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Detection of Methylenetetrahydrofolate Reductase (MTHFR) C677T Gene Polymorphism among Sudanese Hemodialysis Patients

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Abstract

Introduction: Patients with end stage renal disease (ESRD) have the higher rate of mortality than the general population. Cardiovascular disease, a frequent complication in these patients, is a major cause of the mortality. Methylenetetrahydrofolate reductase gene polymorphism determinates the level of homocysteine among other factors and may be consider as one of the cardiovascular risk factor. Several studies have shown that high levels of homocysteine are associated with high risk of cardiovascular disease. The main objective of this study was to examine the distribution of genotype frequencies of C677T MTHFR among ESRD Sudanese patients on maintenance hemodialysis.

Methodology: This case-control study was included 72 samples, genetically unrelated 43 cases collected from two dialysis centers in governmental hospitals in Khartoum and healthy matched 30 controls (matched in age, ethnicity and population), which were collected from community. Blood samples were collected in EDTA containers and genomic DNA was extracted using phenol chloroform method, then C677T MTHFR polymorphism detection was carried out by PCR-RFLP technique.

Results: Sudanese ESRD patients who visiting hemodialysis centers involved in the present study have shown 0% frequencies for the MTHFR gene polymorphism C677T. This indicates that all patients were carried the wild type genotype (CC); no mutant allele (T) was found.

Conclusion: The results obtained in this study revealed that the C677T single nucleotide polymorphism (SNP) for the MTHFR gene found to be very rare among these patients

Keywords: Homocysteine, ESRD, MTHFR polymorphism, hemodialysis, PCR-RFLP technique.

Introduction

Homocysteine (Hcy) is an amino acid derived from dietary methionine after its ATP dependent activation and subsequent trans-methylation of biologically important molecules, it is found in the plasma of all mammals, and the term “total homocysteine” (tHcy) is used to describe a composite of free (sulfhydryl), protein-disulfidebound, and homocysteine-cysteine and other mixed disulfide species (Mudd *et al.*, 2000).

Patients with chronic kidney disease or end-stage renal disease (ESRD) has a high homocysteine levels and they have extensive vascular disease. Hyper-homocysteinemia is been linked with increased risk for both fatal and nonfatal cardiovascular events (Suliman *et al.*, 2000), as well as vascular access thrombosis, the most common cause for hospitalization of hemodialysis patients (Sengupta *et al.*, 2001). Cardiovascular disease (CVD) is common in ESRD patients, and it is responsible for the majority of morbidity and mortality in these patients (Levin *et al.*, 2002).

One of the most common mutations, or polymorphisms, that are associated with a mild increase in plasma homocysteine, is the 677 (C to T) substitution (an alanine to valine change) in the MTHFR gene, which result in a decreased enzyme activity (Margreet *et al.*, 2005).With the MTHFR dysfunctions, an increased plasma homocyseine level is expected, which, in turn, produces a cytotoxic effects (Matetzky *et al.*, 2003). Among the

endothelium-directed factors, a polymorphism of MTHFR gene coding for an enzyme that degrades the endothelium toxic product homocysteine have been associated with ESRD (Grindt *et al.*, 2007).

In Sudan, hyperhomocystenemia, is a new independent risk factor for atherosclerotic vascular disease which has been described in the last years, with the increasing number of end stage renal disease (ESRD) and its association with other cardiovascular risk factors necessitate the study of MTHFR C677T gene polymorphism, the main genetic cause of hyperhomocystenemia in hemodialysis patients.

Objectives

- To detect the presence of MTHFR C677T gene polymorphism among the study population.

- To associate between this mutation and ESRD complications among these patients.

Patients and Methods

A hospital case-control study was included of 72 samples, genetically unrelated 43 cases collected from dialysis centers in governmental hospitals in Khartoum state and healthy matched 29 controls (matched in age, ethnicity and population), which were collected from community.

Inclusion criteria of patients

Sudanese hemidialysis patients, male and female, age > 18 years old.

Exclusion criteria of patients

Patients < 18 years old, Diabetes, pregnant women, Obesity, Hypertension, Smokers, Stroke and Myocardial Infarction.

After informed consents, interviews with study participants were conducted using a structured questionnaire and 5ml of blood samples were collected in EDTA tubes. The questionnaire filled with comprehensive data included information about age, weight, duration of dialysis and family history of some diseases like diabetes, hypertension and cardiovascular disease.

Genetic analysis

C677T MTHFR gene polymorphism investigation was performed on the blood samples as per protocol. DNA was extracted using phenol-chloroform method (Sambrook, 1989).

The point mutation at 677 positions picked up by PCR by relevant probes, this followed by restriction fragments length polymorphism (RFLP) using HinfI (haemophilus influenza) restriction endonuclease enzyme (Micheal *et al.*, 2009). Post digestions, bands of 198bp were visualized under UV light at gel documentation system (DOC-008.XD).

Statistical analysis

Statistical analysis has done by use of SPSS (Statistical Package for the Social Sciences) version l6. The differences between the groups were tested for significance by student's t-test, Onaway ANOVA test and chi-square test. Data were expressed as the mean ± SD. P-values < 0.05 are considered statistically significant.

Results

Demographic Data

A total of 72 samples were included in this study, consisting of 29 males and 14 females in case group and 20 males and 9 females in control group (P=0.89) (Figure 1). All participants in this study classified into two main groups according to their age. In the first age group, which was included subjects at less than 15 years old; there were 24 patients with mean age 10.1 $\pm$ 1.5 and 10.25 $\pm$ 1.7 and 7 controls with mean age 12.8 $\pm$ 1.04 and 12.5 $\pm$ 1.10, for males and females, respectively. The second age group was included subjects at more or equal 15 years old, in which 19 cases with mean age 16.23 $\pm$ 0.78 & 16.67 $\pm$ 1, and 22 controls with mean age 18.93 $\pm$ 0.67 & 17.3 $\pm$1.5, also for males and females, respectively. No significant

difference between cases and controls in the mean age of males and females within either first age group (<15 years old, P= 0.82) (Figure 2), or within second one (≥ 15 years, P= 0.8) (Figure 3).

There was an extremely significant difference in the mean weight between cases and controls in general within both age groups (< 15 & ≥ 15 years old, P< 0.0001) (Figure 4). However, this difference was more obvious among males (P= 0.0001) than females (P= 0.02). Among cases, there were 23% of them with a hypertension, 2% with heart disease and 75% with no other disease (Figure 5).

Genetic analysis

A total of 43 cases with end stage renal disease and 29 controls were included in this analysis. Amplification of the region containing the C677T SNP was resulted in a specific product with 198bp size band (Figure 6).All PCR products digested with *Hinf*1 restriction enzyme resulted in one DNA fragment with size band of 198bp, which identified as homozygous normal genotype (CC). Neither heterozygous genotype (CT), nor homozygous mutant (TT) appeared in the RFLP analysis. Therefore, the frequency of homozygous normal genotype among case and control groups was 100% (Figure 7), while the frequencies of other genotypes (heterozygote & mutant) were 0%.

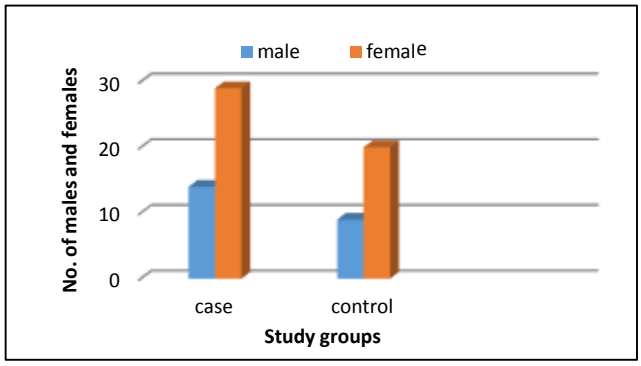

Figure 1: The number of males and females among study groups

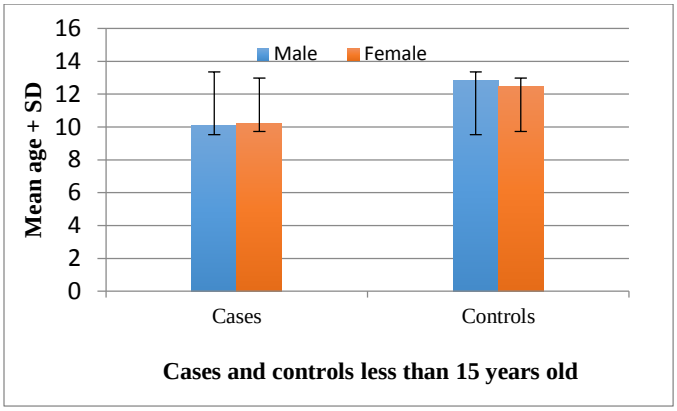

Figure 2: The mean age & SD of the males and females (< 15 years) among cases and controls

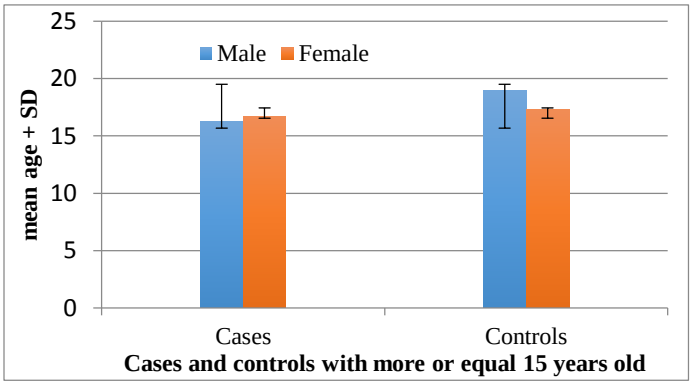

Figure 3: The mean age & SD of the males and females (≥ 15 years) among cases and controls

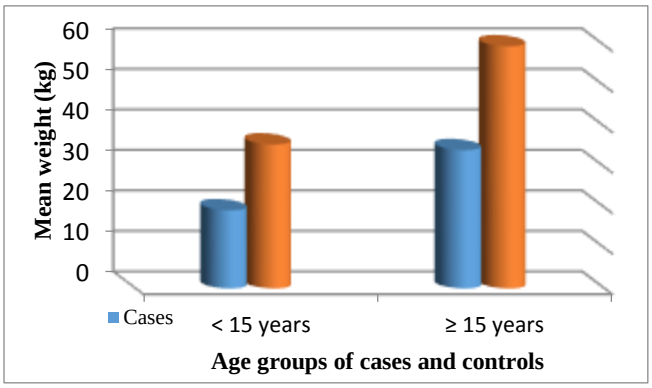

Figure 4: The mean weight of cases and control in general among different age groups

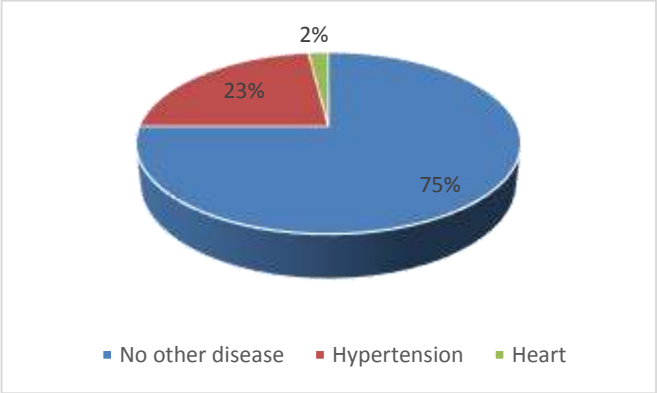

Figure 5: The Percentage Of End Stage Renal Patients With And Without Oher Diseases

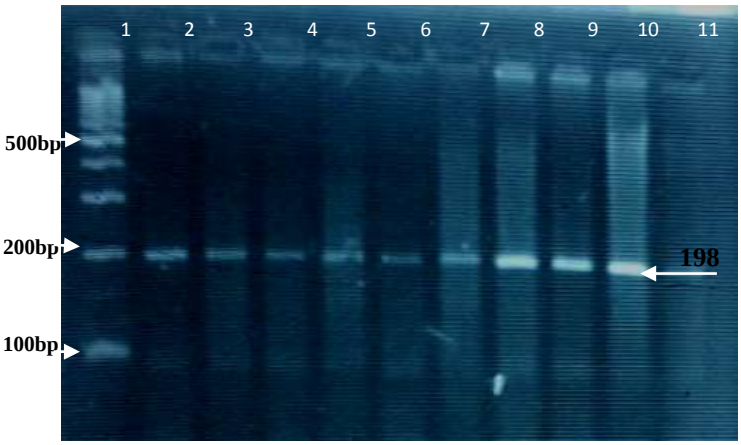

Figure 6: PCR Gel Electrophoresis of the *mthfr* gene containing C677T SNP (band at 198bp) **Lane 1**: 100bp DNA ladder. **Lane 2-11:** PCR products of collected samples, band size 198bp.

Discussion

Patients on dialysis have substantially higher mortality rates compared to the general population. Traditional risk factors such as age, black race, male gender, smoking, hypertension, hyper-lipidemia, diabetes and obesity cannot explain the complete individual susceptibility to development of ESRD and accompanying complications that essentially determine the clinical outcome.

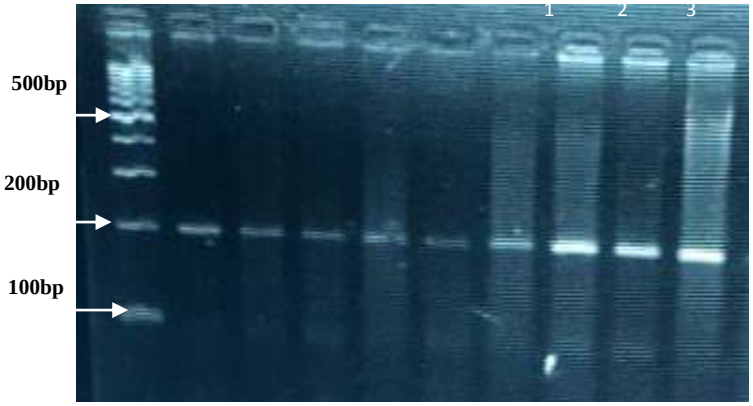

Figure 7: RFLP Gel Electrophoresis for PCR products digested with *Hinf*I restriction enzyme **Lane 1:** 100bp DNA ladder. **Lane 2-10:** Homozygous normal genotype (CC)

Normal kidneys play a prominent role in plasma Hcy handling and homocysteine level increases as renal function declines and progresses to ESRD, with the vast majority (85%) of dialysis patients ultimately experiencing mild-to-moderate hyper-homocysteinemia (Foley *et al*., 1998). Hemodialysis patients have a much higher overall mortality rate than the general population (Nurko, 2006; Herzog *et al*., 2011). Cardiovascular disease, a frequent complication in these patients, is a major cause of the mortality (Astor *et al*., 2008). Methylenetetrahydrofolate reductase gene polymorphism has been associated with increased homocysteine levels, which in turn contribute to cardiovascular disease among ESRD patients (Wu *et al*., 2012).

In the current study, 72 blood samples were collected from ESRD patients to detect the presence of C677T *MTHFR* gene polymorphism. The results revealed that all subjects involved in this study were carried the wild genotype (CC); no mutant allele (T) was reported, which agree with (Saraswathy *et al*. 2012), who has found that the frequency of T allele was 0% in African population; whereas the C677T allele frequency found to be higher in European populations (24.1% to 64.3%).

On the other hand, the absence of this SNP is likely to be a protective factor among patients of ESRD with wild type alleles. This is partly in concordance with a previous study by (Trovato *et al*. 2015), who revealed that the MTHFR gene polymorphisms could have a protective role on renal function as suggested by their lower frequency among their dialysis patients in ESRD. Differences may come from small sample size and most of studies on this SNP have done with a large number of sample size. Other study by Elhassan and Abdalla (2015) recorded low frequency of MTHFR C677T mutant genotypes with low impact in the risk of deep vein thrombosis (DVT) among Sudanese population.

Conclusion

The results obtained in this study revealed that C677T polymorphism is not associated with onset, duration and cause of kidney failure in our hemodialysis population. Sudanese ESRD patients who visiting hemodialysis centers involved in the studied groups have shown 0% frequencies for the MTHFR gene polymorphism C677T, therefore, we have concluded that the C677T SNP for the MTHFR gene found to be very rare among the study population.

Recommendation

Large prospective study that includes specific clinical features and biochemical and genetic markers is necessary to provide precise conclusion about the influence of MTHFR polymorphism C677T on age of onset, duration of hemodialysis and cardiovascular complication among Sudanese population.

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