Frequency of Thrombotic Gene Mutations in Diabetic Patients with and Without Coronary Artery Disease

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Abstract

Although relationship between diabetic atherogenesis and several common risk factors plus non traditional risk markers have been studied extensively and the data is having some controversies. This study aimed to find the relationship between factor V Leiden, prothrombin G20210A and methylene tetrahydrofolate reductase (MTHFR) gene mutations and occurrence of coronary artery disease (CAD) in diabetic patients and to compare with healthy controls. 72 diabetic patients, 29 of them have proven CAD and the remaining 43 have not. Twenty healthy subjects were included in the study as a control group. All patients and controls were subjected to history, physical examination and Electrocardiography. Routine laboratory investigations, as well as plasma homocysteine were done. Factor V G1691A, prothrombin G20210A (PTHR A20210) and MTHFR C\textsuperscript{677} \rightarrow T genotypes were determined by polymerase chain reaction using the commercial thrombotype test. The prevalence's of heterozygotes for F V G1691 A and F II G20210A were not significantly different between both patient groups (6.9% versus 6.8% and 2.3% versus 3.4% among DM without and CAD, respectively), however, no cases were found among control group as regard two polymorphisms. In contrast, the prevalence of MTHFR C\textsuperscript{677} \rightarrow T was significantly higher in CAD patients (23.3% versus 37.9% and 10% among CAD, DM and controls, respectively). There is a strong relationship between MTHFR C\textsuperscript{677} \rightarrow T and presence of hypertension, hypercholesterolemia and hyperhomocysteinemia.

In Conclusion: Diabetic patients with CAD have significantly higher MTHFR gene polymorphism as compared to diabetic patients with no CAD and the presented data suggest a synergistic effect between atherogenic and thrombogenic risk factors in the pathogenesis of CAD. Future study with a large scale population recommend to collaborate these polymorphisms with other risk factors.

Key Words: Diabetic patients – Thrombotic gene – Coronary artery disease.

Introduction

VASCULAR diseases, particularly atherosclerosis, are major causes of disability and death in patients with diabetes mellitus. Diabetes mellitus substantially increases the risk of developing coronary, cerebrovascular, and peripheral arterial disease [1]. Diabetes mellitus appears to be an independent predictor of recurrent stroke in population-based studies, and 9.1% of recurrent strokes have been estimated to be attributable to diabetes [2]. The pathophysiology of vascular disease in diabetes involves abnormalities in endothelial, vascular smooth muscle cell, and platelet function. A better understanding of the mechanisms leading to vascular dysfunction may unmask new strategies to reduce cardiovascular morbidity and mortality in patients with diabetes [3].

The inherited prothrombotic risk factors are supposed to be predisposing to thrombus formation and vascular occlusions [4]. Factor V Leiden (FVL), an arginine to glutamine missense mutation in the factor V (FV) gene at position 506, is the most prominent risk factor for venous thromboembolism [5]. The amino acid substitution in the activated protein C (APC) cleavage site of FV leads to increased thrombin generation due to decreased APC mediated inactivation of FV, and due to decreased FV cofactor activity for FVIIIa inactivation [6]. Factor V Leiden is found in approximately 5% of Caucasians, and increases the risk of thrombosis five- to eightfold for heterozygous carriers and 50- to 80-fold for homozygous carriers [7].

Prothrombin also called coagulation factor II, prothrombin is the thrombin precursor that converts fibrinogen into fibrin monomers. The G20210A mutation is a genetic variation of the prothrombin
Frequency of Thrombotic Gene Mutations in Diabetic Patients

(Factor II) gene consisting of a single nucleotide change (guanine to adenine) at position 20210 of the 3′-untranslated region. The G20210A mutation is the second most frequent genetic prothrombotic mutation after FVL [8]. It is present in approximately 2% of Caucasians, leads to greater prothrombin plasma levels, and increases the risk of venous thrombosis about three fold [9]. Previous studies have found an association between the prothrombin 20210 A/G polymorphism and MI [10,11].

Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme involved in the remethylation pathway of homocysteine metabolism. A common mutation (C677T) has been identified in the MTHFR gene, it is a missense mutation that results from the alteration of an alanine to valine amino acid [12]. This alteration is associated with a reduction in the enzyme activity that results in 10%-20% increases in homocysteine plasma levels (hyperhomocysteinemia) and altered folate metabolism [13]. The population frequencies of homozygous MTHFR C677T vary by ethnic group. In the United States, the reported minor allele frequency in Caucasians is 11.9%, African American 1.2%, and Hispanics 20.7%. The pooled analysis of minor allele frequency in the Japanese population was 11%. The heterozygous MTHFR C677T mutation is more common than the homozygous variant. In the United States, the pooled analysis allele frequency in Caucasians is 44.6%, African American 25.6%, and Hispanics 42% [14].

Although, diabetes is well known risk factor for venous thrombotic events, the association between diabetes and arterial thromboembolism is still matter of debate.

Aim of the study:

This study aimed to evaluate factor V Leiden, prothrombin G20210A and methylene tetrahydrofolate reductase (MTHFR) gene mutations in diabetic patients with and without CAD to compare the prevalence with that found in healthy controls.

Patients and Methods

The current study included 72 diabetic patients (40 males and 32 females, age ranged from 36-65 years), attending Internal Medicine Department of Al Zahraa University Hospital, 29 of them have proven CAD and the remaining 43 have not. All patients had no previous history of thromboembolism or liver disease.

Additionally, 20 healthy subjects (12 males and 8 females, ages range 34-56 years) were included in the study as a control group. Control subjects were free of clinical manifestations of coronary, peripheral or cerebral artery disease by history, physical examination and electrocardiographic findings. A written consent was taken from participants included in the study.

All patients and controls were subjected to history, physical examination and Electrocardiography. Evidence of CAD presence was defined on the basis of at least 50% stenosis in a major coronary vessel. Gensini scoring system was used to determine the CAD severity by assessing luminal narrowing and localization [15]. Multiple lesions in the same vessel were regarded as one-vessel disease.

Laboratory investigations:

Overnight fasting blood samples were collected for patients and controls and analyzed for total cholesterol (TC), Triglycerides (TG), Low density Lipoprotein (LDL), High density lipoprotein (HDL), fasting and postprandial blood glucose by Integra-400 (Roche-Germany). Complete blood cell counts were measured by Sysmix K-21 automatic cell counter (Japan).

Prothrombin concentration (PT) was done by Fibrintimer (Roche-Germany). Protein C and antithrombin III functional activity were measured using a chromogenic assay (Dade Behring, Marburg, Germany). Total and free protein S antigen (ELISA, Diagnostica Stago, Asnieres, France), and anticardiolipin antibodies (ELISA, Byk Gulden, Italy) were measured. The evaluation of anticoagulant response to activated protein C was performed using an activated partial thrombin time (APTT) method (Coatest APC resistance, Chromogenix AB, Molndal, Sweden). Lupus anticoagulant was checked with APTT. Total homocysteine plasma levels were determined via fluorescence polarization immunoassay (Axym; Abbott Laboratories). Blood samples were with drawn in EDTA, centrifuged, and stored within 15 minutes from collection to prevent in vitro homocysteine increase, because of its release by red blood cells. Hemolysed samples were not used for homocysteine analysis. Normal reference homocysteine levels were considered to be 12 or less (g/mol/L) in males and 10 or less in females [16].

Genotyping assay of thrombotic factors:

Genomic DNA was extracted from EDTA peripheral blood samples using standard phenol-
choloroform extraction method salting-out procedure [17]. The extracted DNA can be stored for several months at -20°C.

Factor V G1691A, PTHR A 20210 and MTHFR C 677→T genotypes were determined by polymerase chain reaction using the commercial thrombotype test a reverse hybridization assay for combined molecular genetic analysis that allowed the identification of the point mutations 1691A in the FV and A 20210 in prothrombin genes, respectively, and the genotype MTHFR test a reverse hybridization assay that allowed the molecular identification of C 677→T mutation of the human MTHFR gene (Symbiosis, Asti, Italy).

First, purified DNA is amplified with biotin-labelled primers. The amplified DNA fragments were subsequently used for the reverse hybridization assay which started with the denaturation of amplicon DNA. Subsequently, hybridization buffer was added and strip coated with probes for the wild type and mutated sequence of the gene locus analyzed as well a two additional control zones was added to each sample. During incubation in a shaking water bath the various single-stranded amplicons were hybridized with the respective complementary gene probes. A highly specific washing step removed any non specifically bound DNA. After Addition of streptavidin conjugated alkaline phosphatase to the hybrid consisting of probe and biotin-labelled amplicon, the complex was visualized by the addition of NBT/BCIP (a substrate for alkaline phosphatase). A reading card ensured the easy and fast interpretation of the banding pattern obtained. In the thrombotype test, a total of eight reaction zones were present on each strip: Conjugated control documented the efficiency of conjugate binding and substrate reaction, specific control documented an unspecific hybridization reaction, sensitivity control documented the optimal sensitivity of the reaction, C667 wild type where the wild type sequence (C) was present at nucleotide position 667T mutation where the mutated sequence (T) was present at nucleotide position of the human MTHFR gene.

Statistical analysis:

Data were coded and summarized using SPSS (statistical package for Social Sciences) version 13.0 for Windows. Quantitative variables were described using mean ± standard deviation and categorical data by using frequency and percentage. Comparison between groups was done using Chi square (X^2) test for qualitative variables, t-test for normally distributed variables and Mann Whitney (U) test for non normally distributed variables. Kruskal-Wallis test was done to compare three or more of non normally distributed variables. p-value <0.05 was considered statistically significant.

Results

The baseline demographics and clinical characteristics of the included patients were shown in Table (1). The majority of the patients were men, there was history of smoking in 30.2% of diabetic patients without CAD and 37.9% in diabetic patients with CAD, hypertension, hypercholesterolemia, Hyperhomocysteinemia were observed more frequently in diabetic group with CAD. Table (2) showed comparison of clinical and biochemical data of the patients groups and the control where p<0.001 and p<0.05 showed significance and p>0.5 is statistically non significant. In Table (3) and Fig. (1) the mean levels of protein C, protein S, and AT III among the studied groups were significantly lower in diabetic patients without CAD and diabetic patients with CAD compared to control group where p1=DM without CAD versus controls, p2=DM with CAD versus controls, p3=DM without CAD versus DM with CAD. In Table (4) and Fig. (2), the prevalence’s of heterozygotes for FV G1691A and F II G20210A were not significantly different between both patient groups (6.9% versus 6.8% and 2.3% versus 3.4% among DM without CAD and DM with CAD, respectively no cases were found among control group as regard two polymorphisms. In Table (5) we did not find any relationship between smoking and the MTHFR C677T polymorphism, but in the diabetic patients with CAD there is positive correlations between MTHFR C677→T, Hyper tension, Hypercholesterolemia, Hyperhomocysteinemia, where p>0.5 is statistically non significant, p<0.05 is statistically significant.
Table (1): Demographic and Clinical Information of studied Patient groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>DM (n=43)</th>
<th>DM with CAD (n=29)</th>
<th>X²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>23/20</td>
<td>17/12</td>
<td>2.03</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>4.36±1.87</td>
<td>5.02±2.03</td>
<td>1.15</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Smoking (no%)</td>
<td>13 (30.2%)</td>
<td>11 (37.9%)</td>
<td>1.28</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hypertension (no%)</td>
<td>16 (37.2%)</td>
<td>14 (48.3%)</td>
<td>1.79</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hypercholesterolemia (no%)</td>
<td>9 (20.9%)</td>
<td>12 (41.4%)</td>
<td>4.26</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Hyperhomocysteinemia (no%)</td>
<td>5 (11.6%)</td>
<td>7 (24.1%)</td>
<td>3.98</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

*p<0.5 is statistically non significant.
*p<0.05 is statistically significant.

Table (2): Comparison of clinical and biochemical data of the studied groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>DM (n=43) mean±SD</th>
<th>DM with CAD (n=29) mean±SD</th>
<th>Healthy control (n=20) mean±SD</th>
<th>p-value</th>
<th>DM vs control</th>
<th>CAD vs control</th>
<th>DM vs CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.1±7.21</td>
<td>51.8±9.21</td>
<td>42.8±6.14</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>25.5±9.8</td>
<td>26.9±9.32</td>
<td>24.8±8.42</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132.4±12.9</td>
<td>142.0±17.6</td>
<td>127.0±6.4</td>
<td>&gt;0.05</td>
<td>&lt;0.05*</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80.5±9.6</td>
<td>86.2±11.5</td>
<td>78.4±5.21</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>140.7±19.2</td>
<td>132.5±15.1</td>
<td>92.3±6.4</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>2 hs blood glucose (mg/dL)</td>
<td>281.3±20.1</td>
<td>265.5±11.3</td>
<td>102.5±7.12</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>134.6±23.1</td>
<td>144.6±17.6</td>
<td>115.6±12.4</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>174.6±41.2</td>
<td>176.2±37.2</td>
<td>93.4±9.52</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>24.3±7.6</td>
<td>22.6±5.9</td>
<td>30.2±3.5</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>78.6±7.1</td>
<td>96.4±12.5</td>
<td>68.9±4.2</td>
<td>&lt;0.05</td>
<td>&lt;0.05*</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>29.7±12.5</td>
<td>40.2±11.5</td>
<td>10.49±5.7</td>
<td>&lt;0.01</td>
<td>&lt;0.001*</td>
<td>&lt;0.05*</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.5 is statistically non significant, p<0.001 and p<0.05 are statistically significant.

Table (3): Comparison of protein C, protein S, AT III among studied groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>DM (n=43)</th>
<th>DM with CAD (n=29)</th>
<th>X²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein C (%)</td>
<td>62.4±7.5</td>
<td>59.8±8.7</td>
<td>105.3±18.2</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Protein S (%)</td>
<td>75.6±10.2</td>
<td>77.4±12.6</td>
<td>112.6±20.5</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>AT III (%)</td>
<td>54.7±12.3</td>
<td>61.2±10.4</td>
<td>98.2±7.1</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

*p1=DM versus controls.
*p2=DM with CAD versus controls.
*p3=DM versus CAD.

Table (4): Frequency of MTHFR C<sup>677</sup>→T, PTHR<sup>20210</sup> and FVL among studied groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>DM without CAD (n=43)</th>
<th>DM with CAD (n=29)</th>
<th>Healthy control (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR C&lt;sup&gt;677&lt;/sup&gt;→T</td>
<td>10 (23.3%)</td>
<td>11 (37.9%)</td>
<td>2 (10%)</td>
<td>p&lt;0.05*</td>
</tr>
<tr>
<td>PTHR A20210</td>
<td>1 (2.3%)</td>
<td>1 (3.4%)</td>
<td>0 (0%)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>FVL</td>
<td>3 (6.9%)</td>
<td>2 (6.8%)</td>
<td>0 (0%)</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

*p1=DM versus controls.
*p2=DM with CAD versus controls.
*p3=DM without CAD versus DM with CAD.
Table (5): Correlation of MTHFR C<sup>677</sup>→T with prothrombotic risk factors.

<table>
<thead>
<tr>
<th>Variables</th>
<th>DM without CAD (n=43)</th>
<th>DM with CAD (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Age</td>
<td>1.28</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gender</td>
<td>0.97</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>1.67</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.11</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>1.35</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>3.98</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

*p>0.5 is statistically non significant, p<0.05 is statistically significant.

Discussion

In diabetic patients, the CAD and MI are most common; the possible role of prothrombotic risk factors is not completely calcified. Therefore, we evaluated the effect of several recently described prothrombotic polymorphisms in diabetic patients with and without CAD and estimate their interaction with major atherogenic risk factors.

In this study, the prevalence of homozygous MTHFR C<sup>677</sup>-&gt;T was significantly higher in diabetics with CAD patients (23.3% versus 37.9% and 10% among DM without CAD, DM with CAD and controls, respectively). Several studies have examined the association of homozygous 677T MTHFR and AMI, but the results have been inconsistent. Our results are consistent with previous two studies who showed a significant increase in the frequency of the MTHFR C677T polymorphism among patients with CAD; Almawi et al. [18] in a sample from the Bahrain population; Ghazouni et al. [19] in Tunisians.

In a study including 614 patients, homozygote MTHFR mutation was found to be higher in patients with a history of arterial (n=191) and venous (n=127) thrombo-embolism compared to the control group (n=296), and it was suggested that the presence of homozygote mutations of this gene may be a risk for both arterial and venous thromboembolism [20,21]. In contrast to these results, Yilmaz et al. [22] and Caner et al. [23] did not find an association between MTHFR C677T and increased risk for CAD in Turkish patients.

Many previous studies, demonstrated a higher prevalence of homozygous MTHFR 677T in patients with AMI [24,25,26], whereas others showed no difference in the prevalence of the mutation between coronary patients and controls [27,28].

Although numerous studies identify an association between the 677T allele and atherothrombotic disease, others have failed to corroborate this association, so there is controversy in this respect [22,29]. The diversity of data is probably due to the interaction of Hcy with serum folate concentration, and the latter may mask the interpretation of studies [30].

In addition, our study demonstrated that, the prevalence’s of heterozygotes for FV G1691A and F II G20210A were not significantly different between both patient groups (6.9% versus 6.8% and 2.3% versus 3.4% among DM without CAD and DM with CAD, respectively), however, no cases were found among control group as regard
two polymorphisms. Similar results were reported by other investigators. In an et al. [26] and Yamada et al. [31] found that neither FV G1691A nor F II G20210A was associated with an increased risk of AMI.

The frequency of the factor V-Leiden (G1691A) and prothrombin G20210A gene polymorphisms did not differ between patients and controls in the study of Almawi et al. [18], in accordance with our results. Similarly, Baykan et al. [32] did not find an increased frequency of factor V-Leiden in patients presenting with CAD either. In contrast, Rosendaal et al. [10] did demonstrate an association between F II G20210A and FV G1691A polymorphisms and increased risk of AMI in young women.

In a recent study by Emiroglu et al. [33] FVL and PT G20210A polymorphisms were associated with higher incidence of totally occluded coronary arteries. Also, Settin et al. [34] reported that, factor FVL represents a significant risk for AMI. Conceivably, these differences may stem from variable prevalences of other risk factors in different populations.

Experimental evidence suggests that Hcy may contribute to both atherosclerotic and thrombotic processes by modulating vascular cell proliferation and promoting prothrombotic activity in the vascular wall. Outinen et al. [35], Upchurch et al. [36]. These effects of Hcy may explain the close correlation between the C\(^{677}\)→T mutation of MTHFR which results in hyperhomocysteinemia and the presence of atherosclerotic disease in DM, Sun et al. [37].

Moreover, our study revealed a strong relationship between MTHFR C\(^{677}\)→T and other risk factors of CAD such as hypertension, hypercholesterolemia and hyperhomocysteinemia. Many previous studies were found these associations.

Elevated homocysteine levels have been identified as an independent risk factor for MI and for mortality in patients with confirmed CAD [38]. A common mutation (alanine to valine at position 677) in the MTHFR gene is associated with decreased specific MTHFR activity and elevation in homocysteine.

levels in the homozygous state [39]. Numerous case-control studies have observed an association between the MTHFR C677T polymorphism and homocysteine levels [40,41]. Subsequent studies concluded that the 677 CT mutation is a major cause of mild hyperhomocysteinemia, but it does not increase cardiovascular risk [42].

Previous studies have shown an increase in plasma concentration of homocysteine as an independent factor for the development of arterial thrombosis through the mechanism of endothelial dysfunction and platelet activation [38]. Serious hyperhomocysteinemia relates to congenital errors of metabolism such as deficiency of enzyme cystathionine β synthase, whereas a moderate increase is associated with polymorphism 677 C/T in the gene for the enzyme 5,10 methylenetetrahydrofolate reductase (MTHFR) because it produces a thermolabile enzyme with decreased activity [30].

There is an association between both systolic and diastolic BP and risk of stroke without a clear threshold even at a systolic BP of 115mm Hg [43]. Meta-analyses of randomized controlled trials have shown that BP lowering is associated with a 30% to 40% reduction in risk of stroke [44,45]. There is strong and consistent evidence that cigarette smoking is a major independent risk factor for ischemic stroke. There is also growing evidence that exposure to environmental tobacco smoke or passive smoke increases the risk of cardiovascular disease, including stroke [46,47].

In the present study Although, the high prevalence of cigarette smoking among both groups of patients, we did not find any relationship between smoking and the MTHFR C677T polymorphism. However, reports indicate the interaction of the FVL polymorphism with environmental factors such as smoking increase the risk of AMI [10,48]. More recently, Isordia-Salas et al. [30] reported that, carriers of the 20210A allele have four times greater risk of AMI and this risk is increased in subjects with smoking history.

One of the strength points of this study is the ethnic homogeneity of our sample, including all Egyptian people living in the same city having nearly same environmental factors. Moreover, we tried to estimate the influence of multiple risk factors e.g. obesity, HTN, smoking and total lipid profile with HCY and MTHFR C677T which are not included in most of other studies in Egypt. However, some study limitations need to be addressed. First, folic acid vit B6 and vit B12 plasma levels were not determined in the sample as they are not included in routine analysis in our hospital laboratory and hence, we could not investigate the possible correlation between them and MTHFR C677T Second, the results could be influenced by small size of the study group. Finally.

In conclusion, although the results concerning the association of the MTHFR C677T polymor-
phism with diabetic patients with CAD are still controversial, the factor V-Leiden and prothrombin G20210A polymorphisms do not seem to confer an increased risk for CAD in our studied populations. Given this controversy, further studies involving a greater number of patients to confirm their association are required.

our results do not determine the effect of gene-gene interaction between MTHFR and other corresponding genes (e.g. ACD I/D, Apo E and NOS 3 genes).

References


