Cholesteryl Ester Transfer Protein (CETP) I405V (rs5882) Polymorphism affects plasma lipid parameters and lipoprotein ratio in hyperlipidemic ischemic stroke patients

Authors' contributions

*This work was carried out in collaboration among both authors. Both authors read and approved the final manuscript***.**

ABSTRACT

Background

High-density lipoprotein cholesterol (HDL-C) and other lipoproteins are metabolized in part by the cholesteryl ester transport protein (CETP). Cardiovascular risk and the occurrence of ischemic stroke are linked to polymorphism in the CETP gene.

Methodology

For the study, 100 ischemic stroke patients and 100 controls with matched sexes and ages ranging from 46 to 87 were chosen. Lipoprotein ratios were computed using Excel software, and lipid parameters were evaluated using Randox diagnostic kits. Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) and 2% gel electrophoresis were used to genotype the CETP gene. The genotyping of the CETP gene were performed by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) combined with 2% gel electrophoresis.

Results

There were significant difference ($P<0.0001$) in the genotypic and allelic frequencies of CETP SNP between the healthy and patients with ischemic stroke. The frequencies of I/I, I/V and VV genotypes of the CETP gene were 48%, 37% and 15% for the control and 17%, 33% and 50%, for the stroke subjects, respectively. The frequencies of I and V alleles were 67% and 33% for the control and 37.5% and 62.5% for the stroke subjects, respectively. The V allele carriers of CETP gene had higher plasma TC, TG, VLDL-C, LDL-C, Non-HDL-C, defective HDL-C, HDL_2-C and HDL_3-C when compared to the I allele carriers for both subjects. The V allele carriers were responsible for the increase in dyslipidemia for both subjects.

Conclusion

The results of this study show that mutation of CETP I405V (rs5882) polymorphism causes an increased in plasma TC, TG, VLDL-C, LDL-C, Non-HDL-C, defective HDL-C, HDL_2-C and HDL_3-C concentration and is associated with an increased risk of ischemic stroke.
Keywords: Cholesteryl ester transfer protein, ischemic stroke, lipid parameters, SNP.

1.0 INTRODUCTION

The greatest cause of death worldwide is cardiovascular disease (CVD) [1]. It belongs to a group of conditions that affect the heart, blood vessels (veins, capillaries, and arteries), brain, kidney vascular disorders, cardiac illness, peripheral arterial diseases, and other conditions that have an impact on the cardiovascular system [1]. "The second most frequent cause of death and disability in most countries is stroke. Stroke is a subclass of cardiovascular disease and is the second greatest cause of mortality worldwide" [2]. "A study found that ischemic strokes account for 80% of all stroke types. They happen when a thrombus or embolism blocks a major cerebral artery, which causes reduced blood flow, a significant reduction in the delivery of oxygen, glucose, all other nutrients, and disruption of the nutrition and waste exchange process necessary to support brain metabolism" [2]. "Cerebral ischemia, which results in the death of neurons within the perfusion territory of the damaged blood arteries, will occur if cerebral arterial blood flow is not quickly restored" [2].

"According to different studies, dyslipidemia, which is defined as having high levels of serum or plasma triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and apolipoprotein B (ApoB), and low levels of high density lipoprotein cholesterol (HDL-C) and apolipoprotein A (ApoA), is a risk factor for the development of atherosclerosis, stroke, and other cardiovascular diseases" [1,2]. Research works have shown that plasma lipid parameters are modulated by genetic factors [1,2,] as well as environmental factors such as hypertension [3], demographics [4], diet [5], exercise [6], cigarette smoking [7,8], alcohol consumption [7], and obesity [9]. "Lipid profiles and lipoprotein ratios have been employed to predict the risk of ischemic stroke and other cardiovascular diseases. Examples of some of these lipoprotein ratios are: LDL- C/HDL-C, TC/HDL-C, HDLC/LDL-C, HDL-C/TC, Atherogenic coefficient (AC) = (TC-HDL-C)/HDL-C, TG/HDL-C and Logarithmic transformation of TG/HDL-C (Atherogenic index of plasma i.e AIP)" [1, 2, 10]. In a study carried out by Jeppesen et al. [11], it was observed that "the better ability of different lipoprotein ratios to predict cardiovascular disease compared to single lipid marker is of clinical important and may explain the association of lipid

ratios with a cluster of cardiovascular risk factors that are at least in part unrelated to cholesterol metabolism".

"Cholesteryl Ester Transfer Protein (CETP) is an enzyme that (glycoprotein) contains 476 amino acids with molecular weight of 74 kilo Dalton. CETP helps in the transfer of cholesteryl esters, triglycerides, retinal ester and phospholipids. CETP facilitates the transport of triglycerides and cholesteryl esters between lipoproteins. This enzyme promotes the transfer of cholesteryl esters from high density lipoprotein (HDL) to apolipoprotein B containing lipoproteins, such as low density lipoprotein (LDL) and VLDL in exchange for triacylglycerols" [12, 13]. "Cholesteryl Ester Transfer Protein plays an important role in reverse cholesterol transport metabolism, as changes in CETP activity are inversely correlated with plasma HDL-C concentrations" [14]. "CETP inhibitors significantly raise plasma HDL-C level. HDL-C level has atheroprotective properties and low HDL-C concentrations are the most common lipid abnormality in patients with premature cardiovascular disease" [15].

"Cholesteryl ester transfer protein gene (Gene ID: 1071) is a single gene consisting of a 25 kb genomic DNA and is located on the long arm of chromosome 16 adjacent to the lecithin cholesterol acyltransferase (LCAT) (16q12–16q21). It consists of 15 introns and 16 exons" [16, 17]. "Studies have shown that genetic changes in CETP gene may lead to change in the level of serum or plasma and function of CETP and therefore affect the level of HDL-C and LDL-C in the plasma or serum" [15, 18-20]. "Some examples of CETP gene polymorphisms include: -629C/A, I405V, D442G and Taq1B and may cause diseases due to their influence on serum lipids parameters" [21-25]. "The cholesteryl ester transfer protein I405V (rs5882) polymorphism is located in a known binding domain and it causes an isoleucine/valine shift, leading to decreased protein levels". Takata et al. [26] study observed that "CETP promoter -1337C >T polymorphism may affect plasma CETP concentration and lipid profile, in patients with familial hypercholesterolaemia".

"Two different CETP polymorphisms (R451Q and A373P) located in the coding region of the gene have been clearly studied. The minor alleles of these two different polymorphisms, Q and P, appear at a low frequency in the general population, each having a minor allele frequency of 2–7% in Western European cohorts" [27, 28]. "The minor alleles of these polymorphisms have been associated with lower HDL-C levels" [27] and "higher Cholesteryl Ester Transfer Protein

activity" [29]. The study evaluates the effect of Cholesteryl Ester Transfer Protein I405V Polymorphism on lipid parameters and lipoprotein ratios in patients with ischemic stroke.

2.0 MATERIALS AND METHODS

2.1. Study subjects

For the analysis, the clinical and laboratory data of 100 patients with ischemic stroke who visited the Lagos University Teaching Hospital (LUTH) in southwest Nigeria were gathered. Blood samples from 100 ischemic stroke victims were obtained and analyzed. All of the ischemic stroke patients had cerebral computer tomography performed, which revealed cerebral infarction, and they were all diagnosed as ischemic stroke by neurologists at LUTH. 100 people with the same age range (46–87) and socioeconomic background as the stroke patients make up the control group. Heparin vacutainers and ethylenediaminetetraacetic acid (EDTA) bottles were used to collect blood samples from healthy people and ischemic stroke patients who had fasted for 12 to 16 hours. Patients and healthy people alike received questionnaires and consent papers. Also, ethical approval was attained. The stroke and control subjects not willing to participate in the study were excluded from the study

2.2. Determination of plasma lipid profiles and lipoprotein ratios

EDTA-containing tubes were used to collect blood, which resulted in a final concentration of 0.1%. Red blood cells and plasma were separated by centrifugation at 1500 X g for 15 min at 4^{o}C. Randox kits were used to measure the Total Cholesterol (TC), Triglyceride (TG), and HDL-Cholesterol (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Antrim, United Kingdom).

HDL_2-C = HDL-C – HDL_3-C

VLDL-C equals to TG/5, Non-HDL-Cholesterol is equal to TC minus HDL-Cholesterol and LDL-C=TC - HDL-C –TG/5

The following were used to compute the atherogenic ratios:

TC/HDL-C is the Castellis risk index I (CRI-1)

LDL-C/HDL-C = Castellis Risk Index II (CRI-11)

The ratios of HDL-C/LDL-C and TG/HDL-C were also determined.

AIP = log TG/HDL-C, or atherogenic index of plasma.

Atherogenic coefficient (AC) was calculated as (TC - HDL-C)/HDL-C.

The method outlined by Momoh et al. [2] was used to compute the lipid and lipoprotein ratio. Dyslipidemia was defined according to Momoh et al. [2] and National Cholesterol Education Program 2001[30]. Dyslipidemia was defined by the presence of one or more abnormal plasma lipid indexes.

2.3. Isolation of DNA from blood sample

"Following the manufacturer's recommendations, genomic DNA was extracted from peripheral blood leukocytes using DNA Qiagen kits. Until analysis, the isolated DNA was kept at 4°C. Using the NANODROP *1000*^{R} (Thermo Fisher Scientific, USA) spectrophotometric method, the amount and quality of extracted DNA were determined. The method measured the amount of extracted DNA in nanogrammes per microliter (ng/L) and evaluated the quality (purity) based on the ratio of absorbance at 260nm:280nm for all the samples" [31].

2.4. Amplification of CETP gene

Polymerase Chain Reaction (PCR) technique was used to amplified the DNA samples. The essential PCR reagents and primers were used along with a PCR machine (TECHNE TC-4000) to amplify the CETP genes. Using primer pairs and the polymerase chain reaction, the CETP gene was amplified. The substitution of valine for isoleucine is caused by an A→G mutation at codon 405 in exon 14 of the CETP gene on chromosome 16. Exon 14 was amplified by PCR with primers located in intron 13 (F: 5'-AATGCTTGTCCAGGCCGTGCAGCAT-3') and in intron 14 (R: 5'-CAGTTTCCCCTCCAGCCCACACTTA-3') using methods described by Agerholm-Larsen et al., [28]. The PCR cycling conditions for the CETP SNPs were as follows: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 2 minutes. All PCR reactions were carried out in a total volume of 23 µl containing 2.5 µl dNTPs, 2 nM $MgCl_2$, 1µl of each primer, and 1 unit of AmpliTaq polymerase (Perkin Elmer England), 2.5 µl PCR buffer and 13.8µl grade water. Negative controls were included in each set of reaction.

2.5. Amplification and genotyping of CETP gene

The DNA samples were genotyped by using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. The genotype of the CETP gene was done

by polymerase chain reaction with the use of the primer pairs. Exon 14 was amplified by PCR with primers located in intron 13 (F:5'-AATGCTTGTCCAGGCCGTGCAGCAT-3') and intron 14 (R: 5'-CAGTTTCCCCTCCAGCCCACACTTA-3'). The PCR Cycling conditions for the CETP SNPs were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 2 minutes. The PCR products were then digested with 1 µL restriction enzyme *Fok1* at 37°C. Briefly, 1 µL of restriction enzyme (*Fok1*) was added to 1 µL of PCR amplicon and 10x NEBuffer (5 µL) in a reaction volume of 50 µL. The solution was mixed properly and incubated for 37^{o}C for maximum of 15 minutes. The digested PCR products were followed by electrophoresis on 2% agarose gel containing ethidium bromide. To validate the results, genotyping experiments were repeated for all samples. The genotyping of these samples were completely consistent. The genotypes identified were named according to the absence or presence of the enzyme restriction sites, when an I to V transversion at nucleotide position of the CETP. The I/I genotype is homozygote for the absence of the site (band at 120 and 55 bp), I/V genotype is heterozygote for the presence and absence of the site (bands at 120, 85 and 55 bp), and V/V genotype is homozygote for the presence of the site (bands at 85 bp and 55 bp). A common band of 55 bp was found in all the DNA samples. A negative control was included in each set of the reaction.

2.6. Agarose gel electrophoresis of amplified CETP gene

Amplification was confirmed by electrophoresing of PCR amplicons on 2% w/v agarose gel stained with ethidium bromide, 0.5 mg/mL in Tris-borate EDTA. Electrophoresis was carried out at 120 V, 50 W, and 300 mA for 40 minutes. On completion of electrophoresis, bands were visualized with the gel documentation system (Infinity 3026, France). The sizes of the fragments obtained were estimated by comparison with the 50 bp DNA ladder (Jena Bioscience GmbH, Germany) run alongside with the negative control. The genomic DNA of all the subjects after amplification with PCR and imaged by 2% agarose gel electrophoresis, the purpose gene of 120 bp, 85 bp and 55 bp were found and a common band of 55 bp were found in all the DNA samples.

2.7. Statistical Analyses

The data are shown as Mean SD. The values of lipid parameters and lipoprotein ratios for control and stroke participants were compared between genotypes using GraphPad Prism version 5.01 software. Direct counting was performed to calculate allele frequency, and the Hardy-Weinberg equilibrium was checked using the usual goodness-of-fit test. For both distinct subjects, the significant difference between the wild type and mutant genotypes was compared using the one-way ANOVA Postdoc Turkey's test. Statistical significance was defined as a P-value < 0.05.

3.0. RESULTS

3.1. CETP Genotype Frequencies

This is the first time the research work will be carried out in Lagos, Nigeria. Two hundred subjects were genotyped. The CETP gene polymorphism was highly prevalent in the stroke subjects compared to the controls. For the control subjects: 48 were homozygous for the wild-type (Ile/Ile) and 52 were carriers of the V allele (37 I/V and 15 V/V). The stroke subjects had 17 homozygous for the Ile/Ile genotype and 83 were carriers of the V allele (33 I/V and 50 V/V). The frequencies of I/I, I/V and V/V genotypes for the CETP were 48%, 37% and 15% for the control while 17%, 33% and 50% were for the stroke subjects (P<0.0001) respectively. The frequencies of I and V alleles were 67% and 33% for the control subjects while 37.5% and 62.50% were for the ischemic stroke subject´s respectively. These frequencies did not differ from those predicted from the Hardy-Weinberg equilibrium. The allele frequencies were consistent with Hardy–Weinberg equilibrium for both control and stroke subjects (P = 0.8050 for control and 0.6650 for stroke subjects). The prevalence of the V allele was significantly higher in the ischemic stroke subjects compared to the control (62.50% Vs 33.00%).

Table 1. The number of observed and expected genotype of examined CETP SNP for the control and the ischemic stroke subjects according to Hardy-Weinberg equilibrium.

SNP	Genotype/ Allele	**Control subject**	**Stroke subject**	**P value; OR (95%CI)**

	Frequency					
CETP		Observed Frequency	Expected H-W Frequency	Observed Frequency	Expected H-W Frequency	
	I/I	48 (48%)	44.2225	17 (17%)	11.2225	
	I/V	37 (37%)	44.5550	33 (33%)	44.5550	
	VV	15 (15%)	11.2225	50 (50%)	44.2225	
P Value		0.6650		0.3350		0.33-0.67
X^2 Value		2.8753		6.7258		
	I	133 (66.50%)		67 (33.50%)		0.0001
	V	67 (33.50%)		133 (66.50%)		0.0001

Figure 1. L is DNA Ladder (50 – 500 bp), Lanes 1-5, 9, 11 – 13, and 15 are Ile/Ile genotypes (bands at 120 bp and 55 bp)

Lanes 6-8, and 14 are Ile/Val genotypes with 120 bp, 85 bp and 55 bp respectively. Lane 10 is Val/Val with 85 bp and 55 bp

Table 2: The effect of CETP gene genotypes on plasma lipid parameters for both control and ischemic stroke subjects.

Parameters	Control subjects			Ischemic stroke subjects		
	Genotype			Genotype		
	I/I (48)	I/V (37)	V/V (15)	I/I (17)	I/V(33)	V/V(50)
TC (mg/dl)	140.80 ±12.88[c]	165.60 ±10.38 [b]	173.10 ±9.32[a]	205.42 ±8.38[c]	209.83 ±11.47 [b]	222.13±11.30 [b]
TG (mg/dl)	105.50 ±11.45 [c]	133.20 ±12.84 [a]	108.80 ±11.04[b]	152.33 ±9.24[c]	159.60 ±10.02[b]	174.01±9.94[a]
HDL-C (mg/dl)	100.90 ±5.128 [c]	118.50 ±9.66 [b]	124.90 ±9.58[a]	47.47 ±3.83[b]	49.82 ±3.79[b]	58.61±4.37[a]
HDL_2-C (mg/dl)	27.29 ±0.93[c]	40.67±0.74[b]	42.98±0.66 [a]	16.08 ±0.16[c]	17.78 ±0.13[b]	19.12 ±0.10[a]
HDL_3-C (mg/dl)	73.61 ±2.21[c]	77.83 ±2.09[b]	81.92 ±2.01[a]	31.39 ±0.94[b]	32.04 ±1.03[b]	39.49±1.01[a]
VLDL-C (mg/dl)	21.10 ±2.29 [b]	26.64 ±2.57[a]	21.76 ±2.21[b]	30.47 ±1.85[c]	31.92 ±2.00[b]	34.80 ±1.99[a]
LDL-C (mg/dl)	18.80 ±1.67 [c]	20.46 ±1.87 [b]	26.44 ±1.86[a]	127.48 ±7.42[a]	128.09 ±8.27[a]	128.72.±8.37[a]
Non-HDL-C (mg/dl)	39.90 ±3.02 [b]	47.10 ±3.46 [a]	48.20 ±20.99[a]	157.95 ±7.32[a]	160.01±8.45[a]	163.52 ±8.59 [a]
TC/HDL-C	1.395±0.036[a]	1.397±0.031[a]	1.386±0.028[a]	4.327±0.121 [b]	4.212 ±0.182 [b]	3.790 ±0.106[a]
TG/HDL-C	1.046±0.016 [b]	1.124±0.022[a]	0.871±0.019[c]	3.209±0.091[a]	3.204±0.088[a]	2.969±0.094[b]
AC	0.395±0.042[a]	0.397±0.041[a]	0.386±0.039[a]	3.327±0.284[a]	3.212±0.278[a]	2.790±0.186[b]
AIP	0,020±0.001[b]	0,051±0.002[a]	-0.060±0.001[c]	0.506±0.009[a]	0.506±0.008[a]	0.473±0.008[b]
LDL-C/HDL-C	0.186±0.043[ab]	0.173±0.038[b]	0.212±0.038[a]	2.685±0.114[b]	2.571±0.108[b]	2.196±0.201[c]

HDL-C/LDL-C	5.367±0.328 [b]	5.792±0.336[a]	4.724±0.215[c]	0.372±0.014[c]	0.389±0.019[b]	0.455±0.023[a]

Data are presented as Mean ± SD (n=100). TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; Non-HDL-C, Non-high-density lipoprotein cholesterol, AC, atherogenic index; TC/HDL-C, total cholesterol/high-density lipoprotein-cholesterol; TG/HDL-C, triglyceride/high-density lipoprotein-cholesterol; AIP, atherogenic index of plasma. LDL-C/HDL-C, low-density lipoprotein-cholesterol/high-density lipoprotein-cholesterol; HDL-C/LDL-C, High-density lipoprotein-cholesterol/low-density lipoprotein-cholesterol. One-way ANOVA Posthoc Turkey´s test was used for comparing significant difference between wild type and mutant genotypes for both separate subjects. a=highest, b= medium, c=lowest. Those genotypes that have the same letters are not statistically significant ($P>0.05$) while those that have different letters are statistically significant ($P<0.05$).

4.0. DISCUSSION

The plasma cholesteryl ester transfer protein (CETP) promotes the exchange of triglycerides and cholesteryl esters between HDL-C and apo B-containing particles. It is thought that genetic variation in the CETP gene may be one of the risk factors for the onset of ischemic stroke due to CETP's primary function in the metabolism of lipoproteins. In this study, a nitrogenous base variation of Ile405Val in the CETP gene was determined by PCR-RFLP. The polymorphism in this study was presented in 52% (IIe/Val=37% and Val/Val=15%) of the non-symptomatic control and 83% (IIe/Val=33% and Val/Val=50%) of the ischemic stroke patients subjects. The present study showed that the frequencies of I and V alleles were 33.50% and 66.50% for the stroke and 66.50% and 33.50% for the control subjects respectively ($P<0.0001$). The V allele was significantly ($p<0.0001$) more abundant in the ischemic stroke subjects compared with the control subjects. The Ile405Val was shown to be associated with higher concentration of TG, TC, LDL-C, VLDL-C and dyslipidemia for both the control and the stroke subjects ($P<0.0001$). The Ile405Val genetic mutation of CETP gene is responsible for the significant ($P<0.0001$) increase in the plasma cholesterol and triglyceride levels of the homozygous and heterozygous mutant genotypes for both the control and ischemic stroke subject respectively. We found that the IIe/Val and Val/Val genotypes were responsible for higher HDL-C, HDL_2-C, and HDL_3-C ($P<0.0001$) levels for both the control and the stroke subjects. The SNPs in the CETP gene is responsible for higher ($P<0.0001$) increase in the production of defective HDL-C, HDL_2-C, and HDL_3-C levels as a result of dysfunctional cholesteryl ester transfer protein activity. This mean that increased concentration of these defective lipoproteins may lead to an impaired reverse total cholesterol transport and paradoxically increase the risk of ischemic stroke for both subjects. "The study reported that the common Ile405Val mutation in the CETP gene, was associated with increased levels of HDL-C, HDL_2-C, and HDL_3-C levels and this mutation is one of an independent risk factor for ischemic stroke patience that visited LUTH. HDL particles in the plasma or serum accept cholesterol from non-liver cells; CETP facilitates the transfer of cholesteryl ester onto triglyceride rich lipoproteins as part of the reverse cholesterol transport pathway, ultimately leading to cholesterol excretion by the liver" [32, 33]. When CETP is dysfunctional, cholesterol accumulates in HDL, and the transfer of cholesterol from peripheral cells to the liver is blocked and this may be responsible for the significant increase ($P<0.0001$) in plasma HDL-C, HDL_2-C and HDL_3-C as observed in our study. Different studies have shown

that homozygosity for Val405 was associated with increased HDL-cholesterol levels in 102 Japanese men with hypertriglyceridemia [34], in 234 Dutch men [21] and in 145 Icelandic men [35]. Complete CETP deficiency as seen in Japanese studies lead to massive elevated levels of HDL-cholesterol and apoA-I [36-39] and various studies have demonstrated that the Ile405Val mutation leads to reduced CETP activity [40] and mass [21, 34] in the plasma. “In another study, the association of CETP with HDL levels was observed and the study suggests that CETP is an atherogenic protein increasing the risk of myocardial infarction (MI)” [41]. Agerholm-Larsen et al. [28] study shows that “in a large population sample, it was observed that HDL-cholesterol level increases in both heterozygotes and homozygotes of Val405 in premenopausal women and in postmenopausal women not treated with HRT, whereas in hypertriglyceridemic men, only Val/Val homozygosity is associated with increased in HDL-cholesterol”. Their study also shows that “increased in HDL-cholesterol levels caused by mutations in CETP gene are associated with an increased risk of ischemic heart disease in white women” [28]. ApoA-I is found only in HDL-C and chylomicrons [42], the apoA-I concentration will be affected as a result of Ile405Val polymorphism in the CETP gene. In the present study, there is a clear increased risk of dyslipidemia in the control and the ischemic stroke subjects from Ile/Ile to Ile/Val to Val/Val respectively.

5.0 CONCLUSION

The results of this study show that the mutation of Cholesteryl ester transfer protein (CETP) Ile405Val (rs5882) polymorphism causes an increased in defective HDL-C, HDL_2-C and HDL_3-C concentration and is associated with an increased risk of ischemic stroke.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the author and producers of the products because I do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard or university standard, patient’s written consent was collected and preserved by the authors

ETHICAL APPROVAL

The research ethical approval was obtained from Lagos University Teaching Hospital Research and Ethical Committee with Healthy Research Committee assigned no: ADM/DCST/HREC/100. Control and stroke subjects who were not willing to participate in the research were excluded from the study.

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COMPETING INTEREST

Authors have declared that no competing interests exist.

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