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Role of *TLR4* (C1196T) and *CD14* (C-260T) Polymorphisms in Development of Ischemic Stroke, Its Subtypes and Hemorrhagic Stroke

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Abstract In the present study, we evaluated the association of *TLR4* and *CD14* polymorphisms, i.e. C1196T and C-260T, respectively, with ischemic stroke (n = 700), its subtypes and hemorrhagic stroke (n = 300) in a South Indian population from Telangana. The genotypes were determined using PCR–RFLP, and the strength of association between genotypes and stroke was determined by odds ratio with 95% confidence interval (CI) and chi-square analysis. The results revealed a lack of association for *TLR4* variant with ischemic stroke and hemorrhagic stroke, although a significant association was observed with the subtypes extracranial large artery (p = 0.008), other determined aetiology (p = 0.03) and undetermined aetiology (p = 0.01). Investigations on the variant of *CD14* gene revealed negative association among ischemic stroke patients; however, a significant association was observed for hemorrhagic stroke following dominant and recessive genotypic model (p = 0.05, p = 0.02). Among ischemic stroke subtype, a significant association was observed with intracranial large artery, extracranial large artery, other determined aetiology and undetermined aetiology form of stroke (p < 0.01). Further, analysis of the *CD14* variant between the two major stroke types revealed a significant difference in genotype distribution following the co-dominant genotypic model (p = 0.01).

Keywords Ischemic stroke · Haemorrhagic stroke · TOAST classification · Inflammation · *TLR4* · *CD14*

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Introduction

Stroke is a common cause of mortality and chronic adult disability worldwide, with more than four fifth of all strokes occurring in developing countries (Banerjee and Das, 2016). Stroke has been broadly classified into two types, i.e. ischemic stroke (IS) that accounts for the majority of cases (70–80%) and the least treatable and deadlier form known as hemorrhagic stroke (HS) (15–20%). In understanding the pathophysiology of ischemic and hemorrhagic stroke, inflammation plays a vital role as it leads to inflammatory responses by exacerbating secondary brain injury in acute stage of stroke and also contributes beneficially to the brain in recovery after stroke (Jin et al., 2013). Synthesis of inflammatory cytokines is an important mechanism during ischemic brain injury that determines whether a stroke will lead to reversible defect or permanent damage (Danton and Dietrich, 2003; Rivest, 2009). However, in HS, secondary damage is triggered by presence of intraparenchymal blood that activates cytotoxic, oxidative, innate immunity and inflammatory responses (Xi et al., 2006; Aronowski and Zhao, 2011).

Toll-like receptors (TLRs) are a conserved large receptor family of pattern recognition that demonstrate a critical role in innate immunity and inflammatory responses (Akira et al., 2006; Kong and Lee, 2011). Eleven TLRs have been found in humans of which TLR2 and TLR4 are specifically reported to contribute in brain damage of mice, and studies among IS patients suggests both TLRs to be associated with poor

outcome (Brea et al., 2011; Caso et al., 2008; Yang et al., 2008). TLR4 specifically has been found to be upregulated in conditions like cardiomyopathies, cardiac dysfunction, neurodegeneration and also with differences in lipopolysaccharide (LPS) responsiveness that cause differential production of proinflammatory cytokines. Enhanced expression of TLR4 activates transcription factor, nuclear factor-kβ (NF-kβ) that induces expression of proinflammatory cytokines in conditions like atheroma, atherosclerosis and neuronal damage which are important risk factors for stroke (Frantz et al., 1999; Edfeldt et al., 2002; Lehnardt et al., 2003; Arbour et al., 2000; Shichita et al., 2012; Xu et al., 2001; Markus et al., 2006). Similarly, CD14 is yet another gene involved in inflammatory pathway and is a receptor for bacterial LPS that mediates cell activation by LPS (Kane and Havel, 1999). It is present on cell surface of mature cells of monocyte lineage and macrophages that trigger production of cytokines involved in inflammatory, thrombotic and coagulatory mechanisms (Schumann et al., 1994). 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. It increases the binding affinity of specificity protein (Sp) transcription factors leading to increase in CD14 production. In atherosclerotic disease, following LPS stimulation, the T allele of C-260T has been suggested to affect circulating levels of soluble CD14 (sCD14) (Baldini et al. 1999; Hubacek et al. 1999; Eng et al. 2004; Gu et al. 2008). Similarly, the non-synonymous SNP Thr399Ile (rs4986791, C1196T) of *TLR4* has been associated with reduced cytokine responses following LPS stimulation (Marsik et al. 2005). Therefore, it can be said that the inflammatory responses might get modulated in the bearers of these two variants.

Since stroke is a heterogenous, polygenic and complex disease arising from a combination of environmental, vascular and genetic factors, it becomes important to deduce the role of such factors in different ethnicities. Therefore, studying the role of inflammatory gene *TLR4* (C1196T) and *CD14* (C-260T) variants in IS, its subtypes and HS might be helpful in studying the association of susceptible genes with stroke pathogenesis. Hence, the present study aims to study these two polymorphisms in association with stroke disposition among a South Indian cohort from Telangana.

Materials and Methods

Subjects

Seven hundred IS patients (males to females = 504:196) and 300 HS patients (males to females = 213:87) presenting with new stroke evaluated in the neurology department of Nizam's Institute of Medical Sciences, Hyderabad (Telangana, India) between September 2007 and December 2016 were included in the study. The study was approved by the ethical committee of the study hospital and by the institutional ethics committee. All the patients were examined by a qualified stroke neurologist and stroke types were differentiated by computed tomography (CT) scans and magnetic resonance imaging (MRI). All the patients underwent CT scan as well as MRI. Patients with major cardiac, hepatic, renal, endocrinological disorders, skeletal disorders and cancerous diseases were excluded from the study. IS was classified into subtypes according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification (Adams et al., 1993). As a control group, 700 healthy individuals (males to females = 504:196) matched for sex and age were recruited from the same demographic area. The controls had no clinical evidence of any cerebrovascular disease. Information on demographic features and risk factors was collected using a structured questionnaire. Hypertension, alcohol use, diabetes and smoking were defined as reported previously (Munshi et al., 2008). Subjects included in the study were above the age group of 18 years and all the samples were collected only after obtaining the written informed consent.

DNA Isolation and Genotyping

Two milliliters of venous blood was collected in EDTA-coated tubes after confirmation by neurologists around a varied time interval of 6–24 h (from patient to patient) after onset of stroke. Genomic DNA was extracted using standard phenol–chloroform method, and the polymorphisms in *TLR4* (C1196T) and *CD14* (C-260T) gene were analysed using PCR–RFLP approach (Laine et al., 2005).

Statistical Analysis

Hardy–Weinberg equilibrium was tested, and association between genotypes of IS, its subtypes and HS patients was examined by odds ratio (OR) with 95% confidence interval (CI) and chi-square analysis using OpenEPI6 software. Allelic frequencies were calculated according to the number of different alleles observed and the total number of alleles examined. All statistical tests were done on SPSS 18 and statistical significance was defined as $p < 0.05$.

Results

Seven hundred IS patients, 700 age- and sex-matched controls and 300 HS patients from the same demographic area were included in the study. All the patients belonged to a South Indian population from Telangana. The clinical

Table 1 Clinical characteristics of ischemic stroke patients, hemorrhagic stroke patients and controls

Characteristics	Ischemic stroke patients (n = 700)	Controls (n = 700)	p value (ischemic stroke vs. controls)	Haemorrhagic stroke patients (n = 300)	p value (hemorrhagic stroke vs. csontrols)	p value (ischemic stroke vs. hemorrhagic stroke)
Age	50.2 (24.9)	49.8 (12.6)		55.4 (13.5)		= 0.007
Male:female	504:196	504:196		213:87		
Systolic BP (mmHg) (mean ± S.D)	141.3 (8.6)	123.4 (4.1)	= 0.0001	145.4 (6.6)	= 0.0001	= 0.0001
Diastolic BP (mmHg) (mean ± S.D)	88.2 (5.3)	79.8 (5.6)	= 0.0001	88.6 (5.2)	= 0.0001	= 0.27
Total cholesterol (mean ± S.D)	198.7 (36.4)	195.8 (44.4)	= 0.18	173.0 (40.3)	= 0.0001	= 0.0001
Triglycerides (mean ± S.D)	175.1 (38.0)	138.6 (40.5)	= 0.0001	129.9 (35.5)	= 0.001	= 0.0001
Random glucose (mean ± S.D)	131.4 (36.5)	120.4 (24.4)	= 0.0001	130.7 (24.7)	= 0.0001	= 0.76
HDL cholesterol (mean ± S.D)	53.4 (14.4)	58.8 (15.8)	= 0.0001	74.3 (12.8)	= 0.0001	= 0.0001
Hypertension	58.9%	31.7%	< 0.0001	56.3%	< 0.0001	= 0.44
Diabetes	44.1%	26.6%	< 0.0001	51.3%	< 0.0001	= 0.03
Smokers	43.7%	31.4%	< 0.0001	57.6%	< 0.0001	= 0.0001
Alcohol use	33.7%	26%	= 0.001	56%	< 0.0001	< 0.0001
Family history of stroke	24.3%	10%	< 0.0001	6.7%	= 0.09	< 0.0001

Age, systolic BP, diastolic BP, total cholesterol, high density lipoprotein (HDL) cholesterol, random glucose and triglycerides are given as mean (SD). p values were calculated using Student's paired t test (SPSS 18)

characteristics of IS patients, HS patients and controls have been given in Table 1. Mean age was 50.2 years for IS patients, 55.4 years for HS patients and 49.8 years in controls. Risk factor profile of IS patients revealed

Table 2 Analysis of clinical characteristics between the three study samples

Characteristics	Sample type and size	Mean(SD) or percentage	p value
Age	Controls, n = 700	49.8 (12.6)	0.000
	Ischemic stroke, n = 700	50.2 (24.9)	
	Hemorrhagic stroke, n = 300	55.4 (13.5)	
Systolic BP (mmHg)	Controls, n = 700	123.4 (4.1)	0.000
	Ischemic stroke, n = 700	141.3 (8.6)	
	Hemorrhagic stroke, n = 300	145.4 (6.6)	
Diastolic BP (mmHg)	Controls, n = 700	79.8 (5.6)	0.000
	Ischemic stroke, n = 700	88.2 (5.3)	
	Hemorrhagic stroke, n = 300	88.6 (5.2)	
Total cholesterol (mg/dl)	Controls, n = 700	195.8 (44.4)	0.000
	Ischemic stroke, n = 700	198.7 (36.4)	
	Hemorrhagic stroke, n = 300	173.0 (40.3)	
HDL (mg/dl)	Controls, n = 700	58.8 (15.8)	0.000
	Ischemic stroke, n = 700	53.4 (14.4)	
	Hemorrhagic stroke, n = 300	74.3 (12.8)	
Triglycerides (mg/dl)	Controls, n = 700	138.6 (40.5)	0.000
	Ischemic stroke, n = 700	175.1 (38.0)	
	Hemorrhagic stroke, n = 300	129.9 (35.5)	
Random Glucose (mg/dl)	Controls, n = 700	120.4 (24.4)	0.000
	Ischemic stroke, n = 700	131.4 (36.5)	
	Hemorrhagic stroke, n = 300	130.7 (24.7)	

p values were calculated using ANOVA (SPSS 18)

Table 3 Distribution of *TLR4* (C1196T) and *CD14* (C-260T) genotypes and allelic frequencies in ischemic stroke patients, hemorrhagic stroke patients and controls

	CC	CT	TT	Total	C	T	Total
TLR4 (C1196T)							
Ischemic stroke	527 (75.3%)	109 (15.6%)	64 (9.1%)	700	1163 (0.83)	237 (0.17)	1400
Control	539 (77%)	98 (14%)	63 (9%)	700	1176 (0.84)	224 (0.16)	1400
Hemorrhagic stroke	224 (74.7%)	49 (16.3%)	27 (9%)	300	497 (0.83)	103 (0.17)	600
CD14 (C-260T)							
Ischemic stroke	171 (24.4%)	394 (56.3%)	135 (19.3%)	700	736 (0.53)	664 (0.47)	1400
Control	174 (24.8%)	391 (55.9%)	135 (19.3%)	700	739 (0.53)	661 (0.47)	1400
Hemorrhagic stroke	86 (28.7%)	144 (48%)	70 (23.3%)	300	316 (0.53)	284 (0.47)	600

hypertension in 58.9%, diabetes in 44.1%, smoking in 43.7%, alcohol use in 33.7% and family history of stroke in 24.3% subjects. In the control group, 31.7% had hypertension, 26.6% were diabetic, 31.4% smokers, 26% were alcohol users and 10% had a family history of stroke. Profiles of patients for the various risk factors in HS revealed hypertension in 56.3%, diabetes in 51.3%, smoking in 57.6%, alcohol use in 56% and family history of stroke in 6.7% of patients. Analysis was also done for the clinical characteristics between the 3 study samples for age; systolic and diastolic pressure; total cholesterol, HDL and triglycerides and random glucose. The results showed a significant difference ($p < 0.001$) (Table 2).

Table 4 Analysis of *TLR4* (C1196T) and *CD14* (C-260T) genotypes and alleles among ischemic stroke patients and controls

Genotypes	OR (95% CI)	$\chi 2$	*p* value
TLR4 (C1196T)			
Ischemic stroke with controls			
TT vs. CC	1.03 (0.71–1.50)	0.04	= 0.83
CT vs. CC	1.13 (0.84–1.53)	0.71	= 0.39
TT vs. CT	0.91 (0.58–1.42)	0.16	= 0.68
Dominant			
CC vs. CT + TT	0.90 (0.71–1.16)	0.56	= 0.45
Co-dominant			
CT vs. CC + TT	1.13 (0.84–1.52)	0.68	= 0.40
Recessive			
TT vs. CC + CT	1.01 (0.70–1.46)	0.008	= 0.92
Alleles			
C vs. T	0.93 (0.76–1.14)	0.43	= 0.50
T vs. C	1.07 (0.87–1.30)	0.43	= 0.50
CD14 (C-260T)			
Ischemic stroke with controls			
TT vs. CC	1.01 (0.74–1.39)	0.01	= 0.91
CT vs. CC	1.02 (0.79–1.32)	0.03	= 0.84
TT vs. CT	0.99 (0.75–1.30)	0.002	= 0.95
Dominant			
CC vs. CT + TT	0.97 (0.76–1.24)	0.03	= 0.85
Co-dominant			
CT vs. TT + CC	1.01 (0.82–1.25)	0.02	= 0.87
Recessive			
TT vs. CC + CT	0.96 (0.72–1.27)	0.08	= 0.77
Alleles			
C vs. T	0.99 (0.85–1.15)	0.01	= 0.9
T vs. C	1.00 (0.86–1.17)	0.01	= 0.9

OR odds ratio, *CI* confidence interval

The genotypic distribution and allelic frequencies of *TLR4* (C1196T) and *CD14* (C-260T) in IS patients, HS patients and controls have been given in Table 3. Analysis for the different genotypic models for IS patients with controls revealed non-significant association for both the polymorphisms (Table 4). Similarly, analysis of HS patients vs. controls revealed non-significant association for *TLR4* (C1196T) gene variant. However, for *CD14* (C-260T) variant, a significant difference was observed between HS patients vs. controls following dominant and recessive genetic models [For CC vs. CT + TT, $\chi 2 = 3.76$, $p = 0.05$, odds ratio = 1.40 (0.99–1.99); For TT vs. CC + CT, $\chi 2 = 5.20$, $p = 0.02$, odds ratio = 0.72 (0.55–0.95)] (Table 5). On analysing the genotypic models for IS patients vs. HS patients, a non-significant difference was observed for *TLR4* (C1196T), whereas a significant difference was observed for CT vs.CC and TT vs. CT genotypes ($p = 0.05$ and $p = 0.04$, respectively) and following co-dominant genotypic model for the *CD14* (C-260T) variant [For CT vs. TT + CC, $\chi 2 = 5.79$, $p = 0.01$, odds ratio = 1.39 (1.06–1.83) (Table 6).

These results were further confirmed by multiple logistic regression analysis using all other risk factors and *TLR4* (C1196T) and *CD14* (C-260T) genotypes. It revealed that the most predictive independent risk factors in ischemic stroke cases and controls were age, systolic and diastolic blood pressure, hypertension, diabetes, smoking, family history of stroke and triglycerides ($p = 0.000$). Similarly, for hemorrhagic

Table 5 Analysis of *TLR4* (C1196T) and *CD14* (C-260T) genotypes and alleles among hemorrhagic stroke patients and controls

Genotypes	OR (95% CI)	$\chi 2$	*p* value
TLR4 (C1196T)			
Hemorrhagic stroke with controls			
TT vs. CC	1.00 (0.86–1.17)	0.01	= 0.90
CT vs. CC	1.03 (0.64–1.66)	0.01	= 0.89
TT vs. CT	1.20 (0.82–1.75)	0.92	= 0.33
Dominant			
CC vs. CT + TT	0.85 (0.48–1.51)	0.28	= 0.59
Co-dominant			
CT vs. CC + TT	0.88 (0.64–1.20)	0.63	= 0.42
Recessive			
TT vs. CC + CT	1.19 (0.82–1.74)	0.91	= 0.33
Alleles			
C vs. T	1.08 (0.84–1.40)	0.41	= 0.51
T vs. C	1.08 (0.84–1.40)	0.41	= 0.51
CD14 (C-260T)			
Hemorrhagic stroke with controls			
TT vs. CC	0.91 (0.71–1.18)	0.41	= 0.51
CT vs. CC	1.04 (0.71–1.54)	0.05	= 0.80
TT vs. CT	0.74 (0.54–1.02)	3.22	= 0.07
Dominant			
CC vs. CT + TT	1.40 (0.99–1.99)	3.76	= 0.05
Co-dominant			
CT vs. TT + CC	1.21 (0.89–1.64)	1.58	= 0.20
Recessive			
TT vs. CC + CT	0.72 (0.55–0.95)	5.20	= 0.02
Alleles			
C vs. T	1.00 (0.82–1.21)	0.02	= 0.96
T vs. C	0.99 (0.82–1.20)	0.02	= 0.96

OR odds ratio, *CI* confidence interval

stroke cases and controls, the most predictive independent risk factor were gender (male), systolic and diastolic blood pressure, hypertension, diabetes, smoking, alcoholism, HDL and *CD14* (C-260T) CC genotype ($p = 0.000$) and cholesterol ($p = 0.002$).

Examining the association of *TLR4* (C1196T) with IS subtypes, we found significant association with extracranial large artery (ELA) [$p = 0.008$, OR = 0.60 (95% CI, 0.41–0.88)], other determined aetiology (ODE) [$p = 0.03$, OR = 2.09 (95% CI, 1.04–4.20)] and undetermined aetiology (UDE) [$p = 0.01$, OR = 1.74 (95% CI, 1.10–2.77)], whereas in *CD14* (C-260T), a significant association for TT genotype was observed in intracranial large artery (ILA) [$p = 0.00008$, OR = 1.5 (95% CI, 1.22–1.83)], ELA [$p = 0.0009$, OR = 1.72 (95% CI, 1.24–2.40)], ODE [$p < 0.01$, OR = 0.25 (95% CI, 0.16–0.40)] and UDE [$p < 0.01$, OR = 0.44 (95% CI, 0.32–0.59)] (Table 7).

Discussion

Numerous studies have demonstrated the role of inflammatory markers in influencing cerebrovascular and cardiovascular diseases (CVD). Further, it has been seen that difference in distribution of inflammatory gene variants might increase or decrease the susceptibility to stroke attack. Therefore, profiling of genetic markers is an essential prerequisite for better understanding of stroke pathogenesis (Banerjee et al., 2008). Our earlier association studies on IS and HS involved inflammatory gene SNPs like *SELE* (S128R), *CRP* (1059G>C), *CCL11* (-1382G>A) (Roy et al., 2014a; 2014b, Das et al., 2014a; 2014b) and other genes like *ACE* (I/D), *MTHFR* (677C>T) and *APOE* (E2, E3 and E4) (Das et al., 2015a; 2015b; 2016). However, information on inflammatory gene variants specifically among the Indian population is negligible and therefore, we evaluated the role of *TLR4* (C1196T) and

Table 6 Analysis of *TLR4* (C1196T) and *CD14* (C-260T) genotypes and alleles among ischemic stroke and hemorrhagic stroke patients

Genotypes	OR (95% CI)	$\chi 2$	*p* value
TLR4 (C1196T)			
Ischemic stroke with hemorrhagic stroke			
TT vs. CC	0.99 (0.82–1.20)	0.002	= 0.96
CT vs. CC	1.00 (0.62–1.62)	0.0009	= 0.97
TT vs. CT	1.05 (0.72–1.53)	0.08	= 0.76
Dominant			
CC vs. CT + TT	1.03 (0.75–1.41)	0.04	= 0.83
Co-dominant			
CT vs. CC + TT	0.94 (0.65–1.36)	0.09	= 0.76
Recessive			
TT vs. CC + CT	1.01 (0.63–1.63)	0.005	= 0.94
Alleles			
C vs. T	1.01 (0.78–1.31)	0.01	= 0.89
T vs. C	0.98 (0.76–1.26)	0.01	= 0.89
CD14 (C-260T)			
Ischemic stroke with hemorrhagic stroke			
TT vs. CC	0.96 (0.65–1.43)	0.02	= 0.87
CT vs. CC	1.37 (0.99–1.89)	3.78	= 0.05
TT vs. CT	0.70 (0.49–0.99)	3.93	= 0.04
Dominant			
CC vs. CT + TT	0.80 (0.59–1.09)	1.97	= 0.16
Co-dominant			
CT vs. TT + CC	1.39 (1.06–1.83)	5.79	= 0.01
Recessive			
TT vs. CC + CT	0.78 (0.56–1.08)	2.10	= 0.14
Alleles			
C vs. T	0.99 (0.82–1.20)	0.001	= 0.96
T vs. C	1.00 (0.82–1.21)	0.001	= 0.96

OR odds ratio, *CI* confidence interval

Table 7 *TLR4* (C1196T) and *CD14* (C-260T) genotypic and allelic frequencies in ischemic stroke patients classified according to TOAST classification

TOAST classification	No. of patients	Genotype (%) *TLR4*(C1196T)			Allelic frequencies		Odds ratio	(95% CI)	*p* value
		CC	CT	TT	C	T			
Large artery atherosclerosis	361								
Intracranial large artery	273	188 (68.9)	68 (24.9)	17 (6.2)	444 (0.8)	102 (0.2)	0.82	(0.64–1.07)	= 0.15
Extracranial large artery	88	66 (75)	2 (2.3)	20 (22.7)	134 (0.8)	42 (0.2)	0.60	(0.41–0.88)	= 0.008
Small artery occlusions (lacunar)	91	71 (78)	7 (7.7)	13 (14.3)	149 (0.8)	33 (0.2)	0.86	(0.57–1.28)	= 0.46
Cardioembolism	82	59 (71.9)	20 (24.4)	3 (3.7)	138 (0.8)	26 (0.2)	1.01	(0.64–1.57)	= 0.96
Other determined aetiology	54	46 (85.2)	7 (12.9)	1 (1.9)	99 (0.9)	9 (0.1)	2.09	(1.04–4.20)	= 0.03
Undetermined aetiology	112	98 (87.5)	6 (5.4)	8 (7.1)	202 (0.9)	22 (0.1)	1.74	(1.10–2.77)	= 0.01
TOAST classification	No. of patients	Genotype (%) *CD14*(C-260T)			Allelic frequencies		Odds ratio	(95% CI)	*p* value
		CC	CT	TT	C	T			
Large artery atherosclerosis	361								
Intracranial large artery	273	84 (30.8)	174 (63.7)	15 (5.5)	342 (0.6)	204 (0.4)	1.5	(1.22–1.83)	= 0.00008
Extracranial large artery	88	32 (36.4)	52 (59.1)	4 (4.5)	116 (0.7)	60 (0.3)	1.72	(1.24–2.40)	= 0.0009
Small artery occlusions (lacunar)	91	4 (4.4)	81 (89)	6 (6.6)	89 (0.5)	93 (0.5)	0.85	(0.62–1.16)	= 0.32
Cardioembolism	82	10 (12.2)	70 (85.4)	2 (2.4)	90 (0.5)	74 (0.5)	1.08	(0.78–1.50)	= 0.61
Other determined aetiology	54	8 (14.8)	8 (14.8)	38 (70.4)	24 (0.2)	84 (0.8)	0.25	(0.16–0.40)	< 0.0000001
Undetermined aetiology	112	33 (29.5)	8 (7.1)	71 (63.4)	74 (0.3)	150 (0.7)	0.44	(0.32–0.59)	< 0.0000001

CD14 (C-260T) gene polymorphisms in a South Indian cohort from Telangana.

TLRs are master regulators of innate immunity and play an important role in inflammatory responses during infections and injuries. Majority of studies on role of TLRs in the brain include experiments on rodent models that suggest TLR2 and TLR4 to be involved in cerebral ischemic/reperfusion injury and its activation leading to exacerbation of brain injury (Hyakkoku et al., 2010). Apart from animal studies, there are few clinical reports that have focussed on the association of *TLR2* and *TLR4* polymorphisms with prevalence of stroke. Most of the studies strongly suggest that variations in *TLR4* influence acute ischemic stroke neurological outcome, alterations in systemic markers of inflammation and carotid plaque development (Weinstein et al., 2014; Gardener et al., 2011). In our study involving IS and HS, no significant association for any of the genetic models was observed for C1196T variant of *TLR4* gene. Further, there was no significant difference in the genotypic difference between IS and HS, and in the case of IS subtypes, significant association is reported for ELA, ODE and UDE subtypes. To the best of our knowledge, there are no reports evaluating the association of C1196T variant of *TLR4* with stroke although reports on other variants do exist. A study by Lin et al., 2005 among ethnic Chinese from Taiwan reports positive association for *TLR4* (C119A) variant with overall stroke (Lin et al., 2005). Similarly, a recent study also report rs1927911 to be a risk factor for atherosclerotic cerebral infarction (ACI) among Han population (Song et al., 2015). Other studies, however, have documented negative association among Caucasians, Americans and Chinese (Reismann et al., 2004; Lalouschek et al., 2006; Enquobahrie et al., 2008; Yuan et al., 2010). A recent finding suggests a direct link between immune signalling dysregulation and post-stroke fatigue (PSF) via TLR4. In this experiment, two well-known *TLR4* variants, i.e. Asp299Gly and Thr399Ile, were evaluated to explore the possibility that post-stroke fatigue is related to systemic inflammation and both the SNPs were reported to be associated with lower levels of fatigue (Becker et al., 2015).

As far as the other inflammatory gene *CD14* (-260C/T) variant is concerned, we found no association with IS. However, a significant association was observed with HS following the dominant and recessive genotypic model. A significant difference was observed between IS and HS following the co-dominant genotypic model. In case of IS subtype, there was a significant association with intracranial large artery, extracranial large artery, stroke of other determined aetiology and stroke of undetermined aetiology. Ito et al., (2000) were the first to study this promoter polymorphism and found a negative association for it with CVD. The study group included atherothrombotic, lacunar and transient ischemic attack patients, and measurement of serum concentrations of sCD14 and mCD14 in relation to genotypes showed a non-significant difference (Ito et al., 2000). Following this, a

nested case-control Physician's Health Study (PHA) and three other studies by different groups also documented a lack of association for the *CD14* (-260C/T) polymorphism with stroke (Zee et al., 2002; Lalouschek et al., 2006; Park et al., 2006; Kis et al., 2007. A similar lack of association has also been documented in a South German population cohort. However, the TT genotype significantly associated with risk of atherosclerotic/microangiopathic stroke (Lichy et al., 2002). Similarly, another SNP of *CD14*, i.e. -159C/T, was reportedly found to be associated with increased common carotid artery intima-media thickness among smokers (Risley et al., 2003; Jerrard-Dunne et al., 2004). However, a study among Indian population for the -159C/T variant and meta-analysis studies by Mishra et al., 2016 and Banerjee, 2009 suggest this polymorphism to be non-significantly associated with risk of IS (Banerjee et al., 2008; Misra et al., 2016; Banerjee, 2009). Nevertheless, this polymorphism was reportedly associated with increased inflammation in atherosclerosis and reportedly has less effect on CVD than that on coronary heart disease (CAD) (Giacconi et al., 2007).

Conclusion

In conclusion, our study revealed a significant association for clinical risk factors like age, systolic and diastolic blood pressure, total cholesterol, HDL, triglycerides, hypertension, smoking, alcoholism, diabetes and family history of stroke with ischemic and haemorrhagic stroke development. Genetic association study revealed a lack of association for *TLR4* (C1196T) variant with IS and HS, but on analysing the association of the variant in IS subtypes, a significant association was observed for ELA, ODE and UDE subtypes. Similarly, studying the *CD14* (-260C/T) variant revealed significant association for dominant and recessive genotypic model only in HS, while in IS subtype, it was found to be associated with ILA, ELA, ODE and UDE subtypes. A significant difference was also observed in the genotypes of patients between IS and HS for *CD14* (-260C/T) variant that revealed a significant association following co-dominant genotypic model.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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