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MTHFRC677T Polymorphism and Hyperhomocysteinemia in Ischemic Stroke Patients

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Abstract: *Background*: Homocysteine is an intermediate sulfur amino acid of methionine metabolism. Hyperhomocysteinemia, characterized by increased level of homocysteine, is an independent and modifiable vascular risk factor which metabolic pathway involves vitamins B_6, folate and vitamin B_{12}. *Objective*: We compared the prevalence of *MTHFRC677T* polymorphism, homocysteine folate and vitamin B_{12} in ischemic stroke patient's subgroups. *Methods*: We conducted a cross-sectional analytical study. The study included 128 consecutive ischemic stroke patients associated with hyperhomocysteinemia. The *MTHFRC677T* polymorphism was investigated by TaqMan probes (thermos Fisher Scientific) combined with polymerase chain reaction (PCR). We compared the prevalence of *MTHFRC677T* polymorphism and homocysteine level in ischemic stroke patient's subgroups. We adjusted the variable homocysteine level to the covariates, *MTHFR* polymorphism, folate and vitamin B_{12} with ANCOVA. *Results*: The sex ratio (men/women) was 1.5 with an average age of 60 years. The prevalence of *MTHFRC677T* polymorphism was 19.5% with 18% CT and 1.5% TT. Homocysteine level was 29.89 µmol/l in wildtype patients, 26.54 µmol/l in patients with CT genotype, and 56.17 µmol/l in patients with TT genotype (*t=2.04, p=0.033, CI 95% [0.017; 0.407]*). The *MTHFR* polymorphism prevalence, homocysteine, folate and vitamin B_{12} level did not differ between large brain infarction and multiple small brain infarction patients respectively (chi square: *Qobs=0.05, p=0.94, 95% CI; ttest: t=0.716, df=126, p=0.475, 95% CI*). *Conclusion*: The *MTHFRT677T* genotype increases homocysteine level. *MTHFR* polymorphism and homocysteine did not influence the subtypes of brain ischemic stroke.

Keywords: *MTHFRC677T* Polymorphism, Homocysteine, Ischemic Stroke

1. Introduction

Homocysteine is an intermediate sulfur amino acid of methionine metabolism. Hyperhomocysteinemia, characterized by increased level of homocysteine, is an independent and modifiable vascular risk factor which metabolic pathway involves vitamins B_6, B_9 or folate and vitamin B_{12} [1]. Hyperhomocysteinemia can have many causes, including nutritional, metabolic and/or genetic. Deficiency mainly in folate and in vitamin B_{12}, can cause hyperhomocysteinemia [2]. The primary mutation leads to hyperhomocysteinemia is *MTHFRC677T* [3]. A high prevalence (17.7%) of *MTHFRC677T* polymorphism in the coastal and savannah regions of Togo and Benin, combined with folate deficiency in the entire population was described previously [4].

Objective

We purposed to determine the contribution of the genetic factor notably the *MTHFRC677T* polymorphism in the occurrence of this hyperhomocysteinemia in relation to folate and vitamin B_{12} and other known vascular risk factors. We compared the prevalence of *MTHFRC677T* polymorphism,

homocysteine folate and vitamin B_{12} levels in subgroups of ischemic stroke patients and analyzed other associated clinical and biological vascular risk factors.

2. Methods

We conducted a cross-sectional analytical study. The study took place from July 2, 2012 to July 18, 2013 in neurology department of the teaching Hospital Campus of Lomé (Togo). Togo is a low-income West African country. The local ethical committee approved the study protocol. Patients had given their informed consent for the study. 205 consecutive patients were admitted for recent stroke during the study period. We first excluded 74 (according to our purpose, normal homocysteinemia is not a vascular risk factor) patients of ischemic stroke with normal homocysteine (≤ 15 µmol/l) and 3 patients with renal failure (creatinine > 106 mmol/l). The hospital prevalence of hyperhomocysteinemia was of 61% in 2010 [5].128 ischemic stroke patients (clinical and scanographic diagnosis) which had hyperhomocysteinemia (> 15 µmol/l), normal creatinine (< 106 mmol/l) without anti-epileptic treatment and vitamin B were included in this study. The small infarctions are ischemia less than 15 mm of diameter. Diabetes was defined by the history of the patient or by the presence of an antidiabetic treatment. Hypertension is defined by blood pressure greater than or equal to 140/90 mmHg. An electrocardiogram and a Doppler of supra-aortic trunks were made.

Biological tests: The determination of homocysteine was made by the Fluorescence Polarizing Immunoassay (FPIA) technique on the IMX analyzer. The SimulTRAC-SNB package of INC Pharmaceuticals was used for the simultaneous and quantitative radioimmunological dosage of vitamin B_{12} and folate in serum. Folate deficiency is defined by serum folate rate lower than 11 nmol/l and vitamin B_{12} lower than 147 pmol/l. Low density lipoprotein (LDL) cholesterol and creatinine dosage were measured by Lisa 500 Hycel machine.

Genotyping description:

The DNA was extracted by using the Puregene® DNA purification kit protocol for 4 ml of saliva samples collected in DNA Genotek tubes. The *MTHFRC677T* were genotyped by TaqMan SNP genotyping assays following the manufacturer's instructions. The genotype was called using the QuantStudio™ 7 Flex Real-Time polymerase chain reaction PCR System and Software (v 1.0) (Applied Biosystem).

Statistical analysis: We used ANCOVA of Stata (StataCorp. 2015. Stata: Release 14. Statistical Software College Station, TX: StataCorp LP) to determine the influence of *MTHFR* polymorphism, folate and vitamin B_{12} on homocysteine level. The homocysteine level was considered as the variable adjusted to the covariates: folate, vitamin B_{12}, the *MTHFRT677T* and CT genotype. We used t-test to compare the mean homocysteine, folate and vitamin B_{12} levels in *MTHFRT677T*, CT and wildtype groups and Chi square test to compare the *MTHFR* polymorphism prevalence between large brain infarction patients group and multiple small brain infarction patients group. The results are presented in percentage and mean with standard deviation (sd).

3. Results

The sex ratio of men/women (77/51) was 1.5 with an average age of 60 years. The prevalence of *MTHFR* polymorphism was 19.5% with 18% CT and 1.5% TT. The mean homocysteine level was 29.32 µmol/l (sd: 21.41) (table 1). Homocysteine level was 29.89 µmol/l (sd: 18.22) in wildtype patients, 26.54 µmol/l (sd: 10.06) in patients with CT genotype (*t=-0.23; p=0.816; 95% CI [-0.218; 0.172]*) and 56, 17 µmol/l (sd: 45,56) in patients with TT genotype (*t=2.16, p=0.033, IC 95% [0.017; 0.407]).* Homocysteine level was 29.23 µmol/l (sd: 22.16) in women (table 1) and 29.46 µmol/l (sd: 20.30) in men (*p=0.718*).

Table 1. *Hyperhomocysteinemia related MTHFRC677T polymorphism, arterial hypertension, diabetes, folate and vitamin B_{12}.*

	Homocysteine level (µmol/l)		**Vitamin B_{12} (pmol/l)**		**Folate (nmol/l)**	
Mean value	29.32 (sd: 21.41)		387.42 (sd: 43.99).		6.70 (sd: 5.47)	
Men	29.46 (sd: 20.30)		432.87 (sd: 458.12)		4.02 (sd: 5.20)	
Women	29.23 (sd: 22.16)	*95% CI, p=0.718*	320.32 (sd: 430.99)	*95% CI* p=0.211	5.36 (sd: 5.85)	*95%, CI p=0.876*
MTFHRC677C	29.89 (sd: 18.22)		390.81 (sd: 47.25)		6.77 (sd: 5.65)	
CT	26.54 (sd: 10.06)		364.57 (sd: 121.92)		5.40 (sd: 4.27)	
TT	56.17 (sd: 45.56)*	*p=0.033*	463.8 (sd: 463.7)	*p=0.763*	3.12 (sd: 1.10)	*p=0.36*
Hypertensive	27.75 (sd: 15.19)	*t=1.459*				
No Hypertension	34.30 (sd: 28.79)	*df=126, p=0.147*				
Diabetics	25.58 (sd: 9.94)					
Non diabetics	29.37 (sd: 19.07)	*95%, CI, p=0.358*				

*Homocysteine level was significantly higher in TT genotype than CT and wildtypes patients

Of all patients included in this study, 86.7% were hypertensive and 4.7% were diabetic. The average level of homocysteine was 27.75 µmol/l (sd: 15.19) in hypertensive patients and 34.30 µmol/l (sd: 28.79) in non-hypertensive patients (*p=0.147*). The average level of homocysteine in non-diabetic patients was 29.37 µmol/l (sd: 19.07) and 25.58 µmol/l (sd: 9.94) in diabetic patients (*p=0.358).* CT, TT genotypes and wildtype were associated with hypertension in 87%, 50%, and 86% of the patients respectively. The average level of folate was 6.7 nmol/l (sd: 5.47) and vitamin B_{12} of 387.42 pmol/l (sd: 43.99). Overall, 54% of patients were with folate deficiency and 24% of patients were with vitamin B_{12} deficiency. Folate and vitamin B_{12} had an inverse relationship with homocysteine level, which was statistically significant

in folate and not significant in vitamin B_{12}, respectively *(t=-1.874, p=0.05, 95%, CI [-0.012; 0.422]*; *t=-1.030; p=0.305, 95%, CI [-0.327; 0.103])*. There was a significant positive correlation in folate and vitamin B_{12} (*t=4.64, p=0.000; CI 95% [0.003; 0.007])*. The mean level of LDL cholesterol was 1.4 g/l (sd: 0.52). 58/128 (45.3%) of patients had ischemic stroke in middle cerebral artery, 5/128 (3.9%) had stroke in anterior cerebral artery, 7/128 (5.4%) had ischemic stroke in posterior cerebral artery, 2/128 (1.5%) had stroke in anterior choroid artery and 56/128 (43.7%) of the patients had multiple small infarctions.

Table 2. *MTHFR polymorphism and homocysteine level according to stroke subgroups.*

	Large brain infarction	**Small brain infarction**	***95%, CI***
Frequency	72/128 (56.3%)	56/128 (43.7%)	
CC	61 (61.15%)	42 (14.84%)	
CT + TT	15 (14.8%)	10 (10.15%)	*Qobs=0.05; p=0.94*
TT	1/128 (0.75%)	1/128 (0.75%)	
Mean homocysteine level (µmol/l)	29.60 (sd: 20.75)	27.31 (sd: 12.10)	t=0.716, df=126, *p=0.475*
Mean folate level (nmol/l)	6.99 (sd: 5.70)	5.70 (sd: 4.91)	*t=1.197, df=126, p=0.233*
Mean B_{12} level (pmol/l)	413.84 (sd: 405.93)	349.93 (sd: 505.81)	*t=0.713, df=126, p=0.477*

MTHFR genotype frequency and average level of homocysteine (table 2) were not statistically different in small brain infarction and in large brain infarction respectively (*Chi square test, Qobs=0.05, p=0.94, 95% CI; ttest,* t=0.716; *df=126, p=0.475*). The homocysteine level was 28.02 µmol/l (sd: 22.76) in small brain infarction patients and 29.36 µmol/l (sd: 26.22) in large brain infarcts patients. The Doppler image of supraortic arteries displayed no significant stenosis. In electrocardiogram (ECG) results, 18/128 (14%) of all patients had rhythm disorders, 2/128 (1.5%) had atrial fibrillation, 7/128 (5.4%) had conduction disorders, 5/128 (3.9%) had necrosis sequelae and 39/128 (30.4%) had left ventricular hypertrophy. The ECG was normal in 21/128 (16.4%) of the patients.

4. Discussion

This study has several limitations. The first limitation is the somewhat homogeneous aspect of the patient group. Although ischemic processes are involved in both subgroups, the pathophysiology of small brain infarction is different from that of a large brain infarction, as are the clinical and imagery findings [6]. The second limitation concerns the selection of patients due to the fact that we eliminated subjects with normal homocysteinemia. Genotyping including subjects with normal homocysteinemia would give the real hospital prevalence of the *MTHFRC677T* polymorphism in ischemic stroke patients. However, a normal homocysteine level is not a vascular risk factor despite the diversity of threshold interpretation [7]. Although there are some discrepancies on the reduction of hyperhomocysteinemia by vitamin B_{12} and folate intake, the vascular risk of hyperhomocysteinemia is certain [8]. A previous population-based study highlighted the importance of a high prevalence of hyperhomocysteinemia associated with *MTHFRC677T* and *A1298C* polymorphism and folate deficiency in Togo [4]. The ischemic stroke patients in our study came from this population. These different reasons motivate our objective to focus on patients with hyperhomocysteinemia. We investigated the *MTHFRC677T* polymorphism because it is the primary variant that induces hyperhomocysteinemia. The hospital prevalence of hyperhomocysteinemia was 61% in 2010 [5]. Hyperhomocysteinemia has several factors: genetic factors including the polymorphism *MTHFRC677T*, nutritional factors (folate and vitamin B_{12} deficiency) and metabolc factors including renal failure, antiepileptic treatment. Folate deficiency remains the main vitamin deficiency (54%) in the study population.

The *MTHFRC677T* polymorphism and folate deficiency are the main cause of hyperhomocysteinemia. The folate deficiency is probably nutritional [9]. However, the origin of this deficiency remains to be determined through a nutritional study on the folate content of foods consumed in Togo [4]. Folate deficiency appears to compromise post-stroke recovery probably through hyperhomocysteinemia and folate intake would facilitate post-stroke recovery [10-12]. Vitamin B_{12} plays a major role (co-enzyme) in the conversion of methyl-tetrahydrofolate to tetrahydrofolate (THF), which is the active form of folate. Vitamin B_{12} deficiency thus blocks folate in their inactive form [13]. The *MTHFR* polymorphism remains the main factor that causes a methylene tetrahydrofolate reductase deficiency, an enzyme that allows 5-methyl tetrahydrofolate to be obtained from 5,10 methylene tetrahydrofolate to transform homocysteine into methionine [14]. The MTHFR deficiency leads to hyperhomocysteinemia, which may be of greater impact in addition to folate or vitamin B_{12} deficiency [15]. There are other types of polymorphism, including *A1298C* and *T1317C* (silent), identified in the gene encoding for the MTHFR synthesis. The *MTHFRC677T and A1298C* polymorphism are associated with hyperhomocysteinemia [14].

The *A2756G* polymorphism identified in the methionine synthetase gene has no obvious link to hyperhomocysteinemia [16]. The mechanism of homocysteine is thrombotic by activation of vascular endothelial cells causing platelet adhesion, LDL cholesterol fragmentation and macrophage uptake [17]. We reported a significant proportion of patients with multiple small brain infarctions (43.7%) in the study. Hyperhomocysteinemia

would cause more small brain infarctions than ischemic stroke in large arterial trunks and facilitate the recurrence of ischemic stroke [18, 19]. The small brain infarctions mechanism due to hyperhomocysteinemia may be related to the primacy of vascular endothelial cell activation and platelet adhesion [20]. Hyperhomocysteinemia is considered to be an aggravating factor in arterial hypertension, but we did not note any correlation in the two factors ($p=0.279$) [21, 22]. In this study, we hypothesized that, cardiovascular mortality that particularly elevated in sub-Saharan Africans would be linked not only to undetected and uncontrolled arterial hypertension, but also to other genetically determined vascular risk factors such as *MTHFRT677T* polymorphism, hyperhomocysteinemia and folate deficiency in black patients [23, 24]. Hypertension is particularly severe in black individuals, because it occurs at a younger age and is difficult to control [25].

5. Conclusion

The *MTHFRT677T* and folate deficiency were the main cause of hyperhomocysteinemia among ischemic stroke patients. The *MTHFRT677T* genotype led to a significantly higher homocysteine level than the wildtype and CT genotype. The *MTHFRC677T* polymorphism and hyperhomocysteinemia did not influence the ischemic stroke subtype. However, our analysis did not allow us to underline the main cause of folate deficiency. The possible cause of this folate deficiency may be nutritional. Although arterial hypertension remains the primary cause of stroke, these results showed that hyperhomocysteinemia is an additional vascular risk factor in stroke patients in sub-Saharan Africa.

Statements

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Ethics Approval

This research work has been approved by National Consultative Committee on Bioethics (CCNB) of Togo.

Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Damelan Kombate, Sirui Zhou and Panabalo Waklatsi. The first draft of the manuscript was written by Damelan Kombate and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

All the authors do not have any possible conflicts of interest.

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