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Methylenetetrahydrofolate reductase (*MTHFR)* C677T Gene Polymorphism (rs1801133) and its association with Essential Hypertension in Sudanese Patients.

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Abstract

Background: Hypertension is a multifactorial disease caused by the interaction between genetic and environmental factors. Mutations in the methylenetetrahydrofolate reductase (*MTHFR*) has been known to be associated with the risk of cardiovascular diseases as well as hypertension.
Aim: This study aimed to assess the relationship between the presence of the C677T polymorphism of the *MTHFR* gene (rs1801133) and the risk of hypertension in Sudanese patients.
Method: This is a cross-sectional Hospital based study conducted in Khartoum State–Sudan, during Jan.2018-Oct.2018. The study group consisted of 107 with essential hypertension as patients and 142 age matched normotensive as controls. The genotypes of the methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphism (rs1801133) were detected by Polymerase chain reaction followed by restriction fragment length polymorphism using HinfI restriction enzyme. The statistical analysis of the results was done using SPSS software package. A p-value < 0.05 was taken as significant. Odds ratio with 95% confidence interval was used to assess the risk of the association.
Results:
This study showed that high frequency of hypertension was observed among female (63.6%), with no significant gender difference between cases and controls (P-value=0.58). Ninety-three percent of the patients were at 40 years and above with significant difference between cases and controls (P-value =0.0001). The family history of hypertension was significantly increase the disease risk among hypertensive up to three folds (P-value =0.003, OR=3.103, CI=1.454, 6.626). Smoking and lack of exercise; were significantly associated with disease susceptibility (P-value =0.044, P=0.001 respectively).
We conducted the genotype analysis for the *MTHFR* polymorphism (rs1801133) considering the dominant model: the wild type (CC) versus the recessive mutant (CT+TT). The analysis provided an evidence that the mutant CT+TT genotypes were significantly higher in hypertensive group compared to the normotensive controls (P-value = 0.048) with an increased risk of hypertension of up to two folds (OR=1.90, CI=.954, 3795). Diabetes and heart diseases as a complication of hypertension, showed a significant occurrence among hypertensive (P-value =0.002 and P-value=0.0001respectively) compared to their counterpart controls.
Conclusion: The results of this study indicates that C677T *MTHFR* polymorphism is associated with the increased risk of hypertension in our samples. Family history of hypertension and the

modifiable risk factors such as smoking and sedentary life were also strongly associated with disease susceptibility.

Key words: Hypertension, polymorphisms, *MTHFR* gene.

المستخلص:

ضغط الدم مرض متعدد العوامل: جينية و بيئية. الطفرات في جين إختزال ميثيلين تتراهيدروفولات عرف كعامل خطورة لأمراض القلب وضغط الدم.

الهدف:هذه الدراسة هدفت لمعرفة العلاقة بين وجود متعدد الأليل في جين إختزال ميثيلين تتراهيدروفولات (rs1801133) كعامل خطورة لحدوث ضغط الدم عند المرضى السودانيين.

الطريقة:الدراسة أجريت في الخرطوم على 107 من مرضى ضغط الدم الأساسي و 142 من الأصحاء في الفترة من يناير 2018–أكتوبر 2018. درست الأنماط الجينية ل متعدد الأليل لجين اختزال ميثيلين تتراهيدروفولات بإستخدام تفاعل البلمرة المتسلسل وإنزيم القطع (HinfI).التحليل الإحصائي أجرى بإستخدام برنامج SPSS.

أعتبرت القيمة المعنوية(p–value < 0.05) ونسبة الأرجحية بفاصل ثقة 95٪ لتقييم مخاطر الارتباط.

النتائج: أوضحت الدراسة تردد عالي لمرض ضغط الدم عند النساء وعدم وجود فرق معنوي بين الجنسين عند مقارنة المرضى بالأصحاء. ثلاثة وتسعون بالمئة من المرضى أعمارهم اربعون سنة فأكثر مع وجود فرق معنوي عند مقارنة المرضى مع الأصحاء (P–value =0.0001). التاريخ المرضي للأسرة يزيد عامل الخطورة ثلاثة مرات(OR=3.). التدخين وعدم ممارسة التمارين الرياضية يرتبطان بشكل كبير مع حدوث ضغط الدم.

درست الأنماط الجينية لمتعدد الأليل لجين إختزال ميثيلين تتراهيدروفولات(rs1801133) بالنظر إلى النموذج السائد: الطراز البري (CC) مقارنة بالطراز المتنحي(CT+TT). التحليل الإحصائي أعطى دليل أن الطراز المتنحي (CT+TT) أعلى بكثير عند المرضى مقارنة بالأصحاء مع زيادة عامل الخطورة لمرتين (P–value = 0.048, OR=1.90)

حدوث كبير لمرض السكري والقلب كمضاعفات إرتفاع ضغط الدم بين المرضى مقارنة بالأصحاء

(P–value =0.002 P–value=0.0001) على التوالي.

الخلاصة: أوضحت هذه الدراسة أن متعدد الأليل في جين إختزال ميثيلين تتراهيدروفولات (rs1801133) مرتبط بزيادة مخاطر إرتفاع ضغط الدم. التاريخ المرضي للأسرة، التدخين و عدم ممارسة التمارين الرياضية يرتبط بشدة بالقابلية لمرض إرتفاع ضغط الدم.

Introduction

Hypertension which is known as a silent killer, is considered as a global public health issue (WHO 2013). Its prevalence continues to rise and it is expected to increase to approximately 1.56 billion in the year 2025 (Kearney et al., 2005). The developing countries receive a high burden of hypertension (Seedat 2000, Chockalingam et al., 2006, Ibrahim 2018).

Hypertension is a complex disorder in which multiple factors both environmental and genetics or interaction between them contribute to the development and progression of the disease. The role of the genetic in the predisposition of hypertension is well documented with conflicting results in different ethnic groups worldwide (Newton-Cheh et al., 2009, Ehret and Caulfield, 2013, Priyanga et al., 2015).

The enzyme which is crucial to maintain the homocysteine at healthy levels is the 5,10-Methylenetetrahydrofolate reductase (*MTHFR*) which catalyzes the irreversible conversion of the circulating form of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate that in-turns converts the homocysteine to another amino acid; the methionine; thereby keeps the homocysteine at normal level (Maitland-Van der et al., 2008). Missense mutations in methylenetetrahydrofolate reductase gene have been identified; of these polymorphisms is the C677T; the presence of which results in a thermolabile *MTHFR* that has a lower enzyme activity at 37°C; leading to hyperhomocysteinaemia, which has a negative impact on the vascular system represented in abnormalities including the loss of important endothelial vasodilator, endothelial vascular cell injury, vasoconstriction (Bennouar et al., 2007, Khandanpour et al., 2009, Bhargava et al., 2012, Bansal et al., 2014, Ganguly and Alam 2015). These effects encourage the research to examine the association of the *MTHFR* C677T polymorphism with vascular diseases (Benes et al., 2001, Bhargava et al., 2012, Ganguly and Alam 2015) including hypertension in different population but the results are inconclusive (Rodríguez-Esparragón et al., 2003, Heux et al., 2004, Markan et al., 2007, Niu et al., 2012,Yang et al., 2014, Yi-Le et al., 2014, Nassereddine et al., 2015, Fan et al., 2016,). In this study we aimed to investigate if there is an association between the C677T polymorphism of the *MTHFR* gene (rs1801133) and the risk of hypertension in Sudanese patients.

Methods

One hundred and seven hypertensive patients (cases) and 142 age matched normotensives as controls were enrolled in this cross- sectional Hospital based study conducted during Jan.2018-Oct.2018. Ethical approval was obtained from the Ethical Committee of the Faculty of Medical Laboratories- AlNeelain University. Verbal consent was obtained from each participant prior enrollment in the study.

Inclusion criteria:

Patients with essential hypertension and normotensive of ages ranged from 40- 75 years and both sexes are included. The newly diagnosed patients and the normotensive controls have been confirmed with or without hypertension by an expert physician; according to the criteria of the International Society of Hypertension [systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg] (Whitworth 2003).

Exclusion criteria:

Hypertension that occurs as a result of diabetes were excluded from this research.

DNA extraction and genotyping

Three ml of blood sample were obtained from both cases and controls. Genomic DNA was extracted using Guanidine chloride DNA extraction method. The polymerase chain reaction (PCR) was performed targeting the C677T polymorphism of *MTHFR* (rs1801133), where the alanine is substituted with valine.The primers were designed as Forward primer 5′-CTC GCC TTG AAC AGG TGG AG-3′ -Reverse primer 5′-CTG GAT GGG AAA GAT CCC GG-3′. Primers were then checked using The Basic Local Alignment Search Tool (BLAST) Database. The PCR for the C677T *MTHFR* region was conducted using master mix ready to use from i*NtRON* Biotechnology Company, following the manufacture instructions with final volume 12.5 µl.

The DNA was amplified in a thermos-cycler using the stepdown PCR program as follows:
95˚C for 5 min for initial denaturing, 30 cycles of: denaturing at 94˚C for 40s, annealing at 64,60,59˚C for 40 s, followed by extension at 72 °C for 40 s. Then final extension at 72 °C for 10 min.
The Biolab. Digest® HinfI restriction enzyme recognizes the 5′-GANTC-3′ sequence.
To determine the genotypes of the *MTHFR* C677T polymorphism among case and control groups; 2 µl of the PCR product was incubated for 2 hours at 37°C in 0.25 µl (10U/µl) of Hinf1 restriction endonuclease enzyme and 1 µl of 10X buffer and the final volume was completed to 8µl using double ionized water. For inhibition of the enzyme the temperature was raised to 65°C for 20 minutes according to the manufacturer's instructions. The genotypes were scored according to fragment pattern as wild type homozygous CC (Ala/Ala; 248-bp fragment), TT homozygous mutant (Val/Val; 130, and 118-bp) and heterozygous CT (Ala/Val; 248, 130, and 118-bp).
The restriction pattern was determined by loading the restriction mixture and a 100bp ladder (New England Biolab Inc) into 2% gel electrophoresis, the polymorphism was documented by photographing using gel documentation system.

Statistical analysis:

Statistical analysis was done using SPSS software package version 21. P-value < 0.05 was taken as significant. Odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of the association.

Result

Two hundred and forty-nine subjects were enrolled in this study: included 107 hypertensive and 142 normotensive Sudanese individuals. The comparison between hypertensive patients and normotensives with regard to the non-modifiable, modifiable risk factors and other disease associated with hypertension were shown in (Fig.1,2,3).
The analysis of the demographic data showed that nearly two third of the patients were females (63.6%) with no significant gender difference between cases and controls (P-value=0.58). A high significant difference regarding the age was observed between hypertensive compared to normotensive (P =0.0001). Hypertensive at age >40 years were at an increased risk of hypertension compared to the controls (OR=7). Although we did not get a confident answer about whether the patients or the control individuals have family history of hypertension, however, the obtained data suggested a highly significant association between the family history of the disease and hypertension (P-value= 0.003) and it is significantly associated with the increased risk of hypertension up to three folds (P-value=0.003, OR=3) (Fig.1). The modifiable risk factors examined; smoking and sedentary life were significantly associated with hypertension (P-value= 0.04 and P-value =0.001respectively (Fig.2). The available data regarding the alcohol consumption is not conclusive as many of the participant were unwilling to respond. A significantly high prevalence of diabetes and heart diseases among hypertensive was reported compared to normotensive (P=0.002, P=0.0001 respectively). However, stroke and kidney failure were not significantly associated with hypertension in the examined samples (Fig. 3).

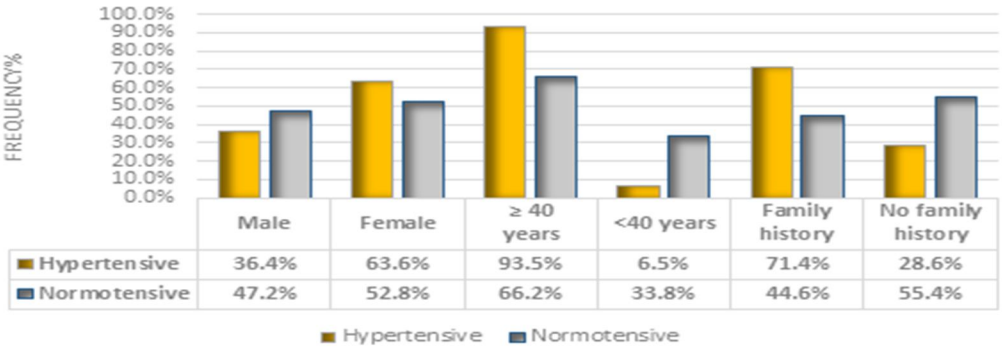

Fig 1: Shows the comparison of the non modifiable risk factors between hypertensive patients and normotensives

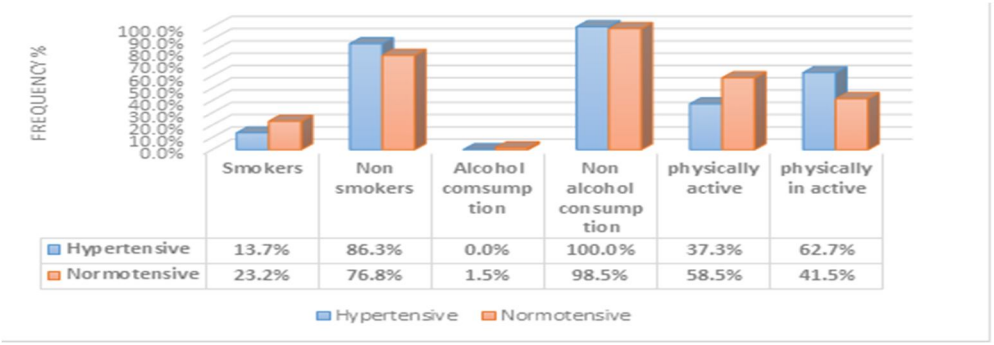

Fig 2: Shows the comparison of the modifiable risk factors between hypertensive and normotensive

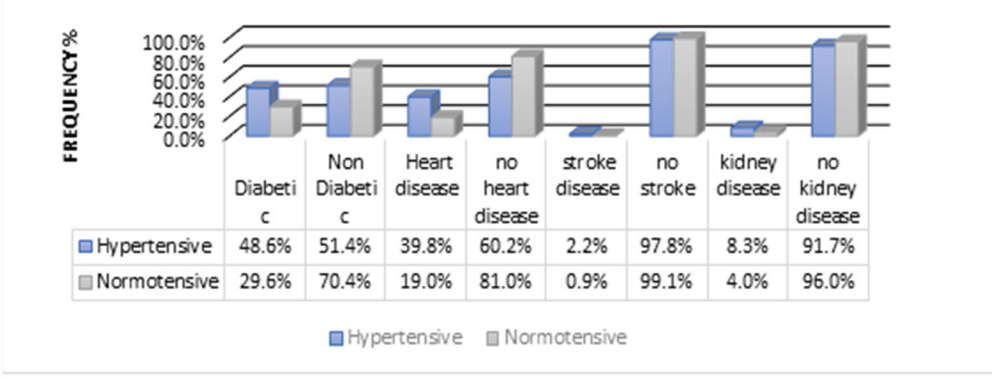

Fig 3: Shows the distribution of hypertension`s complications among hypertensive compared to normotensive

The genotypes of the *MTHFR* polymorphism (rs1801133) was shown in Fig.4. The genotype analysis considering the dominant model genotype (wild type CC) versus the recessive (mutant CT+TT) revealed that the hypertensive group had a significantly higher frequency of the mutant genotype (CT+TT) compared to normotensive (P-value=.048), with about two folds increased risk of hypertension (OR=1.903, CI=.954, 3.795) (Table 1).

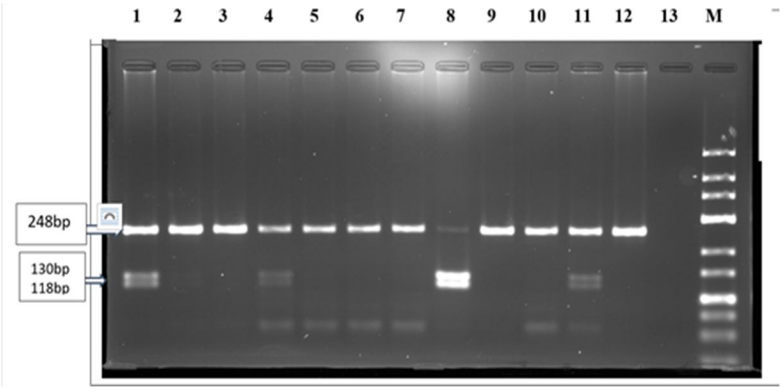

Fig. 4: Shows the gel electrophoresis of the genotypes of the *MTHFR* polymorphism (rs1801133). Lane 1,4,8 and 11 are heterozygous mutant (CT genotype). Lane 2,3,5,6,7,9,10 and 12 un cleaved wild-type homozygous (CC genotype). Lane MΞ 25 bp DNA marker.

Table 1: Shows the genotypes and allele frequency of the C677T *MTHFR* polymorphism among the hypertensive (cases) and normotensives (controls).

Genotypes of the C677T MTHFR polymorphism	Hypertensive (cases) frequency 107 (%)	Normotensive(control) frequency 142 (%)	P-value OR,CI
CC	85 (79.4%)	125 (88.0%)	
CT	20 (18.7%)	15 (10.6%)	P-value=.048
TT	2 (1.9%)	2 (1.4%)	OR=1.903
			CI=.954 - 3.795
Allele frequency			
C	0.89 (89.0%)	0.933 (93.3%)	
T	0 .11 (11.0%)	0.067 (6.7%)	

Discussion

Hypertension is a complex disorder that is known to occur as a result of interactions of genes` polymorphisms with the environmental factors (Newton-Cheh et al., 2009, Ehret and Caulfield, 2013, Fan et al., 2016). Of the genes` polymorphisms that have been extensively studied for the association with hypertension in different ethnic groups is the C677T of *MTHFR* polymorphism (Heux et al., 2004, Bhargava et al., 2012). Yang et al., 2014, Fan et al., 2016).

This is the first study conducted in Sudanese hypertensive patients to investigate the association between C677T *MTHFR* (rs1801133) gene polymorphism with the susceptibility to hypertension. The analysis of the demographic data showed that large scale of the cases (93%) are at ≥ 40 years of which 63% were females. This result agreed the previous finding of (Maric-Bilkan 2005) and could be attributed to a combination of factor including an increased body weight, stress or lack or insufficient exercise that are common among Sudanese women, or might be related to hormonal changes, as females` hormones are known to be protective against hypertension in women before menopause (Sandberg and Hong 2012). This study also showed that having a personal family history of hypertension increases the likelihood three times that an

individual develops hypertension (P=0.003, OR=3.1) This result in accordance with Priyanga et al., 2015 and Miao et al., 2015 finding in Asian ethnic groups; which indicates a real genetic contribution in disease predisposition in different ethnicity.

Smoking and sedentary life were strictly associated with hypertension in our samples (P value= 0.04 and P-value =0.001respectively). This result was supported by the previous studies that provided evidence that both smoking and lack of exercise are associated with the impairment of endothelial function and acutely increases arterial stiffness; thereby increases the possibility of raising the blood pressure (Halperin et al., 2008, Virdis et al., 2010, Keith and Daichi 2013, Pescatello et al., 2015, Kaiye et al., 2017).

Hypertension was known as a risk of developing diabetes and heart diseases among large scale of hypertensive (WHO 2013). In this study diabetes and heart diseases; were observed among hypertensive with a significantly higher frequency compared to normotensive (P-value =0.002 and P-value=0.0001 respectively). Although the stroke and kidney failure were known as serious complications of hypertension (Xin et al., 2019 Jing 2010). However, this study did not show their association with hypertension.

The analysis of the C677T *MTHFR* (rs1801133) gene polymorphism; revealed that the hypertensive group had a significantly higher frequency of the mutant CT+TT genotypes compared to the control group (P-value = 0.04), with two folds increased risk of hypertension (OR=1.903). This finding agreed the previous studies conducted in different ethnic groups including Saudi, Morocan, Czech, Caucasians, Indian (Abdullah et al., 2012, Nassereddine et al., 2015, Benes et al., 2001, Heux et al., 2004, Suchita et al., 2007) and supported by the meta-analysis results which provided evidence that *MTHFR* C677T polymorphism is strongly associated with the risk of hypertension among the widely varied ethnic groups studied (Qian et al., 2007, Yang et al., 2014, Yi-Le et al., 2014).

Although the mutant TT was found with the least frequency among cases, but the presence of the CT genotype among cases must also be considered as a risk of hypertension, as it has been previously confirmed that the mutation in the heterozygous or homozygous state of the *MTHFR* C677T polymorphism correlates with the reduced enzyme activity; and this results in an increased homocysteinaemia level, which is known as a risk of the vascular system abnormalities (Bhargava et al., 2012, Bansal et al., 2014, Ganguly and Alam 2015). This is why in this study we conducted the genotype analysis considering the dominant model genotype (CC versus mutant CT+TT).

The result of this study not only revealed an association of the C677T *MTHFR* polymorphism with the risk for hypertension in our samples, but also might suggest that the controls who have the CT genotype might be at risk of developing hypertension in the presence of other biological risks such as an increased body mass or behavioral risks such as smoking, lack of exercise, or eating unhealthy food.

Conclusions

This study concluded that the C677T *MTHFR* gene polymorphism might be a genetic susceptibility factor and it is associated with the increased risk of essential hypertension in the studied subjects. The association of the other risk factor such as family history, sedentary life and smoking with hypertension was reported in this study.

In addition, hypertension was accompanied by health complications represented by diabetes and heart diseases. Based on this it is better thought of riboflavin that has been previously declared as a modifiable of the *MTHFR* polymorphism (McNulty et al., 2017) to be used as preventive strategy or to delay the development of hypertension and its associated health complications for individuals who carry this polymorphism.

Hypertension is a complex disease. Other candidate genes may contribute to the disease predisposition. Therefore, further studies of large sample targeting certain ethnic group to avoid the population stratification, might be interesting in the understanding of the role of the *MTHFR* and other genes` polymorphisms in the susceptibility to hypertension in our population. Also more attention should be paid to implement programs to raise the public awareness of this silent killer disease is important.

Abbreviations

MTHFR = Methylenetetrahydrofolate reductase

OR= Odds ratios.

SPSS = Statistical package of social studies.

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