Aldose reductase (-106) C/T gene polymorphism and possibility of macrovascular complications in Egyptian type 2 diabetic patients

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ABSTRACT

Introduction: Over the past three decades, the number of people with diabetes mellitus (DM) has more than doubled globally, making it one of the most important public health challenges to all nations. Aldose reductase (AR) is a rate-limiting enzyme in the polyol pathway, which has been implicated in the pathogenesis of diabetic microvascular complications; however, the association of the AR gene with diabetic macrovascular complications has rarely been investigated. Aim: The study aimed to identify the possible association between C(-106) T polymorphism of the AR gene and diabetic macroangiopathy in a cohort of Egyptian patients with type 2 DM. Settings and Design: This study was conducted on 100 Egyptian subjects, the control group (n = 20) and the patient group (n = 80) with type 2 diabetes which were further subdivided into two subgroups with (n = 48) and without macroangiopathic complications (n = 32) as evidenced by carotid intima-media thickness, electrocardiography (ECG) ischemic changes, cerebrovascular insufficiency, and peripheral vascular insufficiency. Subjects and Methods: All studied subjects were subjected to detailed history taking, clinical examination, ECG, carotid ultrasonography, routine laboratory investigations, and molecular studies including the detection of AR C(-106) T gene polymorphisms using the polymerase chain reaction (PCR)/restriction fragment length polymorphism technique. Results: The genotype distribution and allele frequency of AR C(-106) T showed no statistical significance also the genotypes were not associated with any of the different studied parameters. Conclusions: The results suggest that the C(-106) T polymorphism in the AR gene is not involved in the pathogenesis of macroangiopathy in type 2 diabetes.

Key words: (-106) C/T single-nucleotide polymorphism, aldose reductase, diabetes mellitus, gene polymorphism, macroangiopathy

INTRODUCTION

Diabetes mellitus (DM) is one of the most common chronic diseases in nearly all countries and continues to increase in number and significance and is associated with development of chronic complications leading to significant morbidity and mortality. Macrovacular complications are the largest contributor to the direct and indirect costs of diabetes.3

Chronic elevation of glucose alters the biochemical homeostasis of the cells by impacting a number of key biochemical pathways including the polyol pathway.4-6 When intracellular glucose levels are elevated, the polyol pathway of glucose metabolism becomes active.7,8 Aldose reductase (AR, AKR1B1, ALD2) is a member of the aldo-keto reductase superfamily, and it is the first and rate-limiting enzyme in the polyol pathway which sits high
in the biochemical cascade that follows the entry of excess glucose into the cytosol of cells. It is widely distributed in many tissues; it is abundant in human sciatic nerve, lens, testis, cornea, and heart. A recent report suggests that AR protein expression is especially robust in vascular endothelial cells and within macrophages of atherosclerotic lesions.

AR reduces glucose to sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor; sorbitol is then metabolized to fructose by sorbitol dehydrogenase. The fructose produced by the polyol pathway can become phosphorylated to fructose-3-phosphate, which is broken down to 3-deoxyglucosone; both compounds are powerful glycosylating agents that enter in the formation of advanced glycation end products (AGEs). The usage of NADPH by AR may result in less cofactor available for glutathione reductase, which is critical for the maintenance of the intracellular pool of reduced glutathione. This would lessen the capability of cells to respond to oxidative stress. Thus, the activation of the polyol pathway, by altering intracellular tonicity, generating AGEs precursors, and exposing cells to oxidative stress perhaps through decreased antioxidant defenses and generation of oxidant species, can initiate and multiply several mechanisms of cellular damage.

Many studies have reported both increased enzyme activity and expression of the AR gene in patients with diabetic microvascular complication and polymorphisms in the AR gene may be one of the factors that determine genetic susceptibility to diabetic microvascular complications. C(-106) T is a single-nucleotide polymorphism (SNP) which was detected at position 106 in the basal promoter region of the AR gene. An equal or even stronger link with the C(-106) T promoter SNP has been found in Chinese type 2 diabetic patients, genetic polymorphisms of AR independently predicted the onset of cardiorenal complications. Other reports showed a role of hyperactivity of polyol pathway in the development of diabetic macroangiopathy in vivo and in vitro.

This study aimed to investigate whether the C(-106) T polymorphism of the AR gene determines susceptibility to diabetic macroangiopathic complications in Egyptian type 2 diabetic patients.

**Subjects and Methods**

After the approval of the Ethics Committee of the Medical Research Institute, informed consents were obtained from all subjects who participated in the study.

This study included 100 subjects, divided into two main groups: Control group which included twenty apparently healthy volunteers of comparable age and socioeconomic status to the patient group; and patient group which included eighty patients with type 2 DM. The patients were further subdivided according to the presence of atherosclerosis into two subgroups: Group A without macroangiopathic complications, and Group B with macroangiopathic complications as presented by ischemic heart disease (IHD), cerebrovascular insufficiency (CVI), and peripheral vascular insufficiency (PVI).

The studied groups were subjected to the following:

- Detailed history taking with special emphasis on age, duration, and treatment of DM as well as symptoms of IHD, CVI, and PVI
- Clinical examination with the measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure, height, weight, and body mass index (BMI) was calculated; (BMI = weight [kg]/height [m^2])
- Electrocardiography (ECG) and carotid ultrasonography for the measurement of carotid intima-media thickness (CIMT)
- Laboratory investigations:

  Following an overnight fasting period, 10 mL whole venous blood was withdrawn from each subject. One mL of whole blood was mixed with 50 μL of 3.8% ethylenediaminetetraacetic acid for molecular studies and the rest 9 mL whole blood was left to clot and the obtained serum was used for the determination of the concentrations of the traditional analytes for all subjects using an automatic autoanalyzer (Olympus, Germany), including fasting serum glucose (FSG), postprandial serum glucose, HbA1c, serum creatinine, high-sensitivity C-reactive protein (hs-CRP), triglycerides (TGs), total cholesterol (TC), high- and low-density lipoprotein cholesterol fractions (HDL-C, and LDL-C), uric acid level, albumin/creatinine ratio, and calculation of estimated glomerular filtration rate (eGFR).

Molecular studies for detection of AR C(-106) T gene polymorphism were done to all subjects using the polymerase chain reaction (PCR)/restriction fragment length polymorphism technique and included DNA extraction from peripheral blood leukocytes using Gene JET Genomic DNA purification Kit (Fermentas–Thermo, USA). The purity of extracted genomic DNA was detected by agarose gel electrophoresis of the yield on 1% agarose gel. In addition, the genomic DNA concentration was measured on a nanodrop 1000 spectrophotometer (Thermo scientific, USA) at 260 and 280 nm. The mean concentration of the purified genomic DNA was 20.56 ng/μL. After extraction, AR gene promoter region
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RESULTS

The control group included eight males (40%) and 12 females (60%), their mean age was 46.0 ± 12.8 years while patients group included eighty patients with type 2 DM, 36 males (45%) and 44 females (55%), their mean age was 53.1 ± 9.0 years and their duration of diabetes ranged from 2 to 34 years with median 10 years. Group A included 32 patients (14 males [43.7%] and 18 females [56.3%]) without atherosclerosis; their mean age was 48.3 ± 8.1 years and Group B included 48 patients ([22 males [45.8%] and 26 females [54.2%]) with atherosclerosis, their mean age was 56.3 ± 8.3 years. No significant difference was found between any of the groups regarding sex. The mean age, BMI, SBP, DBP, Mean BP, and CIMT were higher in diabetic patients than those of nondiabetic subjects. However, age, duration of DM and CIMT were significantly higher in Group B than in Group A. In Group B, 46 patients (95.8%) had ECG changes of IHD, two patients (4.2%) had PVI, and four patients (8.3%) had stroke [Table 1].

The mean level of serum creatinine, Hba1c, hs-CRP, serum uric acid, and Alb/Cr ratio were significantly higher in diabetic patients than in nondiabetic subjects. eGFR showed no significant difference between both groups. However, FSG, postprandial, Hba1c, and Alb/Cr were significantly higher in Group B than in Group A [Table 2]. The mean level of TGs, LDL-C was significantly higher, and HDL-C was significantly lower in diabetic patients than in nondiabetic subjects. LDL-C was significantly higher in Group B than Group A [Table 3].

Allele C was present in 95% of the control group and 93.8% of the patients group and allele T in 5% of the patients group. The most common genotype of the C-106T polymorphism was CC corresponding to 90%, and 92.5% of the control and patients groups, respectively, followed by CT/TT corresponding to 10%, 0% and 2.5%, 5% of the analyzed control and patients groups, respectively. When the whole study population was analyzed, there was no significant difference in AR C(-106) T genotypes or its allele frequency distributions among the studied groups [Tables 4 and 5].

Because the frequency of CT genotype was 0% in the diabetic patients with macroangiopathy (Group B) and only 6.3% in diabetic patients without macroangiopathy (Group A), subjects with the CT and TT genotypes were combined for further analyses, but no significant difference was found between both groups [Table 6].

Statistical analysis

Statistical assessment was carried out with Statistical Package for Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, IL, USA) for Windows statistical software.

Quantitative variables were tested for normality using Kolmogorov–Smirnov test. Data revealed normal distribution was represented as mean ± standard deviation, and the data revealed deviation from normal distribution was represented as median and range. Analysis of variance or F-test is used for comparison between more than two means when the data are normally distributed. Kruskal–Wallis test was used for testing equality of population medians among groups. The Hardy–Weinberg test was performed using a standard observed-expected Chi-square test. Allele and genotype frequencies among cases and controls were compared with Chi-square test. P < 0.05 was considered statistically significant. [30,31]

FIGURE 1: Agarose gel electrophoresis of polymerase chain reaction products digested with Bfa-I restriction enzyme. Lane 1: 100 bp DNA ladder (100–1000 bp). Lanes 2-6: CC homozygote alleles (bands at 206 bp and 57 bp). Lane 7: TT homozygote alleles (bands at 147 bp, 59 bp, and 57 bp). Lane 8: CT heterozygote allele (bands 206, 147, 59, and 57)
In addition, the genotypes were not associated with any of the different studied parameters [Table 7].

**Discussion**

DM is estimated to affect around 350 million people all over the world,[32] and coronary heart disease which is one of diabetic complications is considered a major cause of mortality and morbidity in Middle East populations.[33]
and occurrence, some pathways are known to be implicated in the pathogenesis of diabetic vascular complications which include activation of the pathway of protein kinase C, the formation of glycation end products, and accumulation of sorbitol through the AR pathway.

Some studies suggested that high-glucose flux via AR pathway in hyperglycemia triggers alteration of glucose metabolism, and vascular perturbation, leading to prothrombotic and pro-inflammatory responses in diabetic atherosclerosis, and high AR expression in mice accelerates diabetic atherosclerosis both globally and in endothelial cells. Results from various cellular and animal models representing a number of inflammatory conditions suggested that inflammation induced by oxidative stress which is a major contributor to diabetic complications could be reduced by the inhibition of AR which can be used for future treatment.

Since Kao et al. identified the AR C(-106) T polymorphism for the first time and found that the CC homozygotes and C allele were obviously higher in those with retinopathy when compared with controls. The AR C(-106) T polymorphism may play more important role in the pathogenesis of microangiopathy and macroangiopathy.

This study investigated the association between AR C(-106) T polymorphism and risk for macrovascular diseases in Egyptian type 2 diabetic patients.

Among the conventional risk factors, in this study, patients with macroangiopathic complications were of older age, longer duration of DM, higher FSG, postprandial glucose, HbA1c, and LDL-C than those without. These results were similar to other previous studies.

No significant difference was found in the genotype distribution of AR C(-106) T among the studied groups. In addition, no significant difference was observed in the frequency of AR C(-106) T alleles among the studied groups. These results were in agreement with Wu et al., who did not find any association in either allele frequency or genotype distribution of AR C(-106) T gene polymorphism and carotid atherosclerosis in Chinese patients with type 2 diabetes. In another study which was done on Japanese population, Watarai et al. also did not find any significant differences in genotypic or allelic distribution in diabetic patients with or without IHDs, but they observed significant increase in the frequency of the CT/TT genotype and T allele among the studied groups.
Although numerous studies have reported that AR C(-106) T polymorphism is a risk factor for the early development of diabetic microvascular complications and stroke, the role of AR may be not so important in the development of macroangiopathy when compared with other complex environmental and genetic factors. The traditional risk factors for atherosclerosis, such as high blood pressure, smoking, elevated LDL-C as well as mixed dyslipidemia classically present in a diabetic state, are playing a prominent role in the development of coronary artery disease in diabetes, with high glucose playing an important, but perhaps less significant role. Some clinical trials support these ideas in which it is noted that the cardioprotective effect through intensive glucose-lowering is comparatively less, in contrast to the remarkable effects of normoglycemia in reducing diabetic vascular complications.

The possible substantial heterogeneity within individual studies, in which age at first diagnosis, strictness of metabolic control, duration of diabetes, and comorbidities such as hypercholesterolemia, hypertension, and others. In addition, there is possible substantial heterogeneity among different studies, due to underlying differences in study populations.

Small sample size may be another cause we failed to find association between AR C(-106) T polymorphism and macroangiopathy. Of course, the involvement of the AR C(-106) T polymorphism in this multigenetic disease needs further study with population with larger sample size. This situation promotes more and more attempts to be made to further assess the associations of these polymorphisms with the disease.

**Conclusions**

No association was found between AR C(-106) T gene polymorphism and macroangiopathy in type 2 diabetic patients, and this result suggests that the polymorphism is not involved in the pathogenesis of this disease.

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**Conflicts of interest**

There are no conflicts of interest.

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