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IS THE C677T Substitution in the MTHFR Gene Associated with PRE-ECLAMPSIA in Indigenous Black Populations?

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Abstract: ***The aim of this study was to determine the association of the Methylenetetrahydrofolate reductase gene (MTHFR) C677T mutation with preeclampsia in an indigenous black population in Bayelsa State, Nigeria. A case-control study was conducted, diagnosis of preeclampsia was made on the criteria of gestational hypertension and proteinuria. The C677T polymorphism was assessed by DNA amplification using polymerase chain reaction and amplicon digestion using Hinfi enzyme. The CC genotype was found in 54 patients and 62 controls, the CT genotype in 2 preeclampsia patients and 6 controls. No participant had the TT genotype. Genotype frequencies of CC, CT and TT were. 0.935, 0.064, 0.000 respectively and allele frequencies were C- 96.8%, T -3.2%. The results showed that the mutant TT genotype implicated as one of the possible causes of preeclampsia was absent in the sampled indigenous black preeclampsia population and probably have no contribution to the high prevalence rate and severity of preeclampsia in indigenous black African women.***

Keywords: MTHFR gene, C677T mutation, Preeclampsia, Hypertension, Indigenous black population

1. Introduction

Preeclampsia is a serious disease of pregnancy characterised by hypertension and proteinuria. (El-Sayed. 2017, Mol *et al*., 2016). The incidence varies from 3-7% in nulliparas and 1-3% in multiparas contributing 10-15% to maternal mortality. (Uzan *et al*, 2011). Risk factors for preeclampsia include nulliparity, multiple gestation, molar pregnancy, age ≥35 years, history of preeclampsia, indigenous Africans, hypertension, diabetes mellitus, renal disease, foetal malformations, maternal history of preeclampsia, antiphospholipid antibodies, autoimmune disorders and high altitude. (Uzan *et al*., 2011, Mutze *et al*, 2008).

The high incidence of preeclampsia in some ethnic groups and in first degree relatives is highly suggestive of a genetic contribution to preeclampsia, but research into candidate genes have yielded very little positive results (Mutze *et al.*,2008, Serrano NC. 2006).

Racial differences in the prevalence and seventy of preeclampsia are well established, and several studies have reported a higher prevalence rate and severity among black women compared to white women (Zhang *et al*., 2003, Caughey *et al.*, 2005, Brown *et al.*, 2007, Tanaka *et al.*, 2007).

A missense mutation in the methylenetetrahydrofolate reductase gene a C to T substitution at nucleotide 677 (MTHFR C-T (667) is associated with a moderately raised plasma homocysteine which is a risk factor for endothelial dysfunction vascular disease and preeclampsia. (Frost *et al*., 1995). The incidence of such genetic polymorphisms, vary amongst ethnic population ((Frost *et al*., 1995)., Marie-Claude *et al*.,1997) and evidence of unique environmental contributions to preeclampsia has been postulated (Hill, 2011). We were interested in investigating the incidence of the MTHFR C-T (667) polymorphism reported in an indigenous African populations and demonstrate a possible association with the severity of preeclampsia in this population.

2. Materials and Methods

Study area: The study area was Yenagoa in Bayelsa state located in the Niger Delta region of Nigeria at Latitude (04^0 15^0 North) (05^0 23^0 South), Longitude 05^0 22^0 West and $06^0 45^0$ East.

Ethical approval: Ethical approval was sought for and obtained from the management of the Federal Medical Center Yenagoa, Bayelsa State, Nigeria

Sample size: A total of 124 subjects were recruited in a case-control study. The subjects were randomly selected from women attending antenatal clinic in the tertiary Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria.

The case group consisted of 56 women with preeclampsia aged 17 – 39 years (mean age 30.36 ± 6.05 SD). The control

group consisted of 68 normotensive women with uncomplicated pregnancies aged from 15-43years (mean age 30.44±5.357 SD). They were matched for age and parity.

Informed consent was obtained from each subject through a user-administered questionnaire.

The diagnosis of preeclampsia was based on the criteria for gestational hypertension in pregnancy and proteinuria. Hypertension in pregnancy was defined as an increase of 30mmHg systolic, and 15mmHg diastolic blood pressure with values obtained before 20weeks of gestation and an absolute blood pressure of 140mmHg, systolic or 90mmHg, diastolic. Proteinuria was defined as ≥ 2+in a voided urine sample.

For the DNA analysis, 5mls of blood samples were collected from the subjects into Ethylene Diamine tetracetic acid (EDTA) bottle. The analysis was carried out in the molecular Genetic Laboratory of the Department of Medical Laboratory science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

DNA Extraction: DNA was extracted using quick gDNA mini prep DNA extraction kit supplied by Inqqaba Biotechnological, South Africa, and pure DNA was stored at -20^{o}c.

DNA Quantification: The extracted DNA was quantified using the Nanodrop 1000 spectrophotometer.

MTHFR Genotyping: The extracted DNA was genotyped by polymerase chain reaction (PCR) using methods described by Frosst*et al.* 1995, using primers 5'-TGAAGGAGAAGGTGTCTGCGGA-3'and 5'AGGACGGTGCGGTGAGAGTG-3' to generate a 198-bp fragment and the genotypes were determined by the length of the PCR products being digested with the *Hinf1* restriction enzyme. Amplification and restriction products were analysed using 2% agarose gel electrophoresis.

3. Result

PCR amplification of the MTHFR gene yielded a 198bp amplicon (Figure 1). The amplicon subjected to enzyme digest with *Hinf1*yielded three bands (198bp, 175bp, 23bp) for the heterozygous (CT) genotype and the homozygous wild type genotype remained uncut (198bp).

Tables 1 and 2 shows the genotyping result for the C677T polymorphism.

A total of 124 samples were analysed, 56 preeclampsia subjects, and 68 normotensive controls. Fifty-four (54) of the pre-eclampsia subjects had the CC genotype (96.4%), 2 had the CT genotype (3.6%), none had the TT genotype (0%).

Of the 68 controls, 62 had the CC genotype (91.2%), 6 had the CT genotype (8.8%) and none had the TT genotype.

The result of the present study showed that the 677 CC homozygous genotype had the highest percentage, both in the preeclampsia and control population while the 677 TT genotype was absent.

Table 1: MTFHR (C677T polymorphism) genotype distribution and allele frequency amongst women with preeclampsia in Yenagoa, Bayelsa State

	Genotypes			Alleles	
	CC	CT	TT	C	T
Observed Number	54	2	0	110	2
Frequencies	0.964	0.036	0.000	0.962	0.017%
Percentage	96.4%	3.6%	0.0%	98.2%	1.7%

Table 2: MTHFR (C677T polymorphism) genotype distribution and allele frequency amongst normotensive pregnant women in Yenagoa, Bayelsa State

	Genotypes			Alleles	
Observed Number	CC	CT	TT	C	T
Frequencies	62	6	0	130	6
Percentage	0.912	0.088	0.000	0.955	0.461
%	91.2%	8.8%	0	9.6%	4.6%

4. Discussion

Preeclampsia is now recognised as a multi-systemic disease causing global vasoconstriction, widespread formation of micro-thrombi and endothelial dysfunction leading to multiple end-organ damage (El-Sayed. 2017, Mutze *et al,* 2008). The placenta also suffers vasoconstriction leading to ischaemia resulting in intra-uterine growth retardation and intra-uterine foetal death (Mutze *et al.,* 2008). Placental hypo-perfusion is associated with low expression of heme-oxygenase, an anti-oxidant causing oxidative stress. Abnormalities in the endothelium precedes the syndrome of HELLP (haemolysis, elevated liver enzymes, and low platelet) that is commonly associated with preeclampsia. It is also responsible for micro-angiopathic haemolytic anaemia (MAHA) and increased vascular permeability causing oedema (El-Sayed. 2017, Mutze *et al,* 2008).

The higher prevalence and disease severity in black populations has made the search for candidate genes even more imperative. Marie-Claude *et al,* 1997, Hill, 2011). Homocysteine, a risk factor for atherosclerosis is higher in black women and these differences are partially related to a low folic acid level. (Thelma *et al,* 2004).

The distribution of the genotype frequencies from our study shows that the CC genotype is by far the commonest and the TT genotype was not present in our study population. This is in agreement with findings from other studies (Frost *et al*., 1995, Marie-Claude *et al,* 1997 which showed a 0% in the TT genotype in African-Americans (Frost *et al*., 1995).

5. Conclusion

This study suggests that the C677T MTHFR mutation probably has little or no contribution to the severity and high rate of preeclampsia noted in indigenous black women. Notably, there was no significant difference in the C677T prevalence in the preeclampsia population when compared with the normotensive women. However, since increase in mean plasma homocysteine in black preeclampsia patients has been reported in several studies, (Thelma et al., 2004, Franco *et al.,* 1998, Setareh *et al.,* 2003) and an inverse

relationship has been noted between the increase in plasma homocysteine and folic acid in blacks with preeclampsia, it might be pertinent to initiate nutritional studies to explore other possible sources of increased homocysteine in black preeclampsia patients.

6. Acknowledgement

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7. Perspectives

The finding of higher homocysteine levels in black pregnant women and its inverse relationship to folic acid levels, have been identified as a possible risk factor for cardiovascular disease in these patients (Franco *et al.*, 1998). Therefore, it is important to explore other racial differences that might be possible risk factors for atherosclerosis as explanations for the increase in homocysteine levels noted in black preeclampsia patients outside a point mutation of the MTHFR gene.

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