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Gene polymorphism of TLR4 (rs10759931) in patients with SARS-CoV-2 and its association with cognitive impairment

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Abstract

This research examined the presence of the rs10759931 single nucleotide polymorphism (SNP) in the TLR4 gene among COVID-19 patients. Additionally, it evaluated how this genetic variation influenced cognitive difficulties in individuals recovering from the disease. This Case–control study (60 patients, 30 recovered, and 50 controls) investigates the G/A genotype of rs10759931 in all studied groups, using a conventional polymerase chain reaction with an allele-specific primer method. Results showed a median of ages was significant raising in age accompanied by severe cases (p =0.003) and more than 50% of the moderate infections in the youth category. Regarding the recovered individual's occurrence six cases along with ages median of 49.5 (45.8-57) males and females with a history of mild to moderate infection. When comparing the clinical severity, allele and genotype frequencies of the G/A rs10759931 TLR4 polymorphism showed no statistically significant differences between COVID-19 patients and controls (OR = 1.22; 95% CI = 0.60–2.49; p = 0.715). In recovered individuals with cognitive impairment, despite the occurrence of 66.7% of cognitive impairment in the GG genotype, there is no significant difference compared to intact recovered individuals (OR = 0.21; 95% CI = 0.03–1.39; p = 0.163). In conclusion, G/A rs10759931 of TLR4 required more information to explore their role in cognitive impairment and infection severity.

Keywords: Iraqi population, polymorphism, allele-specific primer, cognitive impairment, TLR4.

تعدد الاشكال الوراثي لمستويات شبيه التول-4 (*rs10759931*) في المرضى المصابين بفايروس سارس 2 وعلاقته بضعف الذاكرة

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الخلاصة

أختبر في هذا البحث وجود تعدد أشكال النوكليوتيدات المفردة rs10759931 (SNP) في جين TLR4 بين مرضى كوفيد-19. بالإضافة إلى ذلك، تم تقييم كيفية تأثير هذا الاختلاف الجيني على الضعف الادراكي لدى الأفراد الذين يتعافون من المرض. أجريت الدراسة على المرضى والمتعافين والاصحاء جيث تضمنت (60 مريضًا و30 متعافيًا و50 سيطرة) لدراسة النمط الجيني G/A لـ rs10759931 في جميع المجموعات المدروسة، وباستخدام تفاعل سلسلة البلمرة التقليدي مع طريقة تصميم الأليلات المتخصصة. أظهرت نتائج وسيط الأعمار ارتفاعاً معنويا في العمر يصاحب الحالات الشديدة (p=0.003)وأكثر من 50% من الإصابات

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المتوسطة الشدة في فئة الشباب. أما بالنسبة للمرضى المتعافين والتي بلغت ستة حالات مترافقة مع وسيط الاعمارالكبيرة 49.5 (45.8–57) من الذكور والإناث ذو تاريخ مرضي بالإصابة خفيفة إلى متوسطة. عند مقارنة الشدة السريرية، لم تظهر ترددات الأليل والنمط الوراثي لتعدد أشكال TLR4 rs10759931 G/A أي فروق ذات دلالة إحصائية بين مرضى كوفيد–19 والمجموعة الضابطة (OR = 1.22؛ 95% CI = 0.60– 2.49؛ p = 0.715). أعتمادا على شدة المرض، لم تظهر ترددات الأليل والنمط الجيني لتعدد أشكال G/A rs10759931 TLR4فروقات معنوية بين مجموعة مرضى كوفيد–19 (OR = 1.2219 ؛ 95 % CI = 0.60–2.49؛ p = 0.715). اما في المرضى المتعافين الذين يعانون من ضعف إدراكي، على الرغم من ظهور 66.7% من الضعف الإدراكي في النمط الجيني GG، الا انه لا يوجد فرق معنوي مقارنة بالأفراد الذين تم شفاؤهم ولا يعانون من ضعف الادراك (OR = 0.21؛ 95% CI = 0.03–1.39؛ p = 0.163). استنتجت الدراسة، يتطلب التغاير TLR4 من rs10759931 G/A مزيدًا من المعلومات لاستكشاف دورها في الضعف الإدراكي وشدة الاصابة.

1. Introduction

COVID-19 is an acute infectious illness caused by a novel coronavirus known as SARS-CoV-2. This disease can induce a wide range of symptoms, including severe pneumonia and multi-organ failure [1]. Acute respiratory failure, cardiac damage, septic shock, dyspnea, dry cough, high fever, and other consequences might accompany severe cases [2]. At now, the global case fatality rate for COVID-19 is about 3.5%. However, for high-risk populations, such as older persons with preexisting medical issues, this percentage might increase to 15% or even higher [3]. The mechanism could be associated with direct central nervous system viral infection, a widespread inflammatory response brought on by neurotoxicity and cytokine release, tissue hypoxia, and microangiopathy. Cognitive dysfunction may also be caused by COVID-19 [4] infection. During the acute phase, some patients report experiencing headaches, dizziness, and fatigue. Additionally, case reports have revealed abnormal brain imaging findings [5].

During recovery from COVID-19, 19 patients reported experiencing cognitive issues. These problems included difficulties with memory and concentration, as well as sleep disturbances. Some individuals showed irregularities in their neuropsychological test results, suggesting a decrease in their working memory, linguistic expressiveness, and executive function, according to the research [6]. Once the COVID-19 virus penetrates the blood-brain barrier and nasal mucosa, it infects neural cells that help transport the virus throughout the central nervous system, including neurons, astrocytes, and microglia. Structural and functional harm to the brain poses a risk of inducing cognitive decline [7]. Although the majority of COVID-19 patients regain their cognitive abilities on their own, a small percentage, mostly elderly persons and those with preexisting medical issues may suffer from persistent cognitive impairment [8].

Critical for initiating the innate immune response to stress, damage, or infection, various pathogen-associated molecular patterns (PAMPs) activate toll-like receptors (TLRs). Evidence suggests that TLR4 is more strongly bound to the SARS-CoV-2 Spike protein than ACE2 [9]. Among the innate immune cells that express TLR4 are tissue macrophages, DCs, and circulating monocytes. In contrast to resting and activated lymphoid cell subsets, total RNA analyses revealed that TLR4 mRNA was only found in myeloid cells. Adipocytes, microglia, and macroglial cells (including astrocytes, cutaneous microvessel endothelial cells, and umbilical vein endothelial cells) express TLR4 at a constitutive level [10].

While TLR4 is primarily localized in the plasma membrane, the fact that it can be internalized and activate intracellular signaling pathways suggests that it may also be

considered an intracellular Toll-like receptor (TLR) [11]. TLR4-spike protein binding has been predicted by computational modeling [12]. A possible target for regulating excessive inflammatory responses in COVID-19 patients could be to block the spike-TLR4 interaction, as it has been consistently reported in both murine and human macrophages that this protein's S1 subunit binds to TLR4, triggering pro-inflammatory responses and activating transcription factors like NF-κB and AP-1, which encode proinflammatory cytokines and interferons [13]. Therefore, TLR4 is involved in the pathogenesis of several viral illnesses. As a result, Toll-like receptor 4 (TLR4) provides an ideal model for studying how genetic diversity impacts receptor function, susceptibility to infectious illnesses, and the relationship between the two. TLR4 gene polymorphisms are linked to a decreased risk of developing hepatocellular carcinoma and a delayed course of liver fibrosis. Additionally, Sadik *et al.* [14] offered another indirect argument supporting TLR4's involvement in HCV infection by indicating that interleukin-10 (IL-10) is one of TLR4's upstream regulators. TLR4 and IL-10 may interact due to shared single-nucleotide polymorphisms (SNPs) and the fact that their expression levels are predictive of therapy response in chronic HCV patients. Both HBV and HCV infections need a robust initial immune response, mostly caused by IFN α/β, which are byproducts of TLR4 activation, even if the exact mechanism by which these infections trigger TLR4 activation remains unclear [15].

During the coronavirus pandemic in Iraq, we observed several cases where individuals who had recovered from COVID-19 experienced cognitive impairment. Additionally, we noted a scarcity of research examining the relationship between genetic polymorphisms and COVID-19 risk in our population. Thus, this study aims to shed light on how TLR4 single-nucleotide polymorphisms (rs10759931) possibly link to the severity of disease in Iraqi COVID-19 patients and establish their role in recovered individuals with cognitive impairment. Because the SARS-CoV-2 infection is decreasing in our population during the study period, the data in this study are limited in the numbering of samples. They may not provide clear knowledge about the TLR4 SNP rs10759931 role with and SARS-CoV-2 severity, although this study perhaps initially proven the correlation between cognitive impairment after SARS-CoV-2 infection with TLR4 SNP rs10759931.

2. Material and Methods

2.1 Populations studied and sampling

This study evaluated COVID-19 patients previously diagnosed at Baghdad Teaching Hospital between the ages of 18-55 years old, as well as healthy controls aged 22-52 years. Case-control research was conducted on 90 patients and recovered individuals as well as 50 healthy controls during the period starting from September 2023 to December 2023 for testing SARS-CoV-2 infection. WHO Interim Guidance Recognized Criteria. There are two groups of patients (60) moderate and severe (30 cases in each group), and recovered individuals (30) after two to three weeks recovering from infection including 6 cases with cognitive impairments signs like the decline in working memory, language expression, and executive function, and attention and sleep disorder [16].

The present research procedure was authorized by the local ethics committee of Baghdad University's College of Science (Ref: CSEC/0923/0059). The following items are required: an EDTA tube, three milliliters of venous blood collected from patients, and the blood should be frozen at -20 °C until it is needed. The DNA was isolated from blood that included EDTA using a gSYNC DNA extraction kit (Geneaid, Taiwan) according to the manufacturer's instructions.

2.2 Polymorphism of TLR4 gene

Using polymerase chain reaction (PCR)-allele-specific primer analysis, the TLR4 SNP rs10759931 gene was researched. In a 20μL reaction, the following components were used: 2μL of template DNA, 6μL of nuclease-free water, 10μL of PCR PreMix (produced by Pioneer, Korea), 1μL of forward primer (5′-GAGGGTCTGTCTCTAGTTGTCTG/A-3′), and 1μL of reverse primer (5′ CCAAGGTGGAAATCTCTTTGA-3′). The optimized PCR protocol was performed using a Bio-Rad thermocycler (Germany) with the following conditions: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 30 seconds. The process concluded with a final extension step at 72°C for 5 minutes. The PCR result (5 μl) was subjected to electrophoresis in a 1.5% agarose gel in TBE buffer (1x) at 5 volts per square centimeter for 50 minutes to examine the migration of the specified bands. The particular forward primer in a PCR tube was labeled using gel electrophoresis, which showed three genotypes (GG, GA, and AA) associated with two alleles (G and A).

2.3 Statistical Analysis

The median and interquartile range were provided for the continuous non-parametric variables that did not follow a normally distributed distribution. A Mann-Whitney U test was used to see whether there were significant differences. Number and % were used as categorical variables to describe categorical data, and to find statistical significance, either Pearson's Chi-square test or the two-tailed Fisher exact test were used. Utilizing https://www.had2know.org/academics/hardy-weinberg-equilibrium-calculator-2-alleles, the Hardy-Weinberg equilibrium (HWE) was assessed. We used logistic regression to get the odds ratio (OR) and 95% confidence interval (CI). Statistical significance was established when the probability value (p) was less than or equal to 0.05. The statistical research used GraphPad Prism version 8.0.0 (San Diego, California USA) and IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.).

3. Results

As shown in Table 1, the baseline characteristics of the COVID-19 patients stratified to infection severity, and median of ages showed significant a raising in age accompanied with severe cases, (p =0.003). Age in general was divided into three age groups (18-25), (26-30) and (31- >35) years, in the cases of moderate showed a significant increase (p = 0.045) about more than 50% of the infections in the first age group (18-25) youth category. Although there is no significant appearance of infection between age groups in severe cases, the study data recorded the highest infections (80%) in ages over 25 years. With respect to sex, males were observed to have higher rates of moderate (70%) and severe (63.7%) acquired infections compared to females. Significant differences were also observed in ferritin levels (304 ng/ml *vs* 780 ng/ml; p=<0.001), LDH levels (201 IU/L vs 252 IU/L; p=0.002), and D-dimer levels (382 mg/L *vs* 590 mg/L; p<0.001) between moderate and severe COVID-19 patients.

Table 1: Baseline characteristics of COVID-19 patients stratified severity of infection.

Characteristic		COVID-19 Patients		*p*-value
		Moderate (no.30)	Severe (no.30)	
Age; year		25 (22- 28)	28 (25-30)	***p* = 0.003**
Ferritin ng/ml		304 (280-419)	780 (514-946)	***p* < 0.001**
LDH IU/L		201(158-229)	252 (198-285)	***p* = 0.002**
D. dimer mg/L		382(242-530)	590(446-800)	***p* < 0.001**
Age group	18-25	16 (53.3)	6 (20)	***p* = 0.015**
	26-30	9 (30)	13 (43.3)	
	31- >35	5 (16.7)	11 (36.7)	
	p-value	***p* = 0.045**	*p* = 0.273	
Sex	Male	21 (70)	19 (63.3)	*p* = 0.087
	Female	9 (30)	11 (36.7)	
	p-value	***p* = 0.028**	*p* = 0.144	
Diabetes Miletus	Yes	13 (43.3)	18 (60)	*p* = 0.238
	No	17 (56.7)	12 (40)	
	p-value	*p* = 0.465	*p* = 0.273	
Hypertension	Yes	11 (36.7)	12 (40)	*p* = 0.951
	No	19 (63.3)	18 (6 0)	
	p-value	*p* = 0.144	*p* = 0.273	
Ct value	19 – 25	7 (23.3)	11 (36.7)	*p* = 0.102
	26 – 30	10 (33.3)	14 (46.6)	
	31 – 35	13 (43.4)	5 (16.7)	
	p-value	*p* = 0.407	*p* = 0.122	

For continuous variables, the median with interquartile range is provided; for categorical variables, the number and percentage are used; for test statistics, the probability of a Mann-Whitney U test (for comparisons with continuous variables), a two-tailed Fisher exact test, or a Pearson Chi-square test is given; and for non-applicable variables, NA is used. Emphasized for emphasis.

Table 2: characteristics of cognitive impairment for patients and recovered

Characteristics	Patients	Recovered
Infection with SARS-CoV-2	positive	Negative
Severity of infection	Moderate or severe	Moderate
Ages	Different ranges of young and older	Older
TLR4 levels in serum	high	Low
Alleles patterns	AA 20%	GG 66.7%

Among the recovered individuals, six cases were identified. These included both males and females with a median age of 49.5 years (range: 45.8-57 years). All of these individuals had previously experienced mild to moderate COVID-19 infections. Figure 1 shows the results

allele-specific primer Conventional PCR was used to detect SNPs in the gene of TLR4. The outcomes of gel electrophoresis exhibited three genotypes for each SNP rs10759931 (GG, GA, AA), as 312 bp.

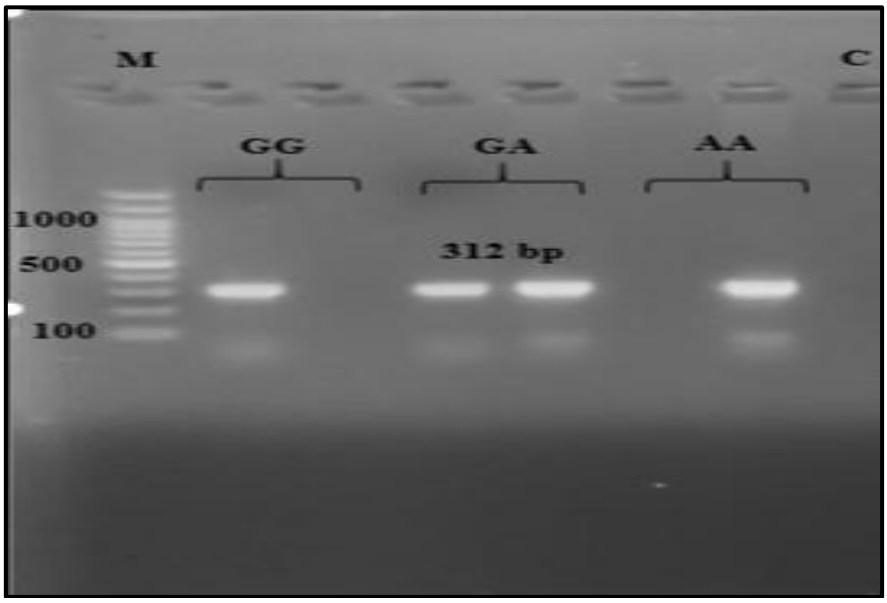

Figure 1: Representative images of agarose gel electrophoresis (1.5%; 5 V/cm2 for 55 minutes) of DNA–PCR products 312 bp for *TLR4 gene* SNP rs10759931 (G/A). M: DNA ladder (100bp), C: healthy control group**s.**

> rs10759931 [G/A]at position 2467 in intron of TLR4, Sequence ID: NM_003266.4
GAGGGTCTGTCTCTAGTTGTCTG**G**tacctggacctgtgatgattagggct
gaataacggtgtctacttgggtgtaaaagccaggtagaggaggtggttca
gaggaagggctctggattgcttagtgtgcataaggcatgctccagagcaa
atcttttgctatttttttagaactaactagccctggtaagtgcagtctctt
cccagatgccagaacatcaagaacacagaaaagaagacaattgggttaat
acatgtttagcatgagaaatgaggaagtaagggaaataaagTCAAAGAGA
TTTCCACCTTGG
Homo sapiens toll-like receptor 4 (TLR4), RefSeqGene (LRG_320) on chromosome 9, Sequence ID: NM_003266.4

Score	**Expect**	**Identities**	**Gaps**	**Strand**
577 bits(312)	7e-160	312/312(100%)	0/312(0%)	Plus/Plus

Query 1
GAGGGTCTGTCTCTAGTTGTCTG**G**TACCTGGACCTGTGATGATTAGGGCTGAATA
ACGGT 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2444
GAGGGTCTGTCTCTAGTTGTCTGGTACCTGGACCTGTGATGATTAGGGCTGAATA
ACGGT 2503

Query 61
GTCTACTTGGGTGTAAAAGCCAGGTAGAGGAGGTGGTTCAGAGGAAGGGCTCTG
GATTGC 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 2504
GTCTACTTGGGTGTAAAAGCCAGGTAGAGGAGGTGGTTCAGAGGAAGGGCTCTG
GATTGC 2563

Query 121
TTAGTGTGCATAAGGCATGCTCCAGAGCAAATCTTTTGCTATTTTTTAGAACTAA
CTAGC 180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2564
TTAGTGTGCATAAGGCATGCTCCAGAGCAAATCTTTTGCTATTTTTTAGAACTAA
CTAGC 2623
Query 181
CCTGGTAAGTGCAGTCTCTTCCCAGATGCCAGAACATCAAGAACACAGAAAAGA
AGACAA 240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2624
CCTGGTAAGTGCAGTCTCTTCCCAGATGCCAGAACATCAAGAACACAGAAAAGA
AGACAA 2683:
Query 241
TTGGGTTAATACATGTTTAGCATGAGAAATGAGGAAGTAAGGGAAATAAAGTCA
AAGAGA 300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 2684
TTGGGTTAATACATGTTTAGCATGAGAAATGAGGAAGTAAGGGAAATAAAGTCA
AAGAGA 2743

Query 301 TTTCCACCTTGG 312
||||||||||||
Sbjct 2744 TTTCCACCTTGG 2755

Table 3: shows that genotype frequencies of SNP rs10759931 in COVID-19 patients, recovered individuals, and control groups all corresponded with the Hardy-Weinberg equilibrium (HWE) with no significant differences.

Table 3: Hardy-Weinberg and Logistic regression analyses of *TLR4* gene SNPs in COVID-19 patients, recovered and healthy controls.

SNP	Allele/ genotype	Patient and recovered		Healthy control		OR	95% CI	*p*-value
		N=90	%	N=50	%			
rs10759931	G	99	55	63	63	Reference		
G/A	A	81	45	37	37	1.39	0.85 – 2.29	0.208
	GG	23	30.3	20	39.7	Reference		
	GA	53	49.5	23	46.6	2.0	0.93 – 4.31	0.111
	AA	14	20.2	7	13.7	1.74	0.60 – 5.05	0.421
HWE-*p*-value		0.072		0.925				

SNP: Single nucleotide polymorphism; HWE: Hardy-Weinberg equilibrium; OR: Odds ratio; CI: Confidence interval; p: the probability of Two-tailed Fisher's exact.

In this investigation, there was no significant difference in the frequency of the rs10759931 TLR4 polymorphism across the groups of COVID-19 patients based on clinical severity.

Table 4: Allele and genotype frequencies of *TLR4* gene SNP stratified by clinical severity in COVID-19 patients.

SNP	Allele/ genotype	Clinical severity				OR	95% CI	*p*-value
		Mild-moderate		Severe				
		N=30	%	N=30	%			
rs10759931	G	30	50	33	55	Reference		
G/A	A	30	50	27	45	1.22	0.60 – 2.49	0.715
	GG	6	25	7	30.3	Reference		
	GA	18	50	19	49.5	1.11	0.32 – 3.76	1.000
	AA	6	25	4	20.2	1.75	0.36 - 8.61	0.680

SNP: Single nucleotide polymorphism; OR: Odds ratio; CI: Confidence interval; *p*: Two-tailed Fisher's exact probability.

In recovered individuals with cognitive impairment, despite the occurrence of 66.7% cognitive impairment in the GG genotype, there is no significant difference compared to intact recovered individuals, as shown in Table 5.

Table 5: Allele and genotype frequencies of *TLR4* gene SNP stratified cognitive impairment in recovered individuals**.**

SNP	Allele/ genotype	Cognitive impairment				OR	95% CI	*p*-value
		Present (N=6)		Absence (N=24)				
		N	%	N	%			
rs10759931 G/A	G	10	83.3	26	54.2	Reference	0.05-1.16	0.100
	A	2	16.7	22	45.8	0.24		
	GG	4	66.7	6	25	Reference	0.03-1.39	0.163
	GA	2	33.3	14	58.3	0.21	0.01-2.78	
	AA	0	0	4	16.7	0.16		0.251

SNP: Single nucleotide polymorphism; OR: Odds ratio; CI: Confidence interval; *p*: Two-tailed Fisher's exact probability; *p*: probability.

4. Discussion

Our findings revealed a statistically significant correlation (p=0.015) between age and severity, with those 50 years and older being more likely to develop severe disease. Specifically, we found evidence that advanced age is associated with increased risk of more serious COVID-19 outcomes. Age groups had a significant impact on the symptoms of the cases of Holly that were reported in Najaf City. A high frequency of cases was reported among young adults (aged 21 to 50). Numerous studies have confirmed that age plays a significant role in COVID-19 infection (21). There were a lot of symptomatic patients in this age group. Across all age groups in this locality, the most common symptoms reported were fever, weakness, and shortness of breath or cough. This indicates that young adults are more likely to be affected by the virus. Still, older people (over 60) with concomitant conditions like diabetes and hypertension had a higher incidence of severe cases and case fatalities [17].

It was discovered that the inflammatory markers ferritin, lactate dehydrogenase (LDH), and D-dimer level were significant predictors of the course of the disease. An elevated

concentration of this inflammatory biomarker signifies systemic inflammation due to the body's stress. Stress levels increase even further in situations where there is greater stress, such as in critical and severe patient states. Because of the severity of the disease, this inflammatory biomarker thus indirectly reflects the body's level of stress*;* the differences were statistically significant (P<0.001). Ferritin levels increased in severity 780 ng\ml compared to moderate state 304 ng\ml. Zhou *et al*. found a correlation between the worsening of COVID-19 and an increase in ferritin levels[17]. The development of acute respiratory distress syndrome (ARDS), which is the primary cause of death if it advances to respiratory failure, is aided by the cytokine storm and the hyperbolic host immune response (i.e., ferritin). According to this meta-analysis, patient groups with severe conditions or ARDS had higher ferritin levels than patient groups with less severe conditions. However, several ARDS-related factors, such as ferritin, are not linked to ARDS-related deaths. This finding is also consistent with our forest plot comparing survivors versus non-survivors. Patients at high risk of death have higher serum ferritin concentrations, as was also noted in this meta-analysis, and its decline suggests that inflammation is under control, thereby enhancing survival [18].

Severe infections can lead to tissue damage and the release of LDH, mediated by the action of cytokines. Patients with severe COVID-19 infections should be expected to release higher levels of LDH into the bloodstream because the disease is characterized by a severe form of interstitial pneumonia, which frequently progresses to acute respiratory distress syndrome (isozyme 3). It is unknown, nevertheless, how much each of the various LDH isoenzymes contributed to the LDH elevation seen in COVID-19 [19].

The current study examines the TLR4 SNP (rs10759931) gene and its role in cognitively impaired patients, especially in recovered individuals from SARS-CoV-2. Allele and genotype frequencies of TLR4 SNP rs10759931 among studied groups of patients recovered, and HC had no significant differences according to HWE analysis. all people are at risk of contracting COVID-19, genotype does not protect against the occurrence of the disease, but immunological factors such as high levels of TLR4 are possible to protect within a certain limit. we observe the prevailing GG pattern, although there is no significant difference, in the patients, recovering it is approximately 30%, and the largest percentage in healthy people is approximately 40%. Perhaps it has a role in immunity, the possibility of genotype GG gives stronger immunity in terms of exposure to individuals to less and resistance to higher permission GG gives strength to immunity [20].

In the current study as for the AA genetic pattern, although there is no significant difference between the studied groups, we note that they are recessive genetic patterns that are more prone to infection. This suggests potentially lower immunity in individuals with this genotype. Specifically, the AA pattern was found in 20% of patients who had recovered from COVID-19 and in 13.7% of healthy controls. Studying more cases may reveal a significant role for the GG polymorphism. A larger sample size could strengthen evidence that the GG genotype confers protection against persistent infections and subsequent cognitive impairment. Also, in the case of clinical severity results revealed no significant differences between COVID-19 patients the proportions appeared close. maybe if we take more numbers, it would have a severity for the disease and take different age groups>45 years may show a significant role and this gene heterogeneity with other factors such as age, and chronic disease [21].

The relatively small sample size of patients and SNPs studied places some limitations on the strength of our results. However, our work shows potential for more extensive translational research with larger cohorts that could yield more conclusive findings. This highlights the need

to expand these trials to include a wider range of individuals experiencing different degrees of cognitive impairment in future research.
Although the GG genotype was present in 66.7% of the sample, the present investigation found no statistically significant difference in genotype among recovered persons with cognitive impairment (six instances).
Using data from a single study, researchers found that people with the TLR4-2604G>A (rs10759931) variant's GG homozygous genotype were more likely to experience cognitive impairment after SARS-CoV-2 infection ($p = 0.0234$, odds ratio [OR] = 1.91), compared to those with the GA genotype, who had a lower risk ($p = 0.0209$, OR = 0.50). This suggests that people with the GG genotype of the TLR4-2604G>A (rs10759931) variant have higher levels of TLR4 expression and are at a higher risk of developing cognitive impairment after SARS-CoV-2 infection compared to those with the GA genotype. There is mounting evidence linking the G allele to an increased risk of many immunologically-based ailments, such as cardiovascular diseases [22].

One of the most important limitations of the study is the size of the studied sample due to the lack of infections at the time of collecting samples also, perhaps one of the reasons for the lack of significant differences because the SNP is located in an area outside the protein expression of the Toll-like receptor 4 and therefore does not share the area of overlap of the Toll-like receptor protein with the Capsid S Protein for the virus and therefore requires more studies that include other SNPs that occur in The Toll-like receptor 4 Protein overlap area with the S capsid protein to find its significant differences with the infections.

Vitamin D is an essential regulator of the immune system. It's well-known that vitamin D deficiency causes a decrease in CD4+ and CD8+ T lymphocyte counts, whereas vitamin D supplementation causes an increase in CD+4 cell counts, Numerous cell types, such as neutrophils, lymphocytes, monocyte dendritic cells, and macrophages, express the vitamin D receptor. T, B, and dendritic cells in circulation contain the VDR as well as the vitamin D-activating enzyme CYP27B1 (1-hydroxylase). These cells utilize the intracranial conversion of circulating 25-hydroxyvitamin D (25D) into the bioactive form, 1,25-dihydroxyvitamin D (1,25D). Vitamin D facilitates the transition of T helper 1 (TH1) cells to T helper αβ (THαβ) cells, and this process influences adaptive immunity. Because vitamin D increases T regulatory cells while decreasing the manufacture of cytokines that promote inflammation and the immunological response to T helper-17, it both boosts the antimicrobial function of these immune cells and has anti-inflammatory effects [23]. Perhaps when we increase the cases and study more of the cognitive impairment with other factors like vitamin D deficiency [24], the history of the disease, and other SNPs from the same gene by the close effect, this study was conducted on COVID-19 patients. Twenty patients participated in the research by Krishnan *et al.* [25], and the results showed changes in executive functions, processing speed, and attention. The cognitive areas of attention, executive processes, episodic memory, and visuospatial abilities were shown to be impaired in the research of 50 Spanish patients carried out by Delgado-Alonso *et al.* [26]. Dressing *et al.* [27] administered a battery of neuropsychological tests to 31 patients, but they were unable to detect any deficits. In our study, there was 20% cognitive impairment out of 30 recoveries associated with older ages and some chronic diseases.

Researchers have shown that even in moderate cases of COVID-19, the TLR4 rs10759931 is linked to cognitive damage in the long run, even in individuals who have fully recovered. Mice lacking TLR4 were able to avoid the memory-damaging effects of S, and individuals with moderate COVID-19 who had the TLR4 single nucleotide polymorphism rs10759931 had a

worse late cognitive prognosis. These results point to TLR4 as a critical target for preventing cognitive impairment and show that S protein has direct effects on the brain. We believe this to be the first animal model to replicate cognitive impairment after COVID-19, which might lead to novel approaches for preventing or treating the virus's neurological effects [28]. Thus, the small number of studies on this topic and the methodological heterogeneity, make it difficult to define a clear pattern of cognitive impairment. Suppose we add to this the fact although the small number of participants in this study prevents proof of significance between patients as well as with cognitive impairment, cases have been documented in association with increased age or other contributing factors.

Many local studies have included different aspects of COVID-19, but there is no local study that touches on this topic such as [29] However, this study is considered the first locally.

Infection with SARS-CoV-2 also affects the brain. Multiple studies have shown that the COVID-19 virus affects the brains of infected individuals [30], which might explain why these people had long-term impacts including cognitive impairment [31] and other chronic dysfunctions. The hallmark of post-COVID-19 syndrome, also called "long COVID-19 or post-COVID-19," is cognitive impairment, even in individuals with modest symptoms. Vaccine research primarily focuses on the SARS-CoV-2 spike protein because of its major involvement in the pathogenesis of COVID-19. Upon binding to its cellular receptor, angiotensin-converting enzyme 2, the spike protein undergoes cleavage, resulting in the formation of two pieces, S1 and S2, on the surface of the virus. In the S1 segment, the binding to ACE2 is located, and in the S2 fragment, the viral and cellular membranes fuse to facilitate cellular entry. Even when the viral RNA is not present, there is evidence that the cells produce a Spike protein fragment during SARS-CoV-2 infection, which reaches several organs, including the CNS. Cells that produce the Spike protein may release extracellular vesicles containing it. This could serve as an additional mechanism for the Spike protein to spread throughout the body [32]. It has been shown that free S1 may penetrate the blood-brain barrier (BBB) and reach several brain areas associated with memory. This indicates that the protein, apart from the viral particles, might influence brain functioning.

Importantly, Swank *et al.* found elevated circulating Spike protein levels in patients with post-COVID-19 diagnoses, but not in those without long-term complications, even months after SARS-CoV-2 infection. However, little is known about the reasons behind post-COVID-19 cognitive impairment, including whether or not the presence of the Spike protein in the brain is a critical event in its development [33].

The working memory quiz showed that COVID-19 patients performed far worse than non-COVID-19 patients, according to the research by Baseler, *et al.* [34]. Retroactive interference disorders made it hard for some COVID-19 patients to remember and repeat the numerical sequences they had just heard. These findings point to the possibility that COVID-19 causes harm to the hippocampus and other brain areas, which in turn impacts the encoding and retrieval processes of working memory [35]. Furthermore, several studies have also shown that COVID-19 patients exhibit reduced vocabulary and expressiveness in their language use. Patients performed more poorly than the healthy control group on language fluency tests, producing fewer words [36]. Possible causes include impairments in language processing and vocabulary recall brought on by injury to areas of the brain associated with language. This is not the first research to use resting-state functional magnetic resonance imaging (fMRI) to assess the default network's functional connectivity in healthy controls and 22 COVID-19 patients. Research showed that the former group had a worse default network connection. Furthermore, there were alterations in the connection of the networks responsible for emotional

regulation and executive control. it seems that COVID-19 can alter the connection between brain regions that are crucial to cognitive function and reshape brain functional networks.

Furthermore, pertinent research has also shown long-term brain damage in COVID-19 individuals. Patients often experienced physical injury, and these injuries were linked to higher cognitive deficits, according to a review of 292 COVID-19 recoveries. Research using a cohort of people followed for one year [37]. They have examined 1,438 senior people (all 60 years old or older) in China who survived the COVID-19 pandemic and 438 who did not. Cognitive tests were administered at 6 and 12 months to examine the two groups' changes in ability. People who had dementia in their family, significant chronic illnesses, or cognitive impairments at the start of the research were not included. Infected individuals, especially those who managed to survive severe COVID-19, had a much higher incidence of cognitive decline compared to the uninfected group. Compared to the control group, this one had a 4.87-fold increased risk of moderate cognitive impairment and a 19-fold increased risk of severe cognitive impairment. Early cognitive decline (1.30-2.27) was associated with non-severe COVID-19 and was 1.71 times higher. These results imply that certain COVID-19 patients may continue to have cognitive impairments beyond recovery, even if the sample size is modest [40]. Similarly, studies following up with 50 patients in Italy who made complete recoveries showed that roughly 25% of those patients had minor cognitive impairment 60 days after their discharge. This impairment was mostly seen as a decline in attention and executive function. According to a pilot study conducted by British researchers on 100 patients, around 81% of COVID-19 patients who were discharged from the hospital had notable and long-lasting symptoms of "brain fog" and exhaustion, which had an impact on their cognitive abilities and overall quality of life. Emerging evidence suggests that individuals who recover from COVID-19 infections may face an elevated risk of developing long-term cognitive deficits or impairments [38]. Understanding the fundamental causes of cancer requires studying gene variants linked to the disease. The same goal guided this study's investigation of the potential relationship between the Iraqi population's susceptibility to breast cancer and the CAT (rs7943316) SNP. Because it plays a protective function in reducing oxidative damage, the antioxidant enzyme catalase is encoded by the CAT (rs7943316) gene, which is special [39].

Conclusions

Older people are more likely to experience more severe SARS-CoV-2 illness, those with chronic medical conditions, and those who have high viral loads (low Ct values) are risk factors that are positively associated with high D-Dimer, LDH, and ferritin concentration. In recovered individuals with cognitive impairment, despite the occurrence of 66.7% cognitive impairment in the GG rs10759931 genotype, there are no statistically significant between cognitive impairment and studied TLR4 SNPs. Polymorphism G/A rs10759931 of TLR4 required more information to explore their role in cognitive impairment and infection severity.

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7. Ethical responsibilities of authors

The present research procedure was authorized by the local ethics committee of Baghdad University's College of Science (Ref: CSEC/0923/0059).

6. Disclosure and conflict of interest

The authors declare that they have no conflicts of interest.

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