Association of LPL Gene Variant and Serum LPL Level with Ischemic Stroke in Iraqi Population

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Abstract

Background: Lipoprotein lipase (LPL) plays an important role in plasma lipoprotein metabolism. An increasing number of studies have suggested an association of LPL gene variants with the risk of cardiovascular and cerebrovascular diseases.

Objective: To analyze whether *Hind III* polymorphism of LPL gene and serum LPL level are associated with ischemic stroke in Iraqi population.

Method: Fifty ischemic stroke patients (clinical diagnosis and x-ray CT) and fifty controls were enrolled in this case–control study. The LPL *Hind III* polymorphism was determined by PCR-RFLP technique and LPL mass level was estimated using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA).

Results: In the present research was not found any association between the *Hindlll* LPL gene polymorphism and acute ischemic stroke in the population studied; the allele and genotypic frequencies of the studied polymorphism was similar in cases and controls and followed the Hardy-Weinberg equilibrium. The relationship between Hind III genotypes and the LPL mass level was analyzed using ANOVA and further confirmed by Post-hoc analysis. there was no significant difference in LPL mass levels between the genotype groups H+H+, H+H-, and H-H- (p value>0.05).

Conclusions: The Hind III polymorphism of LPLis not a genetic marker for the development of ischemic stroke as well as not determinants of serum LPL level in the Iraqian sample used.

Keywords: *Protein lipoprotein, lipase, ischemic stroke, polymorphism association risk.*

Introduction

Cerebrovascular diseases are characterized by a neurologic deficit,due to a focal vascular lesion in the central nervous system, it includes cerebral infarction, intracranial hemorrhage and subarachnoid hemorrhage[1]. Studies have posited the lipoproteinlipase (LPL) gene as the most viable candidate for study due to its contribution to the interindividual variability in lipid levels and their consequent role in atherosclerosis, as well as to the possible correlation between hyperlipidemia and CVD[2,3]. Evidence suggests that variations in the lipoprotein lipase gene may influence stroke risk[4]. LPL has a major role in triglyceride (TG)-rich lipoprotein metabolism by catalyzing the hydrolysis of TG in chylomicrons and very low-density lipoproteins to form chylomicron and very low-density lipoprotein remnants, respectively[5,6]. The human LPL gene is located on chromosome 8p22, spans approximately 35 kb and contains 10 exons encoding a 448 amino acid mature protein[7,8]. Genetic studies have revealed almost 100 mutations and single nucleotide polymorphisms in lipoprotein lipase genein humans[9]. A *HindIII* polymorphism located on intron 8 has been associated

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with subtle alterations in plasma lipids in which a replacement of thymine (T) with glutamine (G) base occurs at position +495 abolishing the *HindIII* restriction enzyme recognition site [10], and has been associated with hyperlipidemia and diabetic nephropathy[11]. Biochemical assessment of LPL function has also frequently been used to assess the role of LPL in atherogenesis. However, these studies are hampered by the need to administer heparin intravenously to release sufficient LPL from the endothelium to measure reliably LPL activity and LPL concentration. Because heparinizationis time-consuming, not standardized, and induces bleeding risk, most investigators have only studied limited numbers of diseased and/or non-diseased individuals. Recently, the availability of a highly sensitive enzyme-linked immunosorbent assay, which can measure accurately freely circulating LPL concentration in non-heparinized serum has provided a tool to more easily assess the relationship between LPL and ischemic stroke. It was recognized that the majority of serum LPL is catalytically inactive and likely represents a mere catabolic product of catalytically active LPL that is bound to the endothelium. Also, it was demonstrated that serum LPL concentration is not associated with post-heparin LPL concentration or LPL activity [12].It has been demonstrated that a reduced concentration of plasma LPL mass is associated with an increased risk of coronary artery disease[13-15].

Materials and Method

Pre heparin LPL mass measurement: Blood samples for LPL mass measurements were withdrawn from subjects and then put in plane tube without anticoagulants to obtain serum. The levels of LPL mass was measured by the sandwich enzyme-linked immunosorbent assay (ELISA).

DNA isolation and genotyping: Five ml of blood was collected in EDTA tubes. Genomic DNA wasextracted from blood samples using the INTRON (korea) kit. The LPL *HindIII* polymorphism in intron 8 was analyzed by PCR-RFLP technique. The primers used for the amplification of the LPL gene bearing the polymorphism are Forward: 5'-TGAAGCTCAAATGGAAGAGT-3' and Reverse5'- TACAAGCAAATGACTAAA-3'. The amplification protocol involved a denaturation of the DNA segment at 94°C for 30 sec, then annealing the segment at 50°C for 30 sec and an extension segment at 72°C for 45 min, this repeated for 35 cycles and the final extension for 5 min. The amplified 715 bp PCR product was digested with *HindIII* restriction enzyme by incubating at 37 °C for 2 hours followed by separation of fragments on 1.5% agarose gelforone hour at 90 volt. *Hind*+ve allelewas detected as fragments of 600 bp and 115 bp base pairs (bp) while as *Hind*−ve allele was detected as fragment of 715 bp.

Results

The variables of diabetes,Hypertension and Smoking were presented with statistically significant difference.

The levels of preheparin LPL mass in ischemic stroke patients and the control group: Serum lipoprotein lipase level (median with interquartile range Q1 and Q3) in ischemic patients was137.8 (83.75,164.8) and not significantly different from that of control subjects 116.0 (80.97,127.1) as shown in the figure 1. The Mann-Whitney U value was 723.5 and the p value was 0.10.

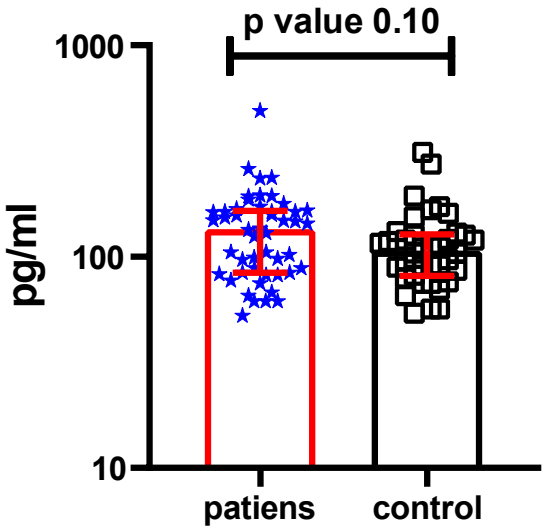


Fig 1: serum lipoprotein lipase (pg/ml) among patient and control groups. median with Q1 and Q3.

Genotyping: Polymorphisms allowed making the allele assignation in each sample for each polymorphism (Fig. 2).

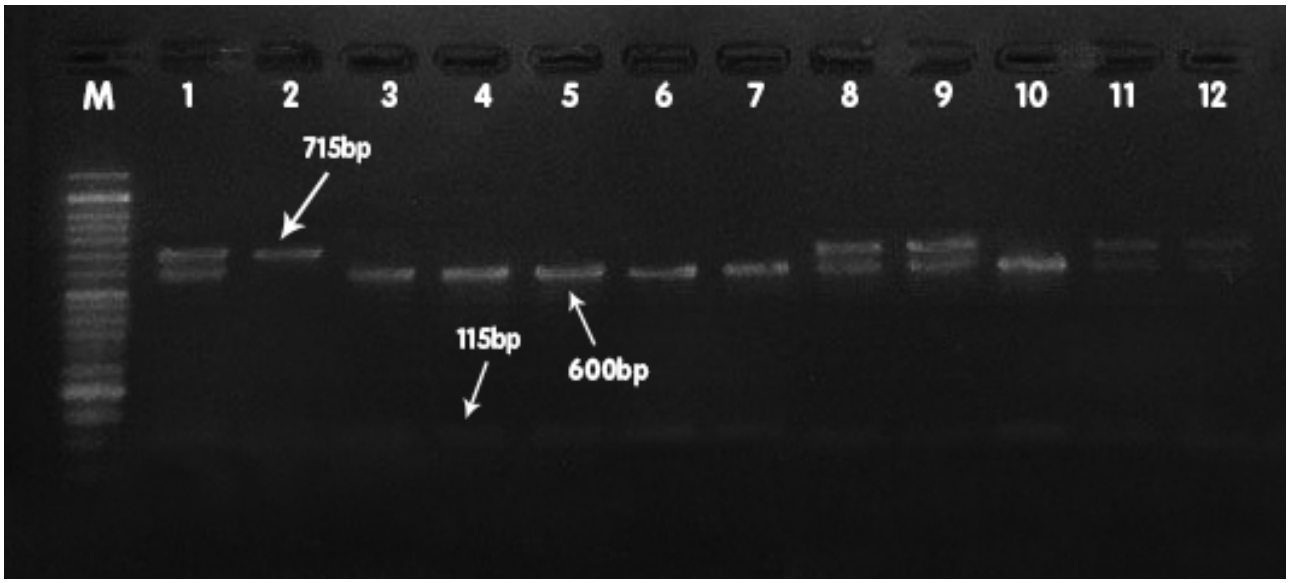


Figure 2: Genotyping of *Hindlll* polymorphisms in lipoprotein lipase gene. Restriction fragments length polymorphisms (RFLP) on 1.5 % agarose gel at 90 volt/hour.DNA 50 bp marker.

Lane 3,4,5,6,7,10 : H+H+ genotype hemozygote for presenting of 600bp and 115bp.Lane 1,8,9,11,12 : H+H-gentype heterozygote for presenting of 715, 600, and 115bp. Lane 2 : H-H- genotype homozygote for absence of cutting site .

Genotype and allele frequencies: The genotypes and allele frequency between the patients with ischemic stroke and the healthy subjects were in agreement with the Hardy–Weinberg equilibrium. The genotype distribution and the allele frequency for LPL gene polymorphisms are summarized in Table 1. Most frequent genotype was H^+H^+ in ischemic patients and control subjects 29(58%), and 34(68%) respectively. H^+H^+ in control is higher than of patients. H-H- was the lowest frequent genotype in both study population, and was in patients 3(6%) lower than of controls 6(12%). H+H- was the intermediate genotype among both patients 18(36%) and control 10(20%) and was higher in patients.

Table 1: The Genotype Distribution and the Allele Frequency of LPL-Hind III gene Polymorphism among Study Groups

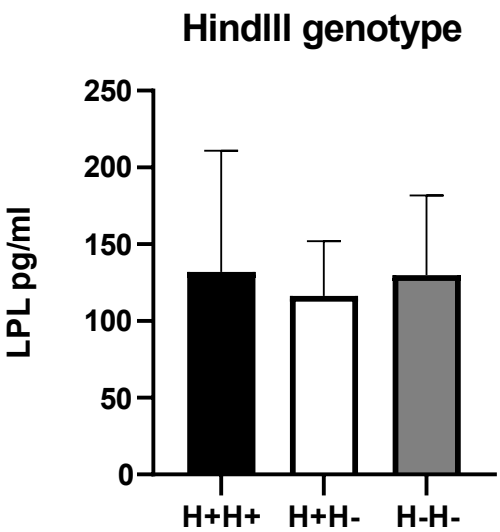
Study group	LPL genotype(%)			Total Allelic frequencies	
	H+H+	H+H-	H-H-	H+	H-
Case	29(58%)	18(36%)	3(6%)	38(0.76)	12(0.24)
Control	34(68%)	10(20%)	6(12%)	38(0.78)	12(0.22)

Association between polymorphisms and stroke: In order to study a possible association between the polymorphisms and stroke develop, we calculate OR value for each of the inheritance patterns. We did not find association by evaluating OR between polymorphisms and stroke development (Table 2).

Table 2: Association for each inheritance patter with stroke

Polymorphism	Model	Genotype	OR	CI 95%	P value
rs320	Codominant	H+H+	1.00		0.159
		H+H-	0.474	0.189- 1.187	
		H-H-	1.706	0.391- 7.43	
	Dominant	H+H+	1.00		0.30
		H+H-, H-H-	0.65	0.287- 1.472	
	Recessive	H+H+,H+H-	1.00		0.295
		H-H-	2.136	0.503- 9.067	

The relationship between HindIII polymorphism and pre heparin LPL level: The relationship between *HindIII* polymorphism and pre heparin LPL levels has been given in figure 3. In present study, no significant difference in serum LPL levels between genotype groups H+H+, H+H-, and H-H-, with mean and standard deviation 132.0 ± 78.87, 116.3 ± 35.76, 129.8 ± 52.05, respectively as shown in figure Fig(3). p value 0.622.



Fig(3): Serum lipoprotein lipase (pg/ml) among H^+H^+, H^+H^-, and H^-H^- genotypes.

Discussion

To the best of our knowledge, this is the first study to evaluate the association between *Hindlll* polymorphisms of the LPL gene and ischemic stroke in Iraqi population. Data showing no significant association between lipoprotein lipase gene polymorphism *Hindlll* and stroke in the Iraqi simple analyzed; suggesting that this polymorphism cannot be used as genetic markers to predict risk for stroke development. A number of studies have suggested the implication of *HindIII* polymorphism located on intron 8 of LPL gene in the pathophysiology of cardiovascular and cerebrovascular diseases[14-15]. Recently, we have noted that *HindIII* variants in the LPLgene have association with atherothrombotic cerebral infarction. However, a study carried out by Xu et al. [16-17] in a Chinese population could not establish an association between *HindIII* polymorphism of LPL gene with atherosclerotic cerebral infarction. Study occur in 2020 demonstrate association between *Hindlll* and coronary artery disease [18]. *Thorn et al*. have reported a significant association of *HindIII*+ve allele with severe coronary atherosclerosis[19-21]. *Chen et al*. reported a significant correlation between carotid artery atherosclerosis and Hind III polymorphism in White male subjects. Furthermore, meta-analysis study suggested that LPL *HindIII* variants were associated with a decreased risk of stroke in the Asian population, but not in the non-Asian population [22]. Another meta-analysis indicated that risk of stroke was decreased in rs320 polymorphisms in the LPL gene [23]. *Munshi et el.* suggests that the HindIII polymorphism of LPL is significantly associated with ischemic stroke risk[24]. However, *Velásquez et el* published that the HindIII polymorphism no associated with ischemic stroke in Latin America [25]. Allele frequencies for the HindIII polymorphism in our study were nearly to those found in in the dbSNP database for east Asia population and genetic studies done in different ethnic groups including (USA)[26], This may be due to LPL levels are controlled by many factors, including differential transcriptional regulation in adipose and skeletal muscle tissue, post-translational modification and translocation over the endothelium, retro-endocytosis, binding to heparan sulfate- containing proteoglycans, lipoproteins, and receptors, and hepatic clearance[27-30] in our knowledge

no previous study has been reported to demonstrate the relationship between the ischemic stroke and this marker. The present study showed no significant differences of LPL levels among LPL genotypes, so *Hindlll* was not be as determinant of LPL level. The genetic determinants of preheparin LPL mass and the separate relationships between LPL gene polymorphism and serum LPL mass with ischemic stroke is unknown. And also information on LPL mass is severely lacking, in particular with respect to interaction with gene polymorphism. Our data in inconsistent with that reported on 640 middle-aged Chinese, in whom LPL-HindIII and PvuII polymorphisms were found to be determinants of preheparin plasma LPL concentration, which was in turn independently modified by smoking and obesity [31].

Conclusion

In our study, the presence of the *Hindlll* polymorphism of the LPL gene was not a risk factor for the development of ischemic stroke and not significantly associated with the LPL mass level as well as the LPL level not associated with increased risk of ischemic stroke.

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

Conflict of Interest: None

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