Occurrence of MTHFR C677T gene polymorphism and its association with atherogenic indices in Mexican women from San Luis Potosí, a preliminary study

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Research Article

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Abstract

Some genetic variants (polymorphisms) of the methylenetetrahydrofolate reductase (MTHFR) enzyme are considered a susceptibility factor in the development of cardiovascular diseases (CVDs). Therefore, this study aimed to investigate the relationship between MTHFR C677T polymorphism and levels of well-recognized atherogenic indices in a female population from San Luis Potosi, Mexico. A total of 340 women participated in the study, and MTHFR C677T genotypification was assessed using a RT-PCR assay. Also, Framingham risk score (FRS), Castelli risk index (CRI), and atherogenic index of plasma (AIP) were estimated. The allelic frequency detected was 0.43 for the MTHFR 677T-allele in the enrolled women. Besides, the mean value of AIP was significantly higher (p<0.05) for individuals with the mutant genotype (TT; 0.29 ± 0.20) contrasted to AIP values detected in people with the wild-type genotype (CC; 0.15 ± 0.20) and heterozygous genotype (CT; 0.16 ± 0.20). Similar findings were observed for CRI through MTHFR C677T genotypes (4.40 ± 1.80; 3.90 ± 1.30; and 3.60 ± 0.90; for CC, CT, and TT, respectively). No significant changes were detected for FRS values across MTHFR C677T genotypes. Lastly, significant statistical associations were identified between the TT genotype and the AIP values (odds ratio: 2.15; 95% CI: 1.95-4.95; p= 0.01). No significant associations were detected between MTHFR C677T genotypes and FRS and CRI indices values. The results found in this research agree with data that support an increased CVDs risk in MTHFR 677T-allele carriers in the human population, as AIP is considered a reliable CVDs risk biomarker.

Introduction

Cardiovascular diseases (CVDs) have focused the attention of health professionals, as CVDs are the principal cause of human deaths worldwide (WHO. World Heart Federation. World Stroke Organization 2011; WHO 2014). Data from the World Health Organization (WHO) have established that CVDs are responsible for approximately 32 % of deaths (17.9 million) each year globally (WHO. World Heart Federation. World Stroke Organization 2011; WHO 2014). Besides, low- and middle-income countries are the most affected by these diseases (WHO. World Heart Federation. World Stroke Organization 2011; WHO 2014). Therefore, a critical challenge for developing countries is to reduce the high mortality caused by CVDs. According to WHO, prevention is the best strategy to combat CVDs (WHO. World Heart Federation. World Stroke Organization 2011; WHO 2014). Consequently, precise and early identification of high-risk individuals is crucial in the exciting battle against cardiovascular diseases.

Numerous risk factors (smoking, unhealthy diets, obesity, physical inactivity, harmful alcohol consumption, high blood pressure, diabetes, hyperlipidemia, and genetic susceptibility) are associated with the onset, establishment, and development of CVDs. In this line, genetic polymorphisms of crucial proteins in different signal pathways have been related to CVDs (Olivi et al. 2015; AlRasheed et al. 2018; Haybar et al. 2018; Shunmoogam et al. 2018). For example, the methylenetetrahydrofolate reductase (MTHFR) enzyme (a key enzyme in the folate cycle) has been implicated as a susceptibility factor in the
development of CVDs (Marosi et al. 2012; Liew and Gupta 2015; Whayne 2015). The MTHFR gene is positioned in chromosome 1 (p36.6 region), and some MTHFR polymorphisms have been detected in this gene (i.e., rs2274976, rs1801133, rs535107, rs4846052, rs1476413, rs4846048, rs4846051, rs1931226, rs2066470, rs3737964, rs7525338, rs1801131, and rs1889292) (Kennedy et al. 2012; Hernández-Guerrero et al. 2013a), two significant functional polymorphisms, C677T (rs1801133) and A1298C (rs1801131) have been associated with CVDs risk (Yu et al. 2017). The C677T polymorphism is found in the exon 4 at nucleotide 677 of the gene (C677T; rs1801133, a substitution of cytosine for thymine). The substitution of cytosine for thymine is associated with the replacement of alanine for valine amino acids in the codon 222 (Ala222Val). As a result, a decrement in the activity of the MTHFR enzyme [approximately 30% for heterozygous (CT) and 70% for homozygotes (TT)] has been observed (Frosst et al. 1995; Weisberg et al. 1998; V. Antonio-Véjar et al. 2014). The protein is a critical enzyme (a rate-limiting enzyme) in the metabolism of folic acid, as MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Crider et al. 2012; Tsang et al. 2015). Additionally, it has been demonstrated that the decrement in the activity of the MTHFR enzyme reduces the serum folate concentration and increases the homocysteine (Hcy) levels (Jacques et al. 1996; Kerkeni et al. 2006). In this regard, Li et al. (2018) in a Chinese population found higher serum concentrations of Hcy in people with the TT genotype (MTHFR C677T polymorphism) compared to levels detected in individuals with CC or CT genotype (Li et al. 2018). Besides, hyperhomocysteinemia (serum Hcy increment) has been related to an increased risk of CVDs (Clarke et al. 1991; Tripathi et al. 2010).

Although relationships between MTHFR C677T polymorphism with an increment of serum homocysteine levels are well recognized, associations with other CVDs biomarkers have been poorly documented. Therefore, this study aimed to evaluate the association between MTHFR C677T polymorphism (considering a genetic predisposition factor to develop CVDs) and renowned atherogenic indices used as early CVDs biomarkers.

**Materials And Methods**

**Population**

This cross-sectional investigation was completed from 2016 to 2019 in several communities from San Luis Potosi (SLP) state in Mexico. A total of 340 healthy women were registered in the study and signed individually informed consent. The registration processes consist of a face-to-face conversation to clarify the aims and goals of the study. In this phase, an increased contribution of women living in the examined areas was observed (95 % women and 5 % men). Then, male individuals were not included in this research (low gender representativeness was achieved). The participants answered a questionnaire to acquire personal characteristics such as age, occupation, education, habits, smoking status, alcohol consumption, diagnosed diseases, marital status, income, and household. Besides, a health condition evaluation was completed (the diagnosis was made by a clinician professional), and medical variables
such as systolic (SBP) and diastolic (DBP) blood pressure were documented as previously specified (Ruiz-Vera et al. 2015; Ochoa-Martínez et al. 2017; Ochoa-Martínez, Ruiz-Vera, Orta-Garcia, et al. 2017; Ruiz-Vera, Ochoa-Martínez, et al. 2019). Women without health complications (apparently healthy) were included in this investigation. Anthropometric measurements were achieved, and body mass index (BMI) was estimated. The study protocol was revised and approved by the Bioethics Committee of Medicine School of the Autonomous University of San Luis Potosi.

**Blood Collection**

Two independent blood samples (after overnight fasting) were obtained from all included women. A blood sample was collected into a tube containing EDTA (Ethylenediaminetetraacetic acid) as an anticoagulant. Also, a second blood sample was obtained using a container without anticoagulant. The anticoagulated blood sample was used for DNA isolation procedures, and the blood sample without anticoagulant was used to perform biochemical analyses. The technique for blood sampling was described previously by our research team elsewhere (Ochoa-Martínez et al. 2019; Ruiz-Vera, Ochoa-Martínez, Zarazua, et al. 2019; Ruiz-Vera, Ochoa-Martínez, Pruneda-Alvarez, et al. 2019).

**MTHFR C677T genotypification**

The genomic DNA isolation was performed following the manufacturer's directions of a commercially available kit (the Wizard Genomic DNA purification; PROMEGA, Wisconsin, USA). After the isolation phase, the obtained DNA genomic was stored at −80°C until their analysis. MTHFR C677T (rs1801133) genotypification was completed using a TaqMan predesigned single-nucleotide polymorphism (SNP) kit (C_1202883_20). The StepOne Real-Time PCR system (Applied Biosystems, California, USA) was used to complete the allelic discrimination. The next PCR amplification protocol was used in the analysis: The total reaction volume was 10μl; denaturation stage (95°C; 10 min); 45 cycles of denaturation (92°C; 15 s); and annealing and extension (60°C; 1 min); DNA concentration (20 ng/μL). Negative controls and duplicate samples were included to check the accuracy of genotyping.

**Biochemical Profile**

Blood samples without anticoagulant were utilized to obtain serum samples after a centrifugation process (1200 x g, 10 min) and stored at −80°C until analyses. The serum biochemical profile of all enrolled individuals was analyzed using standard colorimetric methods, and a detailed description of the techniques was stated previously (Pruneda-Alvarez et al. 2016 Aug; Ochoa-Martínez, Ruiz-Vera, Pruneda-Alvarez, et al. 2017). Commercially available kits were used to quantify serum concentrations of glucose,
total cholesterol, HDL-cholesterol, and triglycerides. The Friedewald equation was employed to estimate serum LDL-cholesterol concentration (Friedewald et al. 1972).

Atherogenic indices

The atherogenic index of plasma (AIP) and Castelli’s risk index (CRI) were estimated using the equations (1) and (2), respectively:

\[
AIP = \log\left(\frac{TG}{HDL-C}\right) \tag{1}
\]

\[
CRI = \frac{TC}{HDL-C} \tag{2}
\]

The Framingham risk score (FRS) was calculated employing the score sheets used to evaluate the multivariable risk of cardiovascular diseases for women from the Framingham heart study (D’Agostino et al. 2008).

Statistical analyses

The \( \chi^2 \) test for Hardy-Weinberg equilibrium was applied to determine the difference between observed and expected genotype frequencies from the allele frequencies. A descriptive analysis of all assessed variables was developed, and mean, median, standard deviation, minimum, maximum, and percentiles were determined. Besides, one-way analysis of variance (one-way ANOVA) for normally distributed variables or the Kruskal-Wallis test for variables with non-normal distribution were carried out to examine differences among groups (through different MTHFR C677T genotypes). Association among the categorical variables was explored using Fisher’s exact test. Finally, logistic regression models were developed to evaluate the association between MTHFR C677T genotypes and estimated atherogenic indices (AIP and CRI) adjusted for the assessed variables; odds ratios were calculated, and wild-type genotype (CC) was established as the reference. For corresponding analysis, a \( p<0.05 \) value was considered statistically significant. Data analysis was completed using GraphPad Prism 5.01 and GraphPad InStat 3 (GraphPad Software Inc. La Jolla, CA, USA).

Results

Sociodemographic characteristics of the study population
Table 1 lists the descriptive statistics of the assessed variables in the enrolled population. The age range was 18.0 to 82.0 years. Approximately 75% of included subjects were overweight or obese (mean BMI=27.9 kg/m²), similar than detected in the ENSANUT (Encuesta Nacional de Salud y Nutrición) survey in Mexico (overweight and obesity were >75.0 % for individuals aged 20 years or more), ENSANUT is a national survey that aimed to evaluate the nutritional status of Mexican population (González Block et al. 2017). Also, nearly 75% of women have a waist-hip ratio >0.8 (data not shown), and both parameters (BMI and waist-hip ratio) are considered important risk factors for CVDs events (Vasan 2006). Mean blood pressure levels were within common values (SBP = 116 mm Hg, DBP = 70 mm Hg) (Table 1). Regarding assessed biochemical measurements, the mean level found was: 103 ± 50.7 mg/dL for glucose in serum, 170 ± 80.3 mg/dL for serum triglycerides, 185 ± 44.2 mg/dL for total cholesterol, 97.3 ± 38.8 mg/dL and 44.5 ± 32.4 mg/dL for LDL- and HDL-cholesterol, respectively (Table 1). Concerning atherogenic indices, a mean value of 6.50 ± 7.20 was estimated for FRS, 3.70 ± 1.30 for CRI, and 0.12 ± 0.22 for AIP (Table 1).

**Frequency of the MTHFR C677T polymorphism**

Afterward, the genotypic and allelic frequency of the assessed polymorphism (MTHFR C677T) were analyzed (Table 2). A sample of 340 women was evaluated, 32 % (n= 109) were homozygous for the MTHFR 677C-allele (CC genotype), 169 enrolled women (50 %) were categorized as heterozygotes for MTHFR C677T polymorphism (CT genotype) and 18 % (n= 62) as homozygous for the MTHFR 677T-allele (TT genotype) (Table 2). Correspondingly, the allelic frequency detected in the study population was 57 % for the MTHFR 677C-allele and 43 % for the MTHFR 677T-allele (Table 2). There was no deviation in C/T genotype frequencies from the Hardy-Weinberg equilibrium ($\chi^2 = 0.969, p > 0.05$).

**Influence of MTHFR C677T polymorphism on anthropometric measurements, clinical evaluations, biochemical profile, and atherogenic indices**

When assessed variables were analyzed according to the MTHFR C677T genotype, significant differences among groups (CC, CT, and TT genotypes) were detected in SBP, DBP, CRI, and AIP values (Table 3). A significant (p<0.05) higher mean level of SBP and DBP was noticed in mutant homozygous (TT genotype) (120 ± 13.0 and 75.0 ± 7.00 mm Hg, respectively) compared to SBP and DBP mean values recorded in heterozygotes (CT genotype) (111 ± 14.0 and 68.0 ± 9.00 mm Hg, respectively). Similarly, the SBP and DBP mean levels obtained from the homozygous for the MTHFR 677C-allele (CC genotype) were significantly lower (111 ± 12.0 and 69.0 ± 8.00 mm Hg, respectively) than those observed for the TT genotype group (Table 3). Regarding CRI data, the mean value of that atherogenic index was significantly higher (p<0.05) for individuals categorized in the TT genotype group (4.41 ± 1.80) contrasted to CRI values detected in CC genotype (3.61 ± 0.90) and CT genotype (3.88 ± 1.30) groups (Table 3). Similar findings were identified when AIP data were examined, significantly higher values for AIP were calculated
for homozygous individuals with the polymorphic genotype (TT genotype; mean AIP values of 0.29 ± 0.19) compared to AIP values detected in heterozygotes individuals (CT genotype; 0.16 ± 0.21) and CC genotype group (0.15 ± 0.21) (Table 3).

Next, women categorized in each group (CC, CT, and TT genotype) were analyzed considering the guidelines for AIP and CRI. Concerning AIP, people with AIP values ≤0.11 are deemed a low-risk population to develop CVDs, and people with AIP values >0.11 are considered a high-risk population (Dobiasova and Frohlich 2001; Frohlich and Dobiasova 2003a; Dobiášová 2004). In this regard, approximately 75 % of women categorized in the TT genotype group (n = 62) have AIP values higher than 0.11 (Table 4). Concerning CC (n = 109) and CT genotype (n = 169), 48 % and 58 % of women, respectively, were considered high-risk populations (AIP values >0.11) (Table 4). As noted, a significant (p < 0.05, Fisher’s exact test) increased proportion of high-risk women was detected in the TT genotype group compared to the CC and CT genotype groups (Table 4). For CRI, a similar analysis was completed, and categorization occurred as follows: ≤3.50 (low risk); >3.50 (high risk) (Kamoru et al. 2017). No significant differences were detected in the proportion of women at low risk (CRI ≤3.5) and elevated risk (CRI >3.5) in the three assessed groups (CC, CT, and TT genotype) (Table 4).

Finally, logistic regression analysis revealed an association between TT genotype (odds ratio: 2.15; 95%CI: 1.95-4.95; P=.03) and AIP (Table 5). Besides, the association remained significant after adjustment for assessed confounders variables (Table 6). As expected, no significant associations between MTHFR C677T genotypes and CRI were detected (Supplementary material, Table S1, and Table S2).

Discussion

Genetic susceptibility is considered a critical risk factor in the progression of several diseases (Abdel Ghafar 2019; Abdel Ghafar 2020; Dagneaux et al. 2020 Jun; Ghafar et al. 2020; J. Liu et al. 2020; Mikhaylenko et al. 2020). In this line, the methylenetetrahydrofolate reductase (MTHFR) enzyme has been associated with the onset and progression of different afflictions, such as cancer, diabetes, metabolic syndrome, and cardiovascular diseases, among others (Liew and Gupta 2015). Concerning CVDs, the MTHFR C677T gene polymorphism (examined in this investigation) is a common mutation that diminishes the activity of this enzyme and is associated with increased susceptibility in the development of cardiovascular events in human populations (Liew and Gupta 2015). The MTHFR 677T-allele (polymorphic allele) frequency found in this study was 43 % (Table 2), comparable to the ones detected in other investigations completed in Mexico (Supplementary material, Table S3) (Hernández-Guerrero et al. 2013b; V Antonio-Véjar et al. 2014; Vazquez-Alaniz et al. 2014; Ramos-Silva et al. 2015; Calderón-Garcidueñas et al. 2017; García-González et al. 2018a). For example, the MTHFR 677T-allele frequency was 51 % for a Mexican mestizo population recruited in Mexico City (Hernández-Guerrero et al. 2013b). Similarly, the 677T-allele was detected in 46 % of Mexican women from the Nuevo Leon state (Calderón-
frequency of 51% for the polymorphic allele (677T) was detected in an adult population from southern Mexico (Yucatan state) (García-González et al. 2018b). Also, allelic frequencies of MTHFR C677T polymorphism in other world regions are shown in the Supplementary material, Table S3 (Heux et al. 2004; Thirumaran et al. 2005; Mazzuca et al. 2015; Basol et al. 2016; El Hajj Chehadeh et al. 2016; Graydon et al. 2019; Silva et al. 2019; Peng et al. 2020), the MTHFR 677T-allele frequency found in European countries ranged from 22.0% to 40.0% (Supplementary material, Table S3).

Earlier scientific investigations have revealed that the MTHFR C677T gene polymorphism is a genetic risk factor associated with an increased human susceptibility to develop CVDs (Hou et al. 2018; Lakkakula 2019; P.-F. Liu et al. 2020). Hou et al. (2018) completed a case-control study on the association between MTHFR C677T polymorphism and the predisposition to ischemic stroke in a Southern Chinese Hakka population (n=4532). The results showed an expanded risk of ischemic stroke in the homozygous polymorphic TT genotype group compared to the homozygous CC genotype and heterozygous CT genotype groups, even after adjusting for traditional risk elements (Hou et al. 2018). Similarly, Al-Ali et al. (2005) demonstrated a strong association between homozygous TT genotype and an amplified risk of CVDs events in a population of the Eastern Province of Saudi Arabia (Al-Ali et al. 2005). In contrast, a prospective cohort in an adult US population (n=6000) revealed low CVDs mortality associated with homozygous TT genotype compared to CVDs deceases in MTHFR homozygous CC genotype and heterozygous CT genotype after adjustment for traditional CVDs risk factors (Yang et al. 2012).

Increased CVDs risk was noted in the enrolled individuals with the TT genotype when AIP was analyzed. In this regard, in an increasing amount of scientific investigations, AIP has been distinguished as a robust biomarker in the prognosis of CVDs events (Bhardwaj et al. 2013; Barua et al. 2019; Fernandez-Macías et al. 2019; Qin et al. 2020). Besides, AIP has been considered a better CVDs risk biomarker in comparison to those commonly used (TC, LDL-cholesterol, HDL-cholesterol, among others) (Edwards et al. 2017). For example, Essiarab et al. (2014), after a cross-sectional investigation demonstrated the utility of AIP as a predictive biomarker of CVDs in a Moroccan women population (n=240), a significant strong relationship between AIP values and an augmented CVDs risk was detected (Essiarab et al. 2014). Similarly, Onat et al. (2010) evaluated the association between this atherogenic index (AIP) with cardiovascular disorders in a Turkish population (n=2676) in a prospective investigation for 7.8 years follow-up. The findings indicated that AIP is a reliable prognostic CVD biomarker in middle-aged adults (Onat et al. 2010).

Furthermore, AIP has been associated with validated biomarkers of subclinical atherosclerosis, such as the intima-media thickness of the carotid artery (cIMT), asymmetric dimethylarginine (ADMA), insulin resistance, the ratio of apolipoprotein B (apoB): apolipoprotein A-1 (apoA1), among others (Tongdee and Nimkuntod 2016; Cure et al. 2018; Caliskan et al. 2019; Fernandez-Macías et al. 2019; Tecer et al. 2019). In this line, Tongdee et al. (2018) found a robust positive correlation between AIP and cIMT values in perimenopausal/ menopausal women (Tongdee and Nimkuntod 2016). Similarly, Fernandez-Macías et al. (2019) exhibited a direct relationship between AIP values and serum ADMA concentrations in middle-aged Mexican women (Fernandez-Macías et al. 2019). Both biomarkers (cIMT and ADMA) have been
recognized as good predictive markers of subclinical atherosclerosis (Surdacki et al. 2007; Urbina et al. 2009). Consequently, it is suggested that the AIP could be used as a reliable biomarker to identify high-risk individuals to develop CVDs, without clinically evident atherosclerotic processes.

The prognostic value associated with AIP is justified by the strong inverse relationship with the low-density lipoprotein (LDL)-cholesterol particle size (Dobiasova and Frohlich 2000; Dobiášová and Frohlich 2001; Frohlich and Dobiasova 2003b; Dobiasova 2006). Dobiášová et al. (2001) have demonstrated that an increment in the AIP level is linked with a reduction in the diameter of LDL-cholesterol particles. Consequently, an increment in the proportion of small dense (sd) LDL particles is observed (Dobiášová and Frohlich 2001); sdLDL is a subclass of LDL particles and are critical molecules in the onset and progression of the atherosclerotic process and CVDs. sdLDL particles are more susceptible to oxidation processes that further expand their atherogenicity (Hoogeveen et al. 2014; Gerber et al. 2017; Santos et al. 2020 Apr). In addition, sdLDL particles are recognized as very important risk factors in the induction of CVDs by the National Cholesterol Education Program (SoRelle 2002). Therefore, sdLDL particles have been suggested as a better biomarker in the prognosis of CVDs than other lipidic biomarkers (Toft-Petersen et al. 2011). However, the accessible methodology used to quantify LDL cholesterol subfractions is expensive, and the application as a routine test in clinical procedures in developing countries such as Mexico is not feasible. Then, AIP could be used as a reliable and economical alternative in the prognosis of CVDs in developing countries, as AIP efficiently indicates the sdLDL level. Recently, our investigation group has developed a study to evaluate different clinical predictive markers to identify specific and affordable biomarkers in the prognosis of CVDs in the Mexican population (Fernandez-Macias et al. 2019). The findings in the Fernandez-Macias et al. (2019) study indicated that AIP was the best tool to detect people susceptible to developing CVDs (Fernandez-Macias et al. 2019).

Despite the exciting results found in this investigation, the AIP use as a predictive CVDs biomarker requires confirmation. Therefore, more studies are necessary to validate the use of AIP as a marker in the evaluation of cardiovascular events in Mexican people. Then, studies that involve different ethnicity, age, regions, gender, and diet, among others, should be implemented.

Several limitations were detected in the execution of this research. Firstly, the cross-sectional design of this investigation is a critical limitation (we cannot affirm causality). Therefore, prospective studies are necessary to verify our results. The sample size was short, and epidemiological investigations with an increased number of enrolled participants are essential to support the findings shown in this study. Also, some other MTHFR polymorphisms (MTHFR A1298C polymorphism, rs 1801131) have been associated with cardiovascular events and were not assessed herein.
Conclusion

The findings in this study are in line with data that support the relationship between the high risk of developing CVDs and the presence of the MTHFR 677T allele in the human population. Hence, using the precautionary principle, the results found in this study will be used as a solid argument to promote risk reduction strategies in the prevention of cardiovascular events in the assessed population. However, additional studies are required to verify the use of AIP as a predictive biomarker of CVDs in Mexican individuals.

Declarations

Acknowledgements. Not applicable

Author Contribution. Methodology: Fernandez-Macias JC and Ochoa-Martinez AC
Formal analysis and Data curation: Perez-Lopez AL and Perez-Lopez AA
Conceptualization and wrote original draft: Perez-Maldonado IN
Approval of final manuscript: all authors.

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Data Availability. The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Declaration of interest. The authors declare no conflict of interest.

Consent for Publication. Enrolled individuals agree to participate in this research.
**Ethical Approval and Consent to Participate.** The study protocol was revised and approved by the Bioethics Committee of Medicine School of the Autonomous University of San Luis Potosi. Written informed consent was signed from all registered individuals.

**References**


Dobiášová M, Frohlich J. 2001. The plasma parameter log (TG/HDL-C) as an atherogenic index: Correlation with lipoprotein particle size and esterification rate inapob-lipoprotein-depleted plasma (FER


Tables

Table 1. Anthropometric and biochemical parameters of assessed population.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Median (Min-Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, (years)</strong></td>
<td>46.0 ± 17.0</td>
<td>46.0 (18.0 - 82.0)</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>27.9 ± 5.60</td>
<td>27.3 (15.9 - 50.3)</td>
</tr>
<tr>
<td><strong>Systolic blood pressure, (mm Hg)</strong></td>
<td>116 ± 18.0</td>
<td>110 (85.0 - 180)</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure, (mm Hg)</strong></td>
<td>70.0 ± 10.0</td>
<td>70.0 (50.0 - 100)</td>
</tr>
<tr>
<td><strong>Glucose, (mg/dl)</strong></td>
<td>103 ± 50.7</td>
<td>88.5 (42.8 - 389)</td>
</tr>
<tr>
<td><strong>Triglycerides, (mg/dl)</strong></td>
<td>170 ± 80.3</td>
<td>156 (16.5 - 549)</td>
</tr>
<tr>
<td><strong>Total cholesterol, (mg/dl)</strong></td>
<td>185 ± 44.2</td>
<td>180 (90.6 - 460)</td>
</tr>
<tr>
<td><strong>LDL cholesterol, (mg/dl)</strong></td>
<td>97.3 ± 38.8</td>
<td>93.6 (10.1 - 325)</td>
</tr>
<tr>
<td><strong>HDL cholesterol, (mg/dl)</strong></td>
<td>44.5 ± 32.4</td>
<td>38.9 (21.5 - 177)</td>
</tr>
<tr>
<td><strong>Framingham risk score</strong></td>
<td>6.50 ± 7.20</td>
<td>7.00 (-8.00 - 22.0)</td>
</tr>
<tr>
<td><strong>Castelli's risk index</strong></td>
<td>3.70 ± 1.30</td>
<td>3.50 (1.20-8.60)</td>
</tr>
<tr>
<td><strong>Atherogenic index of plasma</strong></td>
<td>0.120 ± 0.220</td>
<td>0.135 (-0.950 - 0.815)</td>
</tr>
</tbody>
</table>

Data is represented as arithmetic mean with standard deviation (SD) and median with minimum to maximum.

Table 2. Genotypic and allelic frequencies of MTHFR (C677T) polymorphism of assessed population.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>109</td>
<td>32.0</td>
</tr>
<tr>
<td>CT</td>
<td>169</td>
<td>50.0</td>
</tr>
<tr>
<td>TT</td>
<td>62</td>
<td>18.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>57.0</td>
</tr>
<tr>
<td>T</td>
<td>43.0</td>
</tr>
</tbody>
</table>

F, Genotypic and allele frequencies in the assessed population.

Table 3. Anthropometric and biochemical parameters of assessed population through MTHFR C677T genotype.
<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body mass index</strong></td>
<td>27.8 ± 5.40</td>
<td>29.4 ± 6.20</td>
<td>30.3 ± 8.30</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Systolic blood pressure, (mm Hg)</strong></td>
<td>111 ± 12.0</td>
<td>111 ± 14.0</td>
<td>120 ± 13.0*</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td><strong>Diastolic blood pressure, (mm Hg)</strong></td>
<td>69.0 ± 8.00</td>
<td>68.0 ± 9.00</td>
<td>75.0 ± 7.00*</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td><strong>Glucose, (mg/dl)</strong></td>
<td>98.8 ± 45.0</td>
<td>96.5 ± 36.0</td>
<td>109 ± 52.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Triglycerides, (mg/dl)</strong></td>
<td>201 ± 127</td>
<td>187 ± 85.4</td>
<td>181 ± 62.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Total cholesterol, (mg/dl)</strong></td>
<td>193 ± 41.1</td>
<td>195 ± 50.2</td>
<td>195 ± 35.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>LDL cholesterol, (mg/dl)</strong></td>
<td>130 ± 37.5</td>
<td>134 ± 53.0</td>
<td>128 ± 42.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>HDL cholesterol, (mg/dl)</strong></td>
<td>54.2 ± 18.5</td>
<td>40.6 ± 25.4</td>
<td>38.7 ± 27.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Framingham risk score</strong></td>
<td>7.10 ± 8.00</td>
<td>6.60 ± 7.50</td>
<td>5.90 ± 7.70</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Castelli's risk index</strong></td>
<td>3.61 ± 0.90</td>
<td>3.88 ± 1.30</td>
<td>4.41 ± 1.80*</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td><strong>Atherogenic index of plasma</strong></td>
<td>0.15 ± 0.21</td>
<td>0.16 ± 0.21</td>
<td>0.29 ± 0.19*</td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>

Data is represented as arithmetic mean with standard deviation (SD), ANOVA with Tukey post hoc was performed, *TT vs CC and CT; #TT vs CC.

**Table 4. Proportion of high-risk women according to AIP and CRI through MTHFR C677T Genotype.**
<table>
<thead>
<tr>
<th>MTHFR C677T Genotype</th>
<th>AIP ≤ 0.11 n (%)</th>
<th>AIP &gt; 0.11 n (%)</th>
<th>p</th>
<th>CRI ≤ 3.5 n (%)</th>
<th>CRI &gt; 3.5 n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (n=109)</td>
<td>57 (52 %)</td>
<td>52 (48 %)</td>
<td>&gt;0.05</td>
<td>52 (48 %)</td>
<td>57 (52 %)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CT (n=169)</td>
<td>71 (42 %)</td>
<td>98 (58 %)</td>
<td>&gt;0.05</td>
<td>78 (46 %)</td>
<td>91 (54 %)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TT (n=62)</td>
<td>16 (25 %)</td>
<td>46 (75 %)</td>
<td>0.001*</td>
<td>49 (45 %)</td>
<td>60 (55 %)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Fisher’s exact test.

**Table 5. Logistic regression analysis between MTHFR C677T polymorphism and AIP.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>1.05 (0.85-3.60)</td>
<td>0.09</td>
</tr>
<tr>
<td>TT</td>
<td>2.15 (1.95-4.95)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 6. Logistic regression analysis between MTHFR C677T polymorphism and AIP adjusted for assessed variables.**
<table>
<thead>
<tr>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>1.20 (0.95 - 3.90)</td>
<td>0.06</td>
</tr>
<tr>
<td>TT</td>
<td>2.65 (1.90-5.25)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Age, BMI, SBP, DBP, Glucose, TGS, TC, LDL, and HDL were used as confounders in the logistic regression analysis.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)