New Insight of Methylenetetrahydrofolate Reductase (MTHFR) C677T Gene Polymorphisms, and serum electrolytes in Cardiac Syndrome X Patients

Abstract:

Background: Cardiac Syndrome X (CSX) is a condition affecting the cardiovascular system with a significant degree of morbidity~~. a significant morbidity~~ . Diagnosis and treatment are challenging when the cause is unclear. Subsequently, a molecular marker for screening of people with CSX is highly recommended. The present study evaluated the association between MTHFR C677T gene polymorphism among Sudanese patients with CSX. **Materials and methods:** A total of 100 were enrolled. Venous blood sample was collected from each participant in (EDTA) containers. DNA was extracted from blood samples using guanidine chloride method and MTHFR mutation was detected by PCR-~~-~~restriction fragment polymorphism (PCR-RFLP). Statistical package for social sciences (SPSS) was used to analyze data.

Results: Most patients 30(**60%**) were females, their age ranged between 30-60~~ years old~~ with mean age 44.98±7.34 SD~~,~~ MTHFR 677CT genotype~~s~~ frequency was statistically significant (P~~ value~~≤0.014), where 10(20%) had 677CT and 1(2%) had 677TT among patients group respectively compared to control individuals who had only 2(4%) 677CT. T alleles were significantly more frequent among our participant than C alleles. There is insignificant slightly decreased (2.4 ±2.8, and 2.5±3.2) in serum magnesium levels among patients compared to control respectively, as well as random blood glucose. Elevated mean levels of total cholesterol, and HDL among patients (182 ±18.1, and 49.7±7.1) vs (180 ±20.3, and 46.6 ±11.3) among control group, all findings were statistically non-significant. Slightly decrease in magnesium level (2.2 ±2.1, vs 2.9 ±0.8) among heterozygous CT genotypes compared to homozygous genotypes.

Conclusion: MTHFR C677T is linked to CSX in the Sudanese population, and serum magnesium level was slightly decreased among heterozygous MTHFR C677T. Furthermore, the mutation could be used as a disease molecular screening technique.

Keywords: MTHFR 677CT, Cardiac Syndrome X, Gene Polymorphisms**.**

Introduction:

What is the cohort **:[1s]Comment** eof these enrolled peopl

write in full first **:[2s]Comment**

,write in full first **:[3s]Comment** then use the shortened version afterwards

A general definition of Cardiac syndrome X (CSX) highlights the triad of cardiac pain, ~~a normal~~a normal coronary angiogram with a positive stress testing~~, and~~ [1]. A more restricted definition adds ST-segment depression during angina, absence of spontaneous or inducible epicardial coronary artery spasm upon acetylcholine provocation, and absence of cardiac or systemic diseases [2]. Cardiac syndrome X accounts for 20-30% of patients undergoing coronary angiography [3, 4]. The disease is more common in women than men, furthermore, it is more common pre-menopausal and postmenopausal women pointing to a possible hormonal contribution [5]. The etiology of the disease is not clearly understood. However, some theories have been proposed. The most common theory is that cardiac syndrome X is due to endothelial dysfunction [6]. A properly functioning endothelium necessitates a balance between vasoconstrictors such as endothelin-1, and vasodilators, such as nitric oxide (NO). therefore, an imbalance may result in vasoconstriction of the vessels, leukocyte adherence, plaque activation, mitogenesis, and increases in the oxidative state, thrombosis, activation of coagulation, vascular inflammation, and atherosclerosis [7, 8]. Increased resistance to coronary flow is also incriminated in the pathogenesis of endothelial dysfunction [9, 10]. MTHFR is a 77 kDa flavoprotein involved in folate metabolism. It is essential for the conversion of homocysteine to methionine. Hence enzyme deficiency may be associated with the accumulation of elevated levels of homocysteine in the blood (hyperhomocysteinemia). The gene is present at position 1p36.3 on chromosome 1 [11]. As polymorphism may be associated with reduced enzyme activity, MTFR 677 C-T can cause hyperhomocysteinemia [12]. A point mutation at position 677 of the gene, at which cytosine (C) changes into a thymine (T); is responsible for the replacement of alanine with valine (2, 8). This from of the enzyme is considered as thermolabile [13]. Thus, MTHFR activity is greatly reduced among C677T homozygotes (2). Heterozygotes are in the intermediate range. People who are homozygous for the C677T allele tend to have mildly increased blood homocysteine levels if their folate intake is insufficient but normal blood levels if their folate intake is adequate [14, 15]. The frequency of Methylenetetrahydrofolate reductase 677C-T mutation (MTHFR C677T) is low in Africa (6.6%) compared with Europe and Asia [16]. The current study evaluated the association between MTHFR C677T and cardiac syndrome among Sudanese patients.

Material and Methods:

The study was carried out among Sudanese patients attending outpatients' clinics at Al-shaab hospital, Khartoum Sudan by typical symptoms of CSX. pre-structured questionnaire was used for collection of baseline and clinical data. The study was ethically approved by an institutional committee at faculty of Medicine, Al-Neelain University.

A total of fifty patients with a confirmed diagnosis with CSX were recruited during the study period using nonprobability sampling technique. Any subjects have had coronary heart disease, heart valve diseases, congestive heart failure, current infectious diseases, and malignancy were excluded from the study.

A total of 10 ml of venous blood were collected form each subject. For DNA extraction and molecular analysis, a total of 6 ml of blood was contained -in EDTA tubes and stored at -20^0 C. Another four milliliters of blood were collected in a plain tube and centrifuged for measurement of serum electrolytes, renal and liver functions. Magnesium was measured using the calmagite method. hypomagnesaemia was considered when serum magnesium was less than 1.6 mg/dl. Lipid profile, random blood glucose, and serum creatinine were calculated using standard procedures.

DNA was extracted using guanidine chloride method. DNA was amplified by polymerase chain reaction using Taq DNA polymerase and suitable primers. GeneAmp PCR kit (Perkin-Elmer Cetus) was used to perform PCR using the sequences 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′, as sense and antisense primers, respectively. The 198-bp amplified fragments were cut with HinfI endonuclease, which identifies C-T substitution. Accordingly, The 198-bp fragment derived from the mutated allele was digested by HinfI into 175- and 23- bp fragments. The HinfI-treated fragments were electrophoresed in 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).

Statistical analysis:

SPSS version 20 was to analyze the results. Means were compared using Student t-test and frequencies were calculated using Chi-square test. A P-value equal to or less than 0.05 was considered significant.

Result:

About 100 subjects participates in the study, and categorized as two group: case group, and control group. Most patients 30(60%) were females, their age range between 30-60 years old with mean age 44.98±7.34 SD and compared to 40.38±5.21 SD years for control group. MTHFR 677CT genotypes frequency was statistically significant (P value≤0.014), where 10 (20%) had 677CT and 1(2%) had 677TT among patients group respectively compared to control individuals who had only 2(4%) 677CT. moreover no 677CC genotypes was detected among both group. T alleles were significantly more frequent among our participant than C alleles. All data were illustrated in table 1.

Table 2 displayed the mean levels of all measured mean level of magnesium and lipids profiles among cardiac syndrome X participants and control. There is slightly decreased (2.4 ±2.8, and 2.5±3.2) in magnesium level among patients compared to control respectively, as well as random blood glucose. Conversely there were elevated mean levels of total cholesterol, and HDL among patients (182 ±18.1, and 49.7±7.1) vs (180 ±20.3, and 46.6 ±11.3) mean level among control group, all findings were statistically non-significant.

Correlation between Single Nucleotides Polymorphisms (SNPs) and serum magnesium in patients with cardiac syndrome X were summarized in table 3. There were slightly decrease in magnesium level (2.2 ±2.1, vs 2.9 ±0.8) among heterozygous CT genotypes compared to homozygous genotypes TT respectively, the result was statistically in significant difference (P value ≤0.063)

Figure 1 show an Agarose Gel electrophoresis for MTHFR C677T after digestion with Hinf-I: Lane 1: 25-bp DNA marker, Lanes 3,6,12: (CC) genotype with one fragment 198 bp, Lanes 2,4,5: (TT) genotype with two fragments 175 and 23 (*faint*), Lanes 7-11,13-15: (CT) genotype with three fragments 198, 175 and 23 bp (*not shown*).

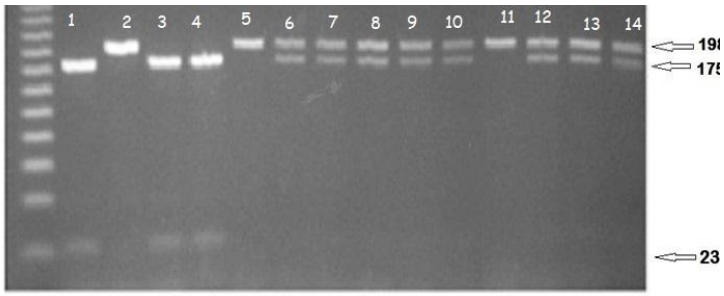

Figure 1: Agarose Gel electrophoresis for MTHFR C677T

Table 1: Frequency of MTHFR Single Nucleotide Polymorphisms (SNPs) among study subjects among

	Patients N=50 (%)	**Control group N=50 (%)**	***P*-value**
Sex			
Male	20 (40%)	23 (45%)	0.921
Female	30 (60%)	27 (54%)	
Age	44.9±8.0	40.3±8.4	0.06
677 CT	10 (20%)	2 (4%)	0.014
677 TT	1 (2%)	0	
677 CC	0	0	
Total	11 (22%)	2 (4%)	
			0.013
C alleles	23 (45%)	0	
T alleles	27 (54%)	1 (100%)	

Table 2: Mean level of Magnesium and lipids profiles among cardiac syndrome X participants and control

Variables	**Patients N=50 (Mean ±SD)**	**Control group N=50 (Mean ±SD)**	***P*-value**
Magnesium mg/dl	2.4 ±2.8	2.5±3.2	0.150
RBG, mg/dl	129 ±39.5	141.3±34.5	0.100
Cholesterol, mg/dl	182 ±18.1	180 ±20.3	0.941
LDL, mg/dl	136.7 ±14.6	136.6 ±16.4	0.971
HDL, mg/dl	49.7±7.1	46.6 ±11.3	0.114
TAG, mg/dl	128.7 ±5	128.1±14	0.712

Urea, mg/dl	32.3±10.7	32.6±8.8	0.976
Creatinine, mg/dl	1.17 ±0.49	1.21±**0**.13	1.000

*Data expressed as mean (SD) or N (%) as applicable.

Table 3: Correlation between Single Nucleotides Polymorphisms and serummagnesium in patients with cardiac syndrome X.

	MTHFR Genotypes		***P*-value**
	CT Genotype	**Wildtype**	
Magnesium mg/dl	2.2 ±2.1	2.9 ±0.8	0.063
RBG, mg/dl	128±37.5	130 ±39.5	0.291
Cholesterol, mg/dl	180 ±13.1	199±11.4	0.050
LDL, mg/dl	136.4 ±4.6	138.7 ±10.6	0.073
HDL, mg/dl	48.3±4.1	44.5±6.8	0.032
TAG, mg/dl	129. 2 ±4	135 .1 ±3	0.743
Urea, mg/dl	29.3±8.5	30.3±9.7	0.912
Creatinine, mg/dl	1.04 ±0.40	1.18 ±0.46	0.123

Discussion:

MTHFR C677T gene polymorphisms was associated with CSX, supporting data obtained from a study linking the enzyme activity to vascular disorders [16]. Current study revealed that there were statistically significant differences of MTHFR C677T genotypes between patients and control group. These -results agreed with Gupta SK et. al [17].

Use same fond **:[4s]Comment** type and size as rest of the text

The frequency of the **heterozygous** allele **677CT was 10**(**20%**) in CSX patients which much higher than **1**(**2%**) detected among the **homozygous** allele **677 TT**, strongly supported data obtained by Alroy S et al. (2007) [18], who find the correlation between the C677T mutation among CSX patients and control, and C677T mutation directly associated with endothelial cell dysfunction, and develop an innovative and affordable therapy for a subset of patients with syndrome X and the C677T mutant.

The low frequency of the homozygous allele TT and CC among CSX participants though it is unexplainable, and it appeared to be less associated with CSX among Sudanese population and had contrasted with what has been proved that TT is associated with a 3-

fold increase in risk for premature cardiovascular disease than CC/CT- corresponded to a report of a family in which two of four family members were affected. Their mother, homozygous for the mutation; was clinically normal. The father, being heterozygous for the polymorphism, exhibited clinical features of CSX. C677T polymorphism effect can be suppressed by addition of folate, which increases enzyme affinity for FAD [19].

This is similar to the globally documented data that conclude that, the occurrence of the wildtype varied from 5.4 percent to 16.0 percent among the control group and from 6.5 percent to 29.7 percent among patients. In all of the investigations, the patterns of genetic background and allele frequencies in the patient and control groups were nearly identical [20].

Regarding allele frequency, T allele was significantly associated with CSX (P value≤0.013), and surprisingly it consistent with that noted with T allele was determined to be the contributing allele for CAD and was likely linked to CAD worse. [21]

With regard gender, the majority of participants (60%) were females, these findings were agreed with the assumption that CSX is middle-aged women's disease and that it is relatively rare among the age extremities. The same conclusion was documented by Kandaz C, et al [19] who stated that the majority of female patients in our study group was substantially higher than the control group (P.05), indicating that CSX is more frequent in women. Numerous studies found an association between MTHFR polymorphism and premature CAD and myocardial infarction (MI) despite normal homocysteine levels. A study by Bouzidi, N et al concluded that there was no association between Hcy levels and CAD severity [22] Other studies, however, did not find a significant relationship between MTHFR C677T and cardiac syndrome X [23].

The current study concluded that serum magnesium levels, as well as random blood glucose levels, are somewhat lower (2.42.8 and 2.53.2) in patients compared to controls. In contrast, mean total cholesterol and HDL values were higher in the patients (182 18.1, and 49.77.1) than in the control group (180 20.3, and 46.6 11.3).

This finding agreed to a by Rassoul F et. al [24], in which LDL cholesterol and triglyceride levels were much greater in CAD patients than in controls, while serum HDL concentrations were considerably lower, and Hcy levels were significantly higher. Triglyceride levels higher than 199 mg/dl may also be a potential risk for CAD in Indians.

use same fond as rest of the text **:[5s]Comment**

When all other parameters were tested, elevated amounts of Hcy, cholesterol, excessive LDL and decreased levels of HDL, and smoking were revealed to be predictive factors of CAD [25].

Interestingly, there was a statistically significant difference (P value≤0.043) in magnesium levels between heterozygous CT genotypes and homozygous TT genotypes (2.22.1 vs 2.90.8), these were satisfied findings as some risk factors for cardiovascular disease and atherosclerosis, such as lipid profile and blood pressure, have been linked to serum magnesium levels [26]. It is seemed that low serum magnesium concertation along with heterozygous CT genotypes of MTHFR play critical role in development of CSX attack. Prospectively the clinical presentation of homocystinuria that caused by severe MTHFR deficiency should be investigated.

Limitation of the study:

The current study was limited with the small sample size. Involving a larger number of patients may increase the level of reliability regarding the obtained results. To our knowledge, this is the first study to look into the genetic relationship between MTHFR and CSX. Another significant limitation is that our research solely evaluated at MTHFR genetic polymorphisms. Furthermore, plasma homocysteine levels and MTHFR enzyme activity must be examined in order to better understand the association between homocysteine metabolism and CSX.

Conclusion:

MTHFR C677T is linked to CSX in the Sudanese population, and serum magnesium level was slightly decreased among heterozygous MTHFR C677T. Furthermore, the mutation could be used as a disease molecular screening technique.

Data and materials availability All data associated with this study are present in the paper.

References:

1. Kaski JC. Overview of gender aspects of cardiac syndrome X. *Cardiovasc Res*. 2002 Feb 15. 53(3):620-6.
2. Kanar BG, Sunbul M. Cardiac syndrome X: An important cause of microvascular angina. *Turk Kardiyol Dern Ars*. 2018 Sep. 46 (6):437-8.)
3. Hurst T, Olson TH, Olson LE, Appleton CP. Cardiac syndrome X and endothelial dysfunction: new concepts in prognosis and treatment. Am J Med. 2006 Jul;119(7):560-6.

doi: 10.1016/j.amjmed.2005.07.009. PMID: 16828624.

4. Singh M, Singh S, Arora R, Khosla S. Cardiac syndrome X: current concepts. Int J Cardiol. 2010 Jul 9;142(2):113-9. doi: 10.1016/j.ijcard.2009.11.021. Epub 2010 Feb 7. PMID: 20138677.
5. Armen Parsyan, Louise Pilote. Cardiac Syndrome X: Mystery Continues. The Canadian Journal of Cardiology. Mar 2012. 28:S3-S6),
6. Kidawa M, Krzeminska-Pakula M, Peruga JZ, Kasprzak JD. Arterial dysfunction in syndrome X: results of arterial reactivity and pulse wave propagation tests. *Heart*. 2003;89(4):422-426. doi:10.1136/heart.89.4.422
7. Hedhli N, Huang Q, Kalinowski A, Palmeri M, Hu X, Russell RR, Russell KS. Endothelium-derived neuregulin protects the heart against ischemic injury. Circulation. 2011 May 24;123(20):2254-62. doi: 10.1161/CIRCULATIONAHA.110.991125. Epub 2011 May 9. PMID: 21555713; PMCID: PMC3104502.
8. Gupta SK, Kotwal J, Kotwal A, Dhall A, Garg S. Role of homocysteine & MTHFR C677T gene polymorphism as risk factors for coronary artery disease in young Indians [published correction appears in Indian J Med Res. 2013 Oct;138(4):574]. *Indian J Med Res*. 2012;135(4):506-512.
9. Tousoulis D, Davies GJ, Asimakopoulos G, Homaei H, Zouridakis E, Ahmed N, et al. Vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 serum level in patients with chest pain and normal coronary arteries (syndrome X). Clin Cardiol, 24 (2001), pp. 301-4.
10. Agrawal S, Mehta PK, Bairey Merz CN. Cardiac Syndrome X: Update. Heart Fail Clin. 2016 Jan;12(1):141-56. doi: 10.1016/j.hfc.2015.08.012. PMID: 26567981.
11. Pereira AC, Miyakawa AA, Lopes NH, Soares PR, de Oliveira SA, Cesar LA, Ramires JF, Hueb W, Krieger JE. Dynamic regulation of MTHFR mRNA expression and C677T genotype modulate mortality in coronary artery disease patients after revascularization. Thromb Res. 2007;121(1):25-32. doi: 10.1016/j.thromres.2007.03.004. Epub 2007 Jul 2. PMID: 17604826.

12. Gaughan DJ, Barbaux S, Kluijtmans LAJ. et al. The human and mouse methylenetetrahydrofolate reductase (MTHFR) genes: Genomic organization, mRNA structure and linkage to the CLCN6 gene. Gene. 2000;257:279–289

13. Zhao M, Li X, Xing C, Zhou B. Association of methylenetetrahydrofolate reductase C677T and A1298C polymorphisms with colorectal cancer risk: A meta-analysis. *Biomed Rep*. 2013;1(5):781-791. doi:10.3892/br.2013.134

14. Kelly PJ, Rosand J, Kistler JP, Shih VE, Silveira S, Plomaritoglou A, et al. Homocysteine, MTHFR 677C-->T polymorphism, and risk of ischemic stroke: results of a meta-analysis. Neurology 2002 Aug 27; 59 (4):529-36.

15. Collings A, Raitakari OT, Juonala M, Rontu R, Kähönen M, Hutri-Kähönen N, Rönnemaa T, Marniemi J, Viikari JS, Lehtimäki T. Associations of methylenetetrahydrofolate reductase C677T polymorphism with markers of subclinical atherosclerosis: the Cardiovascular Risk in Young Finns Study. Scand J Clin Lab Invest. 2008;68(1):22-30. doi: 10.1080/00365510701487735. Epub 2007 Nov 21. PMID: 17934972.

16. Kalina A, Czeizel AE. The methylenetetrahydrofolate reductase gene polymorphism (C677T) is associated with increased cardiovascular mortality in Hungary. Int J Cardiol. 2004 Nov;97(2):333-4. doi: 10.1016/j.ijcard.2003.08.021. PMID: 15458711.

17. Nair KG, Nair SR, Ashavaid TF, Dalal JJ, Eghlim FF. Methylenetetrahydrofolate reductase gene mutation and hyperhomocysteinemia as a risk factor for coronary heart disease in the Indian population. J Assoc Physicians India. 2002 May;50 Suppl:9-15. PMID: 12186157.

18. Alroy S, Preis M, Barzilai M, Cassel A, Lavie L, Halon DA, Amir O, Lewis BS, Flugelman MY. Endothelial cell dysfunction in women with cardiac syndrome X and MTHFR C677T mutation. Isr Med Assoc J. 2007 Apr;9(4):321-5. PMID: 17491230.

19. Kandaz C, Önal B, Özen D, Demir B, Akkan AG, Özyazgan S. Investigation of MTHFR gene C677T polymorphism in cardiac syndrome X patients. *J Clin Lab Anal*. 2018;32(2):e22247. doi:10.1002/jcla.22247

20. Trovato FM, Catalano D, Ragusa A, et al. Relationship of MTHFR gene polymorphisms with renal and cardiac disease. *World J Nephrol*. 2015;4(1):127-137. doi:10.5527/wjn.v4.i1.12

21. Ahmed Zaghloul, Corina Iorgoveanu, Aakash Desai, Kathir Balakumaran, Kai Chen; Methylenetetrahydrofolate Reductase Polymorphism and Premature Coronary Artery Disease, 2019 Cureus 11(6): e5014. doi:10.7759/cureus.5014).

22. Bouzidi, N., Hassine, M., Fodha, H. *et al.* Association of the methylene-tetrahydrofolate reductase gene rs1801133 C677T variant with serum homocysteine levels, and the severity of coronary artery disease. *Sci Rep* **10,** 10064 (2020). https://doi.org/10.1038/s41598-020-66937-3

23. Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ. 2005 Nov 5;331(7524):1053. doi: 10.1136/bmj.38611.658947.55. Epub 2005 Oct 10. PMID: 16216822; PMCID: PMC1283183.

24. Rassoul F, Richter V, Hentschel B, Geisel J, Herrmann W, Kuntze T. Plasma homocysteine levels & 677C-->T methylenetetrahydrofolate reductase gene polymorphism in patients with coronary artery disease of different severity. Indian J Med Res. 2008 Feb;127(2):154-8. PMID: 18403793.

25. Frederiksen J, Juul K, Grande P, Jensen GB, Schroeder TV, Anne TH, et al. *MTHFR* polymorphism (C667→ T) hyperhomocystenemia, and risk of ischaemic cardiovascular disease and venous thromboembolism: prospective and case control studies from Copenhagen City Heart Study. *Blood.* 2004;104:3028–37.

26. Khatami MR, Mirchi E, Khazaeipour Z, Abdollahi A, Jahanmardi A. Association between serum magnesium and risk factors of cardiovascular disease in hemodialysis patients. Iran J Kidney Dis. 2013 Jan;7(1):47-52. PMID: 23314142.