statistically significant relation among different TLR2 genotypes and erythrocyte sedimentation rate (ESR) in Group IA ($p = 0.026$), and between different TLR2 genotypes and O2 saturation and serum alanine transaminase (ALT) in Group IB ($p = 0.03$ and $p = 0.048$, respectively).

With regard to TLR9, we detected a statistically significant relation among various TLR9 genotypes, haemoglobin and total protein ($p = 0.024$ and $p = 0.029$, respectively) in Group IA, and between different genotypes of TLR9 and serum aspartate transaminase (AST) in Group IB ($p = 0.032$). However, we detected no link between various TLR2 genotypes and any of the coagulation indicators. Likewise, there has been no significant association among various TLR9 genotypes and any of the coagulation indicators, except for a highly significant association with D-dimer ($p = 0.006$).

Association Between TLR2 rs5743708 and TLR9 rs5743836 Genotypes and Citrullinated Histone (H3)

Table 3 illustrates the association between different TLR2 rs5743708 and TLR9 rs5743836 genotypes and citrullinated histone (H3). We discovered no link between various TLR2 and TLR9 genotypes and citrullinated histones.

Analysis of Linkage Disequilibrium

The analysis of allelic variants (rs5743708 and rs5743836) showed no evidence of linkage disequilibrium ($X^2=0.062$ and $p > 0.05$).

Table 3 The Association of Different TLR2 rs5743708 and TLR9 rs5743836 Genotypes and Citrullinated Histone

Parameters	TLR2 rs5743708			p-value
	GG (n=71)	AG (n=26)	AA (n=3)	
Citrullinated histone (H3) (ng/mL)	27.25±16.37	27.64±15.72	20.00±7.21	0.8
	TLR9 rs5743836			p-value
	TT (n=63)	TC (n=21)	CC (n=16)	
Citrullinated histone (H3) (ng/mL)	25.67±15.35	27.52±14.20	32.42±19.95	0.3

Abbreviations: TLR 2, Toll-like receptor 2; TLR 9, Toll-like receptor 9; COVID-19, coronavirus disease 2019.

Discussion

Toll-like receptors (TLRs) are a 13-transmembrane receptor family, which is at the forefront of guiding adaptive and innate immune responses to invaders such as viruses, fungi, bacteria, as well as parasites.[20–22]

When β-coronavirus infection occurs, TLR2 is necessary for the release of inflammatory cytokines because it can detect the SARS-CoV-2 envelope protein. Hence, previous study identified TLR2 as a possible target in treatment intervention strategies versus this lethal pandemic and has been shown to play an essential role in the development of COVID-19.[5]

At 4q32, there are two exons of the TLR2 gene. At 2258 bp in the TLR2 rs5743708 variant, a substitution of guanine for an adenine occurred, resulting in the substitution of an arginine-to-glutamine at residue 753.[23]

Our research found that the TLR2 rs5743708 mutant (G/A) genotype and the A allele were significantly related to an elevated risk of COVID-19 infection and were represented as a major risk factor for infection severity.

Ogus et al[24] demonstrated that this 753Q allele was linked to an increased risk of developing TB, also having an impact on the severity of the disease because it resulted in a reduced responsiveness of macrophages to bacterial peptides, leading to a weakened immunological response.

According to Schroder et al,[25] heterozygosity for the Arg753Gln variant exhibited a decreased induction of tumour necrosis factor-alpha as well as interferon-gamma when stimulated with Borrelia lysate in comparison to those not showing this variant. This seemed to protect against the onset of late-stage Lyme illness instead of being a risk factor for the illness.

Kijpittayarit et al[26] showed that this variant has been linked to an elevated susceptibility to cytomegalovirus infection after liver transplantation. In addition, Tabel et al[27] found that this variant was a risk factor for gram-positive pathogen-caused children's urinary tract infections.

In addition, some studies reported that this variant made children more likely to develop rheumatic fever and recurring febrile infections,[28,29] while it also increased the risk of developing familial Mediterranean fever, potentially affecting disease severity and the development of complications in adults.[30]

Similarly, El-Nabi et al[31] reported that the Arg753Gln variant in the TLR2 gene was linked to CMV infection in Egyptian bone marrow recipients. They showed that 85% of the patients screened were G/G homozygous, whereas 15% were G/A heterozygous; there were no patients that were homozygous for (A/A).

Previous studies demonstrated that this variant was also linked to non-infectious diseases, like type 1 diabetes, as this variant might drive the innate immune response to prime and promote an aggressive adaptive immune response;[32] and coronary restenosis, as this variant leads to a loss-of-function mutation that shifts the balance toward a TH1 response, promoting an inflammatory process that results in restenosis.[33] Moreover, this variant was also shown to confer an increased risk of allograft failure and death as it results in poor intracellular signalling and reduced cytokine release, increasing predisposition to microbial sepsis.[34]

TLR9 is mostly found in intracellular compartments and serves as a crucial gatekeeper in the detection and treatment of viral infection.[35] Thus, it was explained that the intensification of hyper-inflammation and thrombotic problems brought on by SARS-CoV-2 may be caused by TLR9 activation, which is a quiet but powerful driving force.[7]

TLR9 gene is encoded via 2 exons, the second of which represents the main coding area and maps to 3p21.3. There are numerous cases of genetic variants that result in TLR9 gain-of-function. For instance, the variant of the C allele of rs5743836 (T-1237C) that causes the activation of pro-inflammatory chemokines and cytokines as well as adaptive immunological responses, has been linked to a higher incidence of ICU-acquired infection and immune-mediated disease.[36,37] The amplification of TLR9/IL-6 signalling by T-1237C was particularly found to result in a deregulation of B-cell stimulation and proliferation in response to CpG stimulation.[38]

The mutant (C/C) genotype and TLR9 rs5743836 C allele were found to be significantly linked with an elevated risk and severity of infection with COVID-19 in our study.

This result was in concordance with the study by Elsherif et al,[39] which found that the TLR9-1237T/C variant was a risk factor for the infection-to-severe-sepsis development in paediatric patients.

This variant was also linked to non-infectious disorders, including a higher risk of asthma in a European-American cohort, a significant increase in the prevalence of Crohn's disease among patients,[40,41] and a higher risk of systemic lupus erythematosus among Asian communities.[42]

Regarding polymorphisms that were found to be associated with COVID-19 disease susceptibility and severity, Delanghe et al, showed that polymorphisms in angiotensin-converting enzyme-1 (ACE-1), complement C3, human homeostatic iron regulator protein (HFE) and cystic fibrosis transmembrane conductance regulator (CFTR) genes were associated with prevalence and severity.[43]

Alseoudy et al showed that TLR3 rs3775290 and TLR7 rs179008 polymorphisms were associated with increased susceptibility to COVID-19 disease.[17]

Recent research illustrated that some genetic mutations increase the severity of COVID-19 disease as D614G which increases the viral load as it enhances the interaction of spike and ACE2. In addition, other mutations increase spike-ACE2 affinity as L452R, S477G and S477N.[44–48]

Our study found that there has been no relation between TLR2 rs5743708 and TLR9 rs5743836 variants and citrullinated histones (H3), which are strong indicators of NETs and thrombosis. Therefore, these variants do not seem to have an impact on the formation and effects of NETs and, hence, thrombus formation. Furthermore, there has been no significant correlation between the two variants and other pro-inflammatory and coagulation indicators.

Thalin et al showed that quantification of citrullinated histone (H3) can be used as a specific, precise, and reliable indicator of NETs.[49]

Among the limitations of this study is that it does not include different age groups as infants, children, and elderly population. More studies involving pregnant females and patients with associated comorbidities are also required.

Conclusion

We concluded that TLR2 rs5743708 and TLR9 rs5743836 variants might be independent risk factors influencing COVID-19 disease susceptibility and severity, but they do not seem to be associated with markers of thrombosis.

This finding might be useful for the early detection of the disease's course and progression as well as for prioritising patients who might profit from TLR2 and TLR9 antagonists. In addition, individuals could be categorized according to their genetic testing of TLR2 and TLR9 SNPs to specify who are in great need for the vaccine.

Abbreviations

TLRs, toll-like receptors; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ssRNA, single-stranded RNA; PAMPs, pathogen-associated molecular pattern; SNPs; single nucleotide polymorphisms; NETs, neutrophil extracellular traps; RT-PCR, reverse-transcriptase polymerase chain reaction; WHO, World Health Organization; IRB, institutional review board; CBC, complete blood count; CRP, C-reactive protein; MGB, minor groove binder; NFQ, non-fluorescent quenchers; AD, allele discrimination; HRCT, high resolution-CT; SPSS, statistical package for social sciences; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; ESR, erythrocyte sedimentation rate; ALT, alanine transaminase; AST, aspartate transaminase.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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