CD14 C(-260)T Polymorphism and Blood Levels of the Soluble Endotoxin Receptor CD14, their Association with Risk of Ischemic Stroke in Iraqi Populations

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Abstract

Background: Stroke is a main cause of death and adult long-term disability worldwide, and stays as an enormous burden for society due to lack of illustrated etiology and effective treatments. **Aim:** the present study was conducted to investigate the association of *CD14* genotype and plasma levels of soluble(s) CD14 with risk of ischemic stroke. **Methods :** A total of 40 patients with ischemic stroke were included in the study who were admitted to hospital from the period between March to August 2019, and other groups consist of 40 apparently healthy individuals. A five ml of blood samples were collected, 2 ml of each sample for polymerase chain reaction amplification and detection of CD14 technique. The remaining (3ml) for CD14 monocyte count by Flow cytometry technique. **Results**: *CD14* C(-260)T genotype was not significantly associated with increased risk of IS (P= 0.367). However, sCD14 plasma levels were higher in subjects with TT genotype compared with those with CT or CC genotype (P= 0.013). The count of CD14 Monocytes, in Ischemic Stroke patients was significantly higher than that of healthy control group. **Conclusion:** a significant correlation between *CD14* genotype and sCD14 levels were higher level in subjects with TT genotype compared with those with CT or CC genotype.

Keyword: *Ischemic Stroke, CD14, sCD14; blood Levels; health; long-term disability*

Introduction

Stroke is a main cause of death and adult long-term disability worldwide, and stays as an enormous burden for society due to lack of illustrated etiology and effective treatments[(1)]. Annually, approximately 800,000 people in the United States have a stroke, and 130,000 die[(2)]. It has accounted for nearly 5.7 million deaths worldwide, and 87% of these deaths occur in low and middle-income countries[(3)]. Ischemic stroke (IS) accounts for 85% of overall stroke and its pathophysiology are regulated by a combination of lifestyle, environmental, and unclear genetic risk factors[(4)]. According to the report from the Centers for Disease Control and Prevention, given in 2013 mortality from stroke was the fourth leading cause of death in the United States in 2008. Therefore, it is important to know the reason for this social burden so that safe and effective therapeutic treatment that could be given at medical services would improve the outcome of millions of acute stroke patients[(5)]. Cluster of differentiation 14 (CD14) acts as a multifunctional high-affinity receptor for the binding of endotoxins, lipopolysaccharides (LPS), and other bacterial wall components that are involved in primary immune and inflammatory responses[(6)], which is an important glycoprotein expressed as membrane CD14 on the surface of monocytes, neutrophils, and macrophages and soluble CD14 (sCD14) in the serum[(7)]. CD14 transfers the LPS and other bacterial signals through the LPS-binding protein/CD14/myeloid differentiation factor 2 (MD-2)/Toll-like receptor (TLR)

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4 complex[8]. This multi-molecule complex triggers cascade signal amplification and activates innate host defense mechanisms, thereby promoting the release of cytokines and increase in antigen presentation, which stimulate an immune response[9]. *CD14* gene located on chromosome 5q23-31, which encodes a 55kDa glycoprotein with 375 amino acids[10]**.** The *CD14* gene contains several polymorphic sites, of which rs2569190 (C-260T, sometimes referred to as C-159T) is the most common polymorphism locus in the promoter region of the *CD14* gene[11]. *CD14* gene (rs2569190) has been associated with differential expression levels of CD14 in monocytes and macrophages. Moreover, in atherosclerotic disease, the T allele of–159C/T has been suggested to affect circulating levels of soluble CD14[12]. In 2000, Ito et al., [13] conducted the first case–control study and reported that rs2569190 C/T polymorphism is not associated with IS susceptibility. Wu et al.,[10] also suggested no significant associations between *CD14* rs2569190 C.T polymorphism and IS risk.

Materials and Methods

The current study was carried on 40 patients (18 males, 22 females) age range between 45-85 years from March to August2019. Other groups consist of 40 apparently healthy individuals (20 male and 20 female) without any history of systemic disease were clinically considered as healthy also included in this study as a control group. We excluded patients with hemorrhagic stroke, stroke associated with surgery, severe trauma or organ ischemia. A five ml of blood samples were collected by vein puncture using disposable syringes under aseptic technique 2 ml of each sample were transferred into with EDTA tube and immediately frozen at -20 C till further use to avoid repeated thawing and freezing for polymerase chain reaction amplification and detection of *CD14* (RFLP-PCR) technique. The remaining (3ml) were transferred into tube with EDTA maintained at room temperature until analysis sample at same day for CD14 monocyte count by Flowcytometry kit for Thermo Fisher/ Bioscience™ USA. This study was in agreement with ethics of Al-Diwaniya Teaching Hospital and verbal informed consent was obtained from all participants.

Results

Distribution of *CD14* C(260)T polymorphism was detected by RFLP-PCR technique, at this locus there are three genotypes, CT,CC and TT with band sizes 360/204/201/156 bp, 204/201/156 bp and 360/201 bp respectively, table (1), figure (1). The genotype distribution had no deviation from Hardy-Weinberg equilibrium in all study groups and agree with the reports of Xu et al[14]. Both groups were comparable in mean serum concentration CD14 expression table (2). Mean serum concentration of CD14 expression in ischemic stroke patients was significantly higher than that of healthy control 3.91±2.3versus 1.906±4.16 and P-value was ($P < 0.001$). Figure (2 and 3) showed the flow cytometric analysis of CD14 expression on Monocyte cells in ischemic stroke patients (A) and healthy control (B). Mean soluble CD14 levels were statistically significantly higher in patients bearing the TT genotype (p=0.013) compared with heterozygotes (CT) and those homozygous for the CC genotype, and this indicate significant correlation between soluble CD14 levels (pg/ml) and *CD14* Polymorphism in patients with IS, table(3).

Table (1): Distribution of *CD14* (−260C/T) Genotype and Alleles Frequency

Genotype	Controls (n=40)		Patients (n=40)		OR	95% Confidence interval		p	PF	EF
						Lower	Upper			
CD14 C(-260) T	N.	%	N.	%						
CT	15	37.5	18	45	1.466	0.3002	1.7915	0.496	……	0.141
CC	18	45	12	30	0.632	0.7612	4.7878	0.026	0.285	…..
TT	7	17.5	10	25	1.273	0.2150	1.8835	0.041	……	0.190

Cont... **Table (1): Distribution of *CD14* (−260C/T) Genotype and Alleles Frequency**

Overall P value		0.367								
Alleles Frequency										
C	51	63.75	42	52.5	0.795	0.3451	2.9957	0.149	0.204	….
T	29	36.25	38	47.5	1.256	0.3338	1.1833	0.149	….	0.204

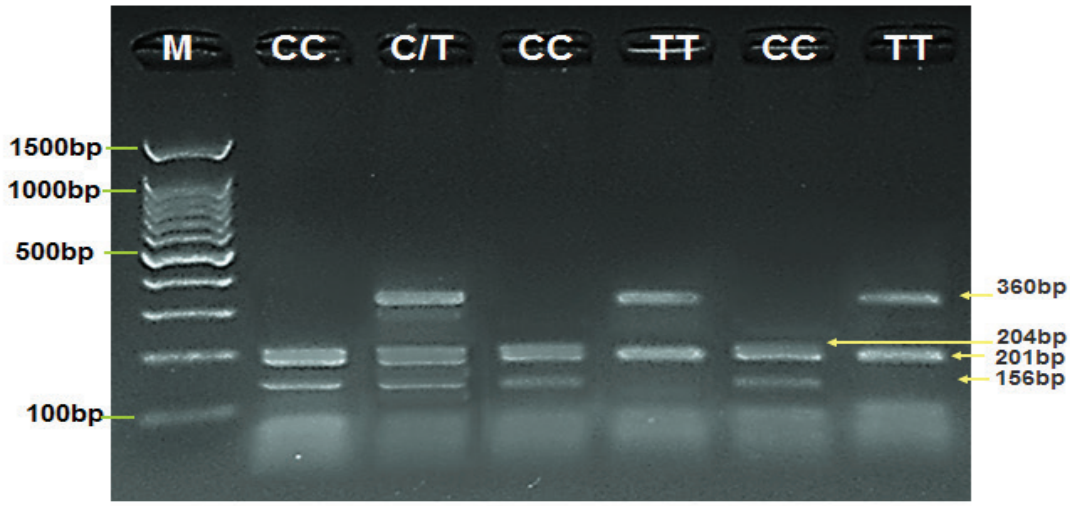

Figure (1): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of *CD14* −260C/T polymorphism by using HaeIII restriction enzyme. Where M: marker (1500-100bp), lane (CC) wild type homozygote products were digested by restriction enzyme at 204bp, 201bp and 156bp bands, lane (TT) mutant type homozygote, the products were digested by restriction enzyme into 360bp and 201bp bands. So that (C/T) heterozygote was showed as 360bp, 204bp, 201bp and 156bp bands.

Table(2):The compassion between the study groups regarding CD14 expression

	Patients	**Healthy control**	**P value**
Range	(0.49- 7.9)	(0.48- 4.2)	0.001 (S)
Mean	3.91	1.906	
SD	2.3	4.16	
SE	0.36	0.65	
N	40	40	

Table (3): Correlation between soluble CD14 levels (pg/ml) and *CD14* C(-260) T genotype in patients with IS

	CD14 genotype				
	CT Genotype	**CC Genotype**	**TT genotype**		
CD14 conc.pg/ml				F	P
Range	(0.55 - 7.9)	(0.49 – 6.7)	(1.18 – 7.8)	4.85	0.013 (S)
Mean	2.73	3.43	5.5		
SD	2.30	2.30	2.15		
SE	0.54	0.66	0.68		
N	18	12	10		

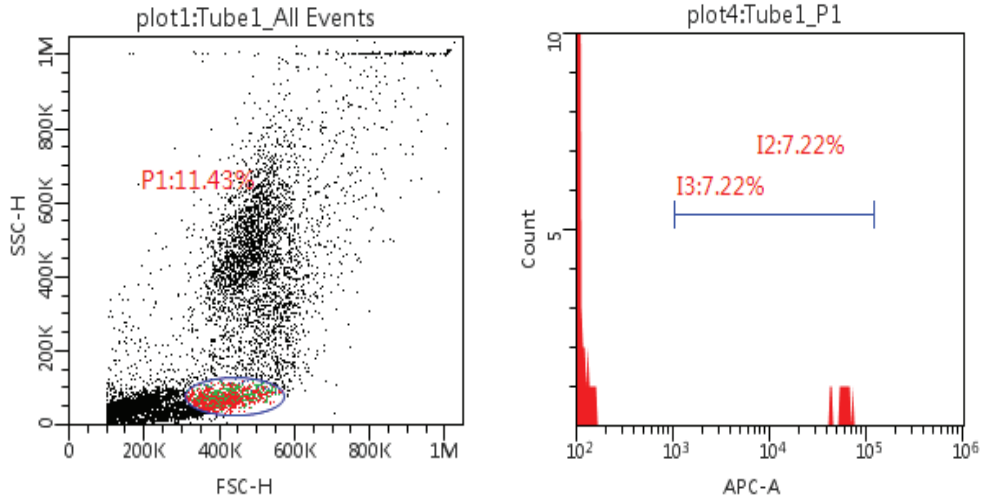

Figure (2): Results of Flow cytometry Analysis for CD14 detection on Monocytes of patients (APC (Allophycocyanin (Red color), FSC-H (Forward Scatter Height) and SSC-H (Side Scatter Height).

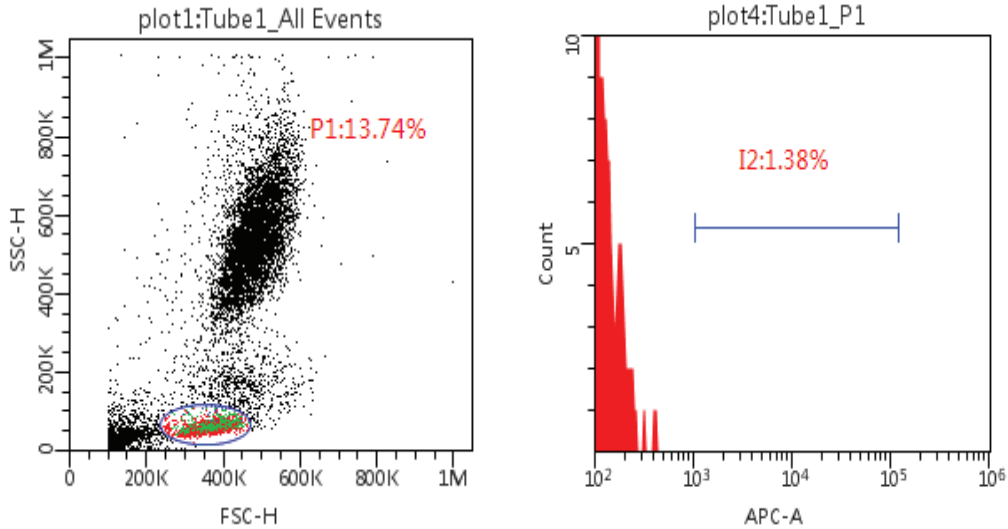

Figure (3): Results of Flow cytometry Analysis for CD14 detection on Monocytes of healthy control (APC (Allophycocyanin (Red color), FSC-H (Forward Scatter Height) and SSC-H (Side Scatter Height).

Discussion

Numerous studies have demonstrated the role of inflammatory markers in influencing cerebrovascular. Further, it has been seen that difference in distribution of inflammatory gene variants might increase or decrease the susceptibility to stroke attack[14]. *CD14* is another gene involved in inflammatory pathway. The C-260T (rs2569190) polymorphism is present in the promoter region of the gene and has been associated with differential expression levels of CD14 on monocytes/ macrophages[15]. Overall, the present result indicate no significant association between *CD14* (−260C/T) polymorphism and risk of ischemic stroke (p=0.367). The possible explanation may be that *CD14* rs2569190 C/T polymorphism is not involved in ischemic stroke susceptibility directly but plays a pathogenic role in synergy with other abnormally expressed proteins or gene polymorphisms[10]. Our results agree with Ito et al[13]; Xu et al[16], which investigated whether rs2569190 C/T polymorphism contributes to a disposition to ischemic stroke and did not find any significant association between rs2569190 C/T polymorphism and ischemic stroke susceptibility.

The results of present study showed high frequency of (TT) genotype among patients when compared to healthy controls, these result identify (TT) genotype is associated with increased risk of ischemic stroke with statistically significant (P=0.041). and may be considered as the etiological factor for IS. The results of present study consistence with result of Lichy et al[17], which found that the TT genotype is associated with a risk of the stratified micro- or macro-angiopathic ischemic stroke. Moreover, the present result revealed frequency of allele C was more in healthy control compared to ischemic stroke patients. Also showed that (CC) genotype frequency in patients (30%) lower than in healthy controls (45%) and this results agree with results Park et al[18], which found that (CC) frequency in patients (15.2%) lower than controls (23.2%). So CC genotype and C allele may be considered as a preventive fraction to give a protection from IS.

The present study revealed that the level of immune markers (CD14) became significantly increased among cases with ischemic stroke (3.91± 2.3pg/ml) when compared with healthy controls (1.906± 4.16 pg/ml), these results indicate significant association between Monocytes (CD14) and ischemic stroke (p<0.001). Monocytes are characterized by the expression of several clusters of differentiation, such as CD14 in human[19]. The authors have analyzed the potential role of monocytes in the development of the pathology of ischemic stroke[20]. Several studies have indicated that due to the breakdown of tissue, numerous danger-associated molecular patterns (DAMPs) are generated by the brain to induce this type of immunosuppression, which eventually results in stroke-associated infection (SAI) with increased mortality[21]. Following ischemic stroke, microglia, which are brain resident macrophages, are activated and circulating immune cells, such as monocytes, neutrophils and lymphocytes are recruited to injured site[22]. There may be specialized roles for those monocyte subsets in stroke pathophysiology and they might become targets for therapeutic intervention if their mechanisms are more precisely elucidated[23]. Our results agree with Klimiec et al[24], which found that high levels of plasma sCD14 are reported in patients with ischemic stroke compared to heathy control (1330 [1140–1500] vs 1070 [921–1220] ng/mL, p<0.001) and these level closely associated with the risk of death and Mendel et al[25], who showed high CD14 levels may be associated with vascular endothelial cell damage, which facilitates atherosclerosis formation and increases the risk of ischemic stroke subsequently.

Results showed significant correlation between soluble CD14 levels (pg/ml) and *CD14* Polymorphism in patients with ischemic stroke (p=0.013), were we found higher soluble CD14 levels in the TT genotype (5.5± 2.15 pg/ml) as compared with CT or CC genotype (2.73± 2.3 pg/ml and 3.43± 2.3 pg/ml respectively). The polymorphism C260T of the *CD14* promoter has been shown to increase transcriptional activity. This enhanced transcriptional activity has been associated with higher concentrations of sCD14[26]. A potential functional role of rs2569190 on *CD14* has been suggested as it alters a Sp1 transcription factor binding site and modulates the activity of promoter[27]. The T allele of rs2569190 decreases the strength of the bond between the *CD14* promoter GC box and Sp consensus sequence, changes the transcriptional capacity, and increases the protein expression level of CD14[28]. Our results agree with results of many previous studies which had suggested that the *CD14* (rs2569190) polymorphisms will increase

sCD14 levels in homozygous carriers of T allele[29][30].

Conclusions

Our results do not confirm an independent and clinically relevant relationship of *CD14* gene polymorphism with risk of IS. Patients who undergone IS showed a higher level of CD14 expression than healthy control. In this study we found a significant correlation between *CD14* genotype and sCD14 levels were higher level in subjects with TT genotype compared with those with CT or CC genotype.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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