Original Research Article

Is *MTHFR*C677T Gene Polymorphism Associated with Hypertension in Nigerians?

Running Title: Association of MTHFR*C677T Gene Polymorphism* with hypertension in Nigerians.

ABSTRACT

Background: Essential hypertension is very common in Nigeria. The cause is unknown. Genetic factors have been postulated by some authors as a possible risk factor. Such genetic factors include the mutation of methylenetetrahydrofolate reductase (MTHFR) gene.

Aim: This study aimed to document the allelic and genotype frequencies and distribution among hypertensive and healthy Nigerian population.

Materials and Methods

This was a cross-sectional study involving 75 consenting subjects (50 cases and 25 controls) at the Cardiology Clinic of the Lagos University Teaching Hospital, Lagos, Nigeria. Structured interviewer administered questionnaire was used to obtain socio-demographic and clinical history of subjects. About 5mls of venous blood was collected from each subject by a trained phlebotomist into EDTA bottle and stored at 4ºC until ready for analysis. Genomic DNA extraction was done after which polymerase chain reaction was carried out. This was followed by restriction enzyme digestion and agarose gel electrophoresis. The digestion products were then visualized with SYBR Safe (Monitagen) using Sygene bio-imaging system.

Results

When compared with hypertensive subjects, normotensive subjects had more CC (84% vs 74%) and CT (16% vs 12%) genotypes. Hypertension was significantly associated with mutant *MTHFR* genotypes (14% vs 0.0%) (OR = 3.995, 95% C.I: 1.101- 10.034; p=0.033). Except for age (OR= 1.771, 95% CI: 1.036 – 3.029; p=0.037), smoking (OR= 0.000; p=0.999), alcohol consumption (Or= 0.000; p=0.999), and sex (OR= 15.052, 95% CI: 0.196- 115.028; p=0.139) did not attain statistical significance.

Conclusion: The 677TT homozygous mutant had the highest risk of association with hypertension in Nigerians.

Key Words: Hyperhomocysteinemia; mutant; polymorphism; hypertension

INTRODUCTION

Many multi-organ complications arise from hypertension. The prevalence of hypertension is said to be lowest in India (males: 3.4%; females: 6.8%) and highest in Poland (males: 68.9%; females: 72.5%) [1] A Nigerian review on hypertension published in 2015 revealed a prevalence of 28.9% (males 29.5%; females 25.0%) with a geographical prevalence of 30.6% in the urban population and 26.4% among the rural dwellers, respectively. [2] In 95% of subjects, the cause is unknown and is referred to as essential hypertension. [3] However, many authors now believe that genetic factors such as the mutation of methylenetetrahydrofolate reductase (MTHFR) gene play prominent roles in the pathogenesis of essential hypertension. [4-9]

The *MTHFR* gene has been shown to have many polymorphisms. A study in the Israeli women population reported an increase in the diastolic blood pressure in women with *MTHFR* C677T population irrespective of their blood lipid levels. [10] A recent study also reported the association between *MTHFR* C677T gene polymorphism and essential hypertension which is closely related to the increased level of homocysteine (Hcy). [11]

The C677T polymorphism (in which valine substitutes alanine at the 222nd codon) is one of the clinically significant mutations. [9] *MTHFR* C677T is thermolabile because its activity decreases when the temperature is above 37°C. [12] The enzyme activity of the *MTHFR* 677TT genotype varies depending on whether it is homozygous (30%) or heterozygous (60%). [7] Whereas some authors have reported that the *MTHFR* C677T mutation did not increase cardiovascular risk, others have shown that the homozygous mutation was linked with diseases such as hypertension and stroke, [5,13] with different prevalences in different parts of the world. [14-16]

This study aimed to document the allelic and genotype frequencies and distribution among hypertensive and healthy Nigerian populations. The study also aimed to evaluate MTHFR 677CT SNP as a possible independent predictor of hypertension in relation to other traditional markers of hypertension such as age, gender, alcohol use and smoking habits among Nigerians.

MATERIALS AND METHODS

Study site and population

This cross-sectional study involving 75 subjects (50 subjects and 25 controls) was carried out at the Cardiology Clinic of the Lagos University Teaching Hospital after obtaining ethical approval from the institutions Ethics and Research Committee with assigned number ADM/DCST/ HREC/1889. Twenty-three males and 27 females were recruited as study group with 11 males and 14 females serving as controls.

Inclusion criteria

Male and female subjects diagnosed with primary hypertensive disorders and normotensives of 18 years and above, were included in the study.

Exclusion criteria

The following subjects were excluded from the study: Subjects below 18years; those with clinical or laboratory evidence of secondary hypertension; extradural and subdural haemorrhages. Subjects that survived from TIA or stroke events in cases of blood disease (e.g. leukaemia, polycythaemia vera); brain tumours or brain metastases.

Subject Selection, Sample Collection and Handling

Structured interviewer administered Questionnaires were used to obtain socio-demographic and clinical history of subjects. Verbal and written informed consents were obtained from the subjects after clear explanation of study objectives, benefits and limitations. Subjects were aged 18 years and above with the diagnosis of essential hypertension confirmed by

cardiologists according to the International Society of Hypertension's criteria [systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg]. [17] The hypertensive subjects were attending the Cardiology Clinic of the hospital regularly. Before recruitment and at each visit, blood pressure readings were taken twice in the sitting position with a mercury sphygmomanometer (Accoson MDF800 desk Mercury Thermometer, UK), 30 minutes apart and subjects with mean blood pressure readings above 140/90.

The control group were normotensive subjects (SBP < 140 mmHg or DBP < 90 mmHg) without any family history of diabetes, cardiovascular and thyroid diseases.

Approximately 5mLs of venous blood was collected by a trained phlebotomist into EDTA bottle and was stored at 4ºC until ready for analysis.

DNA Extraction and Restriction Enzyme Digestion

After genomic DNA extraction with the QiagenDNeasy Kit according to the manufacturers' protocols, polymerase chain reaction (PCR) was done, followed by restriction enzyme digestion. A 25µl PCR reaction mixture comprised of 2µl genomic DNA, 16 µl PCR water, 0.8µl dNTP (2.5mM), 0.2µl $MgCl_2$ (1.5mM), 0.5µl 5^1-AAGATCAGAGCCCCCAAAGC-3^1 forward primer, 0.5µl 5^1- ACTCAGCGAACTCAGCACTC-3^1 reverse primer and 5.0µl Tag DNA polymerase, constituted inside the PCR tube. The genotyping protocol for the detection of the MTHFR C677T polymorphism was adapted from Frosst*et al*. [18] The Hinf I restriction digestion and agarose gel electrophoresis were carried out according to standard protocols. The digestion products were visualized with SYBR Safe (Monitagen) using Sygene bio-imaging system.

DATA ANALYSIS

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0 Chicago, IL. For descriptive statistics, χ^2 test was used as univariate statistics to compare proportions in the distribution of demographic variables and MTHF C677T genotypes among the subjects in the study, and the student t-test was used to compare mean values of systolic and diastolic blood pressures among the two groups of subjects. Logistic regression model was adopted to describe associations between MTHFR genotypes and some socio-demographic factors with dichotomous response on presence or absence of hypertension among subjects, and determine

independent predictor variables for hypertension in the study. The critical level of significance was set at $p<0.05$.

RESULTS

Socio-demographic characteristics of subjects

The socio-demographic characteristics of the study and control subjects with respect to their age range, sex and blood pressure are shown in Table 1. In both groups (study and controls), the age group 60 years and above (study 40%; Control 48%) constituted the bulk of the subjects in this study. This was followed by the age group 50-59 years (20% in both study group and control). Subjects ≤30 years (10.0%) in the study group and 30-39 years (8.0%) in the control group had the lowest frequency. The blood pressure readings of the study group (systolic 147±18; diastolic 92±15) were significantly higher than in the control (systolic 115±5.8; diastolic 76±5.3) (p=0.000).

Distribution of *MTHFR* C677T genotypes

Normotensive subjects had more CC genotypes than the hypertensive subjects (84% vs 74%). The normotensive group also had more CT genotypes than the hypertensive group (16% vs 12%). There was a significant association of homozygous mutant genotype TT with hypertension; while 7 of the hypertensive subjects had T genotype, none of the normotensive subjects had the TT genotype (14.0% vs 0.0%; *p*=0.049). Table 2.

The logistic regression for variables associated with hypertension

In Table 3, hypertension was significantly associated with mutant *MTHFR* genotypes (OR = 3.995, 95% C.I: 1.101- 10.034; p=0.033) among subjects. However, other traditional socio-demographic risk factors for hypertension such as smoking (OR= 0.000; p=0.999), alcohol consumption (Or= 0.000; p=0.999), and sex (OR= 15.052, 95% CI: 0.196- 115.028; p=0.139) did not attain statistical significance except age (OR= 1.771, 95% CI: 1.036 – 3.029; p=0.037) when evaluated with *MTHFR* mutation as a potential risk factor for hypertension among study group.

DISCUSSION

This case–control study demonstrated that the *MTHFR*C677T gene polymorphism was significantly associated with increased risk of hypertensive disorders in Lagos, South-Western Nigeria. Previously, studies have been conducted to determine the association between *MTHFR* C677T polymorphism and hypertensive disorders, however, the conclusions were largely controversial. In a Moroccan study, the homozygous mutant SNPs for the *MTHFR* gene (677TT/CT) were associated with significant risk of hypertension similar to the findings in this study [5] A meta-analysis report by Kumar *et al*., 2015 also found significant association between *MTHFR* C677T polymorphism and the risk of developing cerebrovascular accident or ischemic stroke [19]. Other published reports include studies from Spain. [20] Chinese Han [21,22], Taichung, China [23] and Turkey [24]. Yang *et al*., also reported a strong association of *MTHFR* C677T with the risk of hypertension among Asians, Caucasians and Chinese subjects in a meta-analysis [25] However, few reports have observed no significant association between mutant *MTHFR* genotypes and hypertension. These included studies carried out on Chinese Caucasians [26] and Japanese [27] where the presence of the T allele did not predispose subjects to increased risk of hypertension.

A significant relationship between gender and mutant *MTHFR* C677T was not established in this study. In a similar study carried out in South West Cameroon, which considered *MTHFR* C677T genotype distribution by gender in both hypertensive and normotensive subjects, reported no gender preference in the genotype distribution for the *MTHFR* 677 SNP [28] The *MTHFR* genotype frequencies for CC, CT and TT among control group subjects were 84.0%, 16.0% and 0.0% respectively. The hypertensive group had 74.0%, 12.0%, 14.0 % for CC, CT and TT

respectively thus showing a significant difference in *MTHFR* 677CT genotype distribution between control and hypertensive subjects. (P=0.049).This finding is in agreement with reports from a study by Nasseredine *et al*., in Morocco as further evidence that the homozygous mutant 677TT was significantly associated with the risk of hypertension in an African population.[5] Markan *et al.* also reported similar observations in the Indian population[29] A number of studies from Sub-Saharan Africa have suggested that genotype frequencies for *MTHFR* mutant variants are extremely low among African populations; suggesting that the *C677T* allele is less common in African populations than other ethnic groups[28] Therefore, studies indicating marginal elevations in the genotype frequencies of *MTHFR* C677T mutant SNP in a pathologic entity among African populations, should arouse strong suspicion for disease association studies.

Conclusion: The 677TT homozygous mutant had a high risk of association with hypertension in Nigerians.

Ethical Approval: As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent: As per international standard or university standard, patients' written consent has been collected and preserved by the authors.

Recommendation: MTHFR screening should be encouraged early in life or as part of routine health check. This may help identify individuals with increased risk of hypertensive heart diseases, stroke and metabolic syndrome for early life style modifications in those at risk.

Study Limitation: Limitation to this was the inability to obtain serum folate levels of the subjects. It was difficult to control all other factors that might have contributed to variations in homocysteine levels among participants.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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 authors and producers of the products because we 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.

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TABLES

Table 1 : Demographic Characteristics of Subjects

Characteristics	Hypertensive(%)	Normotensive (%)	Frequency	χ2	*p* value
Age Group (Yrs)					
≤30	5(10)	3(12.0)	8	0.972	0.808
30-39	8(16)	2(8.0)	10		
40-49	7(14)	3(12.0)	10		
50-59	10(20)	5(20.0)	15		
≥60	20(40)	12(48.0)	32		
Sex					
Male	23(67.6)	11(32.4)	34(100)	0.246	0.619
Female	27(65.9)	14(34.1)	41(100)		
Mean BP					
Systolic (Mean±SD)	147±18	115± 5.8			
Diastolic (Mean±SD)	92±15	76±5.3			

Table 2: Subjects and Gender Distributions of C677T Genotypes

	CC(%)	**CT(%)**	**TT(%)**	**Total (%)**	**χ2**	***p* value**
Hypertensive	37(74.0)	6(12.0)	7(14.0)	50(100)	5.759	0.049
Normotensive	21(84.0)	4(16)	0(0.0)	25(100)		
Total	58	10	7	75		
Sex						
Male	30(83.4)	3(8.3)	3(8.3)	36(100)	2.727	0.256
Female	28(71.8)	7(17.9)	4(10.3)	39(100)		
Total	58	10	7	75		

Table 3 : The Logistic Regression for Independent Predictors of Hypertension among Subjects

Variables	**B**	**OR**	**95% CI**	***p* value**
Presence of Mutant MTHFR genotypes	1.939	3.955	1.101- 10.034	0.033
Age	0.572	1.771	1.036 – 3.029	0.037
sex	5.011	15.052	0.196 - 115.028	0.139
Alcohol use	-21.747	0.000	0.000 - 0.000	0.999
Smoking	-21.809	0.000	0.000 - 0.000	0.999