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Archives of Medical Research 41 (2010) 246–250

Archives of Medical Research

ORIGINAL ARTICLE

C677T Polymorphism of the 5,10 MTHFR Gene in Young Mexican Subjects with ST-Elevation Myocardial Infarction

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Received for publication January 15, 2010; accepted April 13, 2010 (ARCMED-D-10-00023).

Background and Aims. The C677T polymorphism of 5,10 methylenetetrahydrofolate reductase (MTHFR) gene has been associated with hypertension and coronary artery disease in several populations worldwide, but results are still controversial. The aim of this study was to examine the possible association of C677T polymorphism with ST-elevation myocardial infarction (STEMI) in young Mexican subjects.

Methods. In a case-control study, 167 unrelated patients ≤45 years of age with diagnosis of STEMI who were admitted to a cardiovascular intense care unit and 167 unrelated controls subjects matched by age and gender were recruited from January 2006 and June 2009. The C677T polymorphism was determined in all participants by a polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP).

Results. There was no significant difference in the genotype distribution between groups ($p = 0.69$) or allele frequency ($p = 0.40$). There were independent factors for STEMI: smoking (OR 4.9, 95% CI 3.0–8.1, $p = 0.001$), hypertension (OR 1.8, 95% CI 1.0–3.3, $p = 0.03$), family history of atherothrombotic disease (OR 2.3, 95% CI 2.0–4.6, $p = 0.02$), and dyslipidemia (OR 3.2, 95% CI 1.8–5.6, $p < 0.001$). Diabetes mellitus did not represent an independent risk factor for STEMI (OR 1.2, 95% CI 0.2–2.2, $p = 0.82$).

Conclusions. The TT genotype from the C677T of 5,10 MTHFR gene is not an independent risk factor for STEMI in the Mexican population. However, more studies are needed to determine the possible "protective effect" of the C677T polymorphism in our population.

Key Words: Acute myocardial infarction, C677T polymorphism, Atherothrombosis, Risk factors.

Introduction

The major thrombosis complication of CAD is ST-elevation myocardial infarction (STEMI) (1), representing a very important care health issue in our country (2). It is estimated that ~10% occurred in young individuals (2). The pathogenic mechanism of myocardial infarction (MI) is complex and involves the interaction of multiple environmental and genetics factors. Environmental risk factors such as diabetes mellitus, hypertension, hyperlipidemia, obesity and smoking account for ~50% of the risk for development of myocardial infarction (MI). The remaining risk is attributed to genetic factors. Previous studies demonstrated a higher proportion of heavy smoking, dyslipidemia, family history of atherothrombotic disease, with a low prevalence of diabetes and

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0188-4409/$ - see front matter.
doi: 10.1016/j.arcmed.2010.04.008

hypertension as risk factors for MI in young individuals. In addition, high levels of homocysteine (Hcy) have been associated with increased risk for MI (3). Plasma Hcy levels are modulated by nutritional and genetic factors, among which the methylenetetrahydrofolate reductase (MTHFR) gene has received special attention, in particular, the C677→T in the gene coding the MTHFR, which is characterized by a point mutation at position 677 of the MTHFR gene resulting in Val→Ala substitution responsible for enzyme reduced activity (4). Subjects who were homozygous for 677T polymorphism had a mildly increased risk for hyperhomocysteinemia, especially if subjects have low plasma folate levels (5). Previous studies found an association between the MTHFR 6777CT polymorphism and the risk of myocardial infarction (6–9), hypertension (9), and cerebrovascular disease (10–11). However, these findings have not been confirmed by others (12–17). A meta-analysis (18) of a case-control observation of 40 studies reported a higher risk of MI of individuals with the MTHFR 677TT genotype, although a significant heterogeneity between Europe and North America was observed. In contrast, another later meta-analysis (19) did not support this association in Europe, North America or Australia. Although a high frequency of the T allele has been demonstrated in the general Mexican population compared with individuals from other genetic backgrounds (20), this polymorphism exhibited a negative correlation with some disease such as preeclampsia/eclampsia (21), gastric cancer (22), and neural tube defects (23). In contrast, we previously demonstrated that C677T represents an independent risk for other atherothrombotic diseases such as stroke in Mexican subjects (24).Therefore, the aim of this study was to examine the possible association of C677T polymorphism in young Mexican subjects with STEMI.

Materials and Methods

Study Group

This case-control study included 167 consecutive patients who survived their first STEMI. Diagnosis of STEMI was based on an electrocardiogram and clinical and laboratory data in accordance with the European Society of Cardiology and American College of Cardiology (25,26). Patients were admitted to the Intensive Coronary Care Unit of the Cardiology Hospital, Centro Médico Nacional Siglo XXI in Mexico City between January 2006 and June 2009. Clinical and demographic data were recorded. Other risk factors for STEMI were noted. Patients 45 years of age or younger were included to minimize the effect of long-term environmental influences on disease etiology. Hypertension was defined if a subject fulfilled the European Society Cardiology (ESC) criteria (27) or if they were already being treated with antihypertensive drugs. A family history of atherothrombotic disease was defined as MI, stroke or sudden death in a first-degree male relative <55 years of age or a female relative <65 years of age. Patients were considered smokers if they were currently smoking or had ceased within the last 12 months (3 cigarettes/day). Subjects were considered to be dyslipidemic if they had a cholesterol level of 200 mg/dL or if they were being already treated for the condition (28). Patients were considered to be diabetic if they had fasting glycemia >126 mg/dL or if they were already diagnosed with the disease (29). A total of 167 Mexican subjects selected from blood bank donors and apparently healthy volunteers without personal history of atherothrombotic disease, age- and gender-matched, and with similar socioeconomic background were included in the control group. The study protocol was reviewed and approved by the Human Ethics Committee and the Medical Research Council of the Instituto Mexicano del Seguro Social and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all subjects before study enrollment.

DNA Isolation and Genotyping of MTHFR C677T Polymorphism

Genomic DNA was isolated from frozen packed white blood cells according to manufacturer's instructions using a commercial kit QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification of the DNA samples was performed following the method of Frosst et al. (4). A 198-bp fragment encompassing the region around nucleotide 677 was amplified by PCR with the following primers: 5′-TGAAGGAGAAGGTGTCTGC GGGA-3′, and 5′-AGGACGGTGCGGTGAGAGTG-3′. The C677T polymorphism created a HinfI (Promega, Madison, WI) restriction site, so digestion of the PCR product of the mutant allele by this enzyme generates two fragments (198 and 175 bp) that could be fractionated on 3% agarose gel electrophoresis (BioRad Laboratories, Hercules, CA) and visualized under ultraviolet light following ethidium bromide staining (Figure 1). Genotyping results were confirmed by direct sequencing of the PCR product with a DNA sequence.

Statistical Analysis

Continuous data are expressed as the mean ± standard deviation (SD); categorical data are expressed with percentages. The significance of differences between continuous variables was determined by Student's t test. Differences between categorical variables were determined with the χ^2 test. We tested the allele frequencies conformed to Hardy-Weinberg equilibrium proportions using χ^2 test. Adjusted odds ratios (ORs) were calculated by multivariate logistic regression analyses for C677T polymorphism and traditional cardiovascular risk factors; p value <0.05 was considered as statistically significant. All statistical analyses were performed using SPSS statistical software package v. 15 (SPSS Inc., Chicago, IL).