Role of Methylenetetrahydrofolate Reductase (MTHFR) C677T Mutation in Cardiac Syndrome X

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Abstract

Background: Cardiac syndrome X (angina-like chest pain, positive stress - ECG, and normal coronary angiogram) has serious medical complications. Methylenetetrahydrofolate reductase (MTHFR C677T) may be an important factor associated with the disease.

Objective: The present study aimed to evaluate the association between MTHFR C677T mutation and cardiac syndrome X (CSX) in Sudanese population.

Materials and methods: A total of 50 patients with CSX and their matching controls were enrolled in this study. Venous blood sample was collected from each participant in Ethylene Diamine Tetra Acetic acid (EDTA). DNA was extracted from blood samples using guanidine chloride method and MTHFR mutation was detected by polymerase chain reaction- restriction fragment polymorphism (PCR-RFLP). Data were analyzed using statistical package for social sciences (SPSS), version 18.

Results: The mean age for patients was 44.98 years and for controls was 40.38 years. MTHFR C677T was significantly associated with CSX (20% versus 4% in control group; P.value: 0.014); the frequency of the heterozygous allele was higher than the homozygous allele (20% vs. 2%)

Conclusion: MTHFR C677T is associated with CSX in Sudanese population. The mutation may be used as a molecular screening tool for the disease.

Keywords: Cardiac syndrome X, Methylenetetrahydrofolate reductase; C677T mutation

Introduction

Cardiac syndrome (CSX) is defined as a typical angina-like chest pain, a positive response to stress testing and normal coronary arteriography (CAG) [1]. CSX is a serious medical condition associated with increased frequency of myocardial infarction, stroke, Congestive cardiac failure and death. Subsequent functional disability secondary to the syndrome was also reported. The frequency of Methylenetetrahydrofolate reductase 677C-T mutation (MTHFR C677T) is low in Africa (6.6%) compared with Europe and
Asia [2]. The 677C-T mutation may represent an important genetic risk factor in vascular disease [3].

Unfortunately, CSX is very difficult to diagnose on clinical basis because it depends on excluding other diseases. A molecular marker for early detection of people with CSX is highly needed. Association between MTHFR C677T and CSX was established earlier [4].

The present study aimed to evaluate the association between MTHFR C677T and CSX in Sudanese population.

**Materials and methods**

Fifty patients with CSX and their matching controls, residing in Khartoum State, Sudan were involved in this study after signing a written consent. The study was carried out during the period from February 2011- June 2012. Demographic, social, and clinical data were collected from all participants using a pre-structured interview questionnaire directly from the patient and/or from hospital records. A total of 10 ml of overnight fasting venous blood were collected in EDTA tubes and kept frozen at -20°C for DNA extraction and molecular analysis.

Ethical approval was obtained from a local institutional committee at Al Neelain University.

DNA was extracted using guanidine chloride method. DNA was amplified by polymerase chain reaction using Taq DNA polymerase and suitable primers. PCR was performed with a GeneAmp PCR kit (Perkin-Elmer Cetus). The sense and antisense primers were as follow:

5'-TGAAGGAGAAGGTGTCTGCGGA-3'
5'-AGGACGGTGCGGTGAGAGTG-3'

Thirty-five cycles (95°C for 60 seconds, 62°C for 90 seconds, 72°C for 60 seconds) were used to amplify 198-bp products. The amplified fragments were cut with Hinfl endonuclease, which can recognize the C-to-T substitution in the fragments. Because this one nucleotide substitution corresponds to a conversion of Ala-to-Val residue in the MTHFR encoding region, the two different alleles were designated A (Ala) and V (Val). The 198-bp fragment derived from the A allele is not digested by Hinfl, whereas the fragment of the same length from the V allele is digested by Hinfl into 175- and 23-bp fragments. The Hinfl-treated PCR fragments were electrophoresed in 9.6% polyacrylamide gels and stained with ethidium bromide. The wild type (CC) appeared as 198 bp band, TT as 175 bp band, and CT appeared as two bands (175 and 198 bp). Data was analyzed using SPSS (version 18). Student t-test was used to compare means. P-value is considered significant when =/< 0.05.

**Results**

The present study included 50 patients with CSX, 30 females (60%), and 20 males (40%). The mean age for patients was 44.98 years compared to 40.38 years for control group. Ten patients (20%) had MTHFR C677T mutation compared to two control individuals (4%). The difference
was significant \( (P.\text{value}:0.014) \). The frequency of the heterozygous allele was extremely higher than the homozygous allele (20\% vs.2\%).

**Discussion**

The present study investigated whether MTHFR C677T is associated with CSX. The majority of patients were females, the assumption that CSX is women’s disease (4). Middle-aged individuals were more commonly affected with CSX. This finding matched with the theory that CSX is middle-aged disease and it’s relatively rare among the age extremities [1]. The frequency of the heterozygous allele (20\%) was higher than the homozygous allele (2\%). The frequency of the allele in the normal population was 4\%. These data agreed, in general, with a global study which found that allele frequency was 0.07 in sub-Saharan Africans. In addition, none of the Africans examined was homozygous for the mutation[6].

MTHFR C677T was associated with CSX, these data strongly supported data obtained by Alroy et al., (2007); who revealed a 47\% frequency of patients with CSX having the heterozygous allele compared to 33\% for the homozygous allele [7].

The current study supported data obtained from a meta-analysis of the risk of coronary heart disease related to the 677C-T polymorphism [8]. It concluded that individuals with the 677CT genotype have a significantly higher risk of coronary heart disease. Moreover, the study agreed with data obtained by Morita et al., who reported a significantly higher frequency of the rarer 677C-T allele, corresponding to a valine substitution, in the disease group [9].

The lowest frequency of the homozygous allele among CSX patients can be explained based on Tonetti’s report of a family in which two of four children were affected with MTHFR. Their mother, homozygous for the mutation, was clinically normal. The father, being heterozygous for the polymorphism, exhibited clinical features of CSX. MTHFR C677T was more frequent than T677T among Sudanese Patients having CSX. The present study contrasts with what has been investigated by Kluijtmans’s study which concluded that, homozygosity for this mutation is associated with a 3-fold increase in risk for premature cardiovascular disease [10]. The present study did not agree with Schwartz research who concluded that, this polymorphism was not a risk factor for myocardial infarction in their population [11]. Others also did not find such a relationship in their studies [12, 13].

It appears that, other factors; including ethnic and racial variations, as well as genetic and environmental factors, may play a critical role in deciding susceptibility of individuals with different MTHFR alleles to get affected with the disease.

**Conclusion**

MTHFR C677T is associated with CSX in Sudanese population. The use of the mutation as screening tool for CSX should be considered.
References


