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Original Article

Role of aldose reductase C-106T polymorphism among diabetic Egyptian patients with different microvascular complications

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Abstract. The aldose reductase pathway proves that elevated blood glucose promotes cellular dysfunction. The polyol pathway converts excess intracellular glucose into alcohols via activity of the aldose reductase. This enzyme catalyzes the conversion of glucose to sorbitol which triggers variety of intracellular changes in the tissues. Among diabetes, activity is drastically increased in association with three main consequences inside the cells. The aim of this study was to detect the association of the C-106 T polymorphism of the aldose reductase gene and its frequency among a sample of 150 Egyptian adults with type 2 diabetic patients having diabetic microvascular. The detection of the aldose reductase C-106 T polymorphism gene was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genotype distribution of the C-106 T polymorphism showed that CC genotype was statistically significantly higher among patients with retinopathy compared to nephropathy. Patients with nephropathy had significant association with the TT genotype when compared with diabetic retinopathy patients. Follow up study after the genotype detection among recently diagnosed diabetic patients in order to give a prophylactic aldose reductase inhibitors; studying the microvascular complications and its relation to the genotype polymorphisms. The study may include multiple gene polymorphisms to make the relation between the gene and the occurrence of these complications more evident.

Keywords: Advanced glycation end products, polymerase chain reaction-restriction fragment length polymorphism, aldose reductase

Introduction

The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Aldose reductase (EC: 1.1.1.21; alcohol: NADPH oxidoreductase, ALR2) belongs to the aldo-keto reductase (AKR) superfamily. Human ALR2, encoded by AKR1B1 gene, present on chromosome 7, is a monomeric protein of 315 amino acids with a molecular mass of about 36 kDa. Since the active site of ALR2 is highly plastic and flexible, inhibitors with diverse chemical structures are shown to interact with its enzyme active site [1].

ALR2 catalyzes the NADPH dependent reduction of a variety of aldehydes and ketones to their corresponding alcohols. ALR2 is a cytosolic enzyme and is widely distributed in eye lens, retina, kidney, adrenal gland and various reproductive organs. Under normoglycemic conditions, a minor part of nonphosphorylated glucose enters the polyol pathway because glucose is not a preferred substrate for ALR2. Thus, the significance of the polyol pathway may be quite limited under normoglycemic conditions: it supplies fructose as an energy source for sperm in the seminal vesicle and is involved in osmotic regulation by producing an osmolyte sorbitol in the renal medulla [2].

Elevation of intracellular glucose levels can cause an increased flux through the enzyme aldose reductase (ALDR2), which is only activated when intracellular glucose concentrations rise to hyperglycemic levels. ALDR2 uses nicotinamide adenine dinucleotide phosphate (NADPH) to reduce glucose to sorbitol, which is then oxidized to fructose via sorbitol dehydrogenase (which uses NAD^+ as a cofactor). The decline in cellular NADPH secondary to the increases in polyol pathway flux may depress the generation of nitric oxide in endothelial cells and the cellular redox balance. Increased flux through sorbitol dehydrogenase can lead to an increased NADH/NAD^+ ratio that may alter enzyme activities and also contribute to the development of complications. Aldose reductase inhibitors (ARIs) have been shown to prevent some of the pathologic changes in diabetic retinopathy, nephropathy, and neuropathy [3].

Recent studies suggest that ALR2 is an important component of the detoxification system in the removal of

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reactive aldehydes and glutathione conjugates. ALR2 catalyzes the reduction of biogenic aldehydes derived from the catabolism of steroids, catecholamines and phospholipids. ALR2 is particularly efficient in reducing medium-to long chain (C6 - C18) aldehydes generated in high abundance during lipid peroxidation. The reduction of glutathione conjugates by ALR2 may serve as a protective mechanism in minimizing the reactivity of the aldehyde function unquenched by glutathiolation. ALR2 acts as an extrahepatic detoxification enzyme in various tissues. ALR2 is shown to be involved in key signaling events associated with cell growth and proliferation under specific circumstances [4].

The aldose reductase polymorphisms are the (CA)*n* dinucleotide repeat (Z = 138 bp) with different alleles: Z-10, Z-8, Z-6, Z-4, Z-2, Z, Z+2, Z+4, Z+6, and Z+8. From the promoter region, the 263-bp fragment containing two single nucleotide polymorphisms: -106 C→T with the alleles C and T and -12 C→G with the alleles C and G.

In the C (-106) T polymorphism, the thymine replaced the cytosine at the position 106 upstream of the transcription initiation site within the promoter region of the gene.

Activation of the polyol pathway in hyperglycemia leads to perturbation of metabolic homeostasis, particularly depletion of NADPH and accumulation of sorbitol in tissues where glucose uptake is not dependent on insulin. Thus increased ALR2 mediated flux of glucose into the polyol pathway in target tissues such as eye lens and retina has been implicated as a major cause in the aetiology of diabetic cataract and diabetic retinopathy. Because sorbitol does not easily cross cell membranes and its subsequent conversion to fructose is slow, it accumulates inside the cell, resulting in altered osmotic pressure and redox state of pyridine nucleotides (increased NADH/NAD ratio). Increased membrane leak of glutathione and myo-inositol due to hyperosmotic swelling results in oxidative damage to the lens and eventually results in the cataract. Similar events are implicated in the aetiology of ocular complications of diabetes such as retinopathy.

Polymorphism in ALR2 gene, particularly C106T substitution and (CA)n repeat, has been associated with susceptibility to retinopathy in type 2 diabetes. These studies together substantiate the role of ALR2 in diabetic complications. Polyol pathway mediated alterations in pyridine nucleotides have been linked to diverse metabolic changes such as synthesis of nitric oxide and activation of protein kinases. Specifically, increase in NADH due to elevated polyol pathway activity could increase the synthesis of diacylglycerol (DAG) from dihydroxyacetone phosphate. DAG is an essential activator of the protein kinase C, and DAG dependent activation of kinase is thought to play a key role in mesangial expansion and smooth muscle cell proliferation induced by high glucose. These findings of the pathophysiological significance of ALR2 suggest that the inhibition of ALR2 can be used as a potential target for therapeutic intervention of diabetic complications in general and ocular complications in particular [5].

The aldose reductase enzyme of the polyol pathway was first found to be implicated in the aetiology of the long term diabetic complications as well as certain other pathological conditions such as ischemia, abnormal vascular smooth cells proliferation, cancers and mood disorders [5].

This study aimed to detect the association of the C (-106) T polymorphism of the aldose reductase gene and its relation to the occurrence of different diabetic microvascular complications: Retinopathy, nephropathy and neuropathy, among a sample of Egyptian type 2 diabetic patients having proved diabetic microvascular complications by standard clinical guidlines.

Materials and Methods

This study was carried out on 150 subjects recruited and categorized into five groups: Group I: Thirty patients suffering from type 2 diabetes mellitus proved to have diabetic nephropathy by microalbuminuria and renal function tests. Group II: Thirty patients suffering from type 2 diabetes mellitus proved to have diabetic retinopathy by fundus examination. Group III: Thirty patients suffering from type 2 diabetes mellitus proved to have diabetic neuropathy proved by CNS examination. Group IV: Thirty newly diagnosed and well controlled cases of type 2 diabetes mellitus, proved not to have any evident microvascular complications. Group V: Thirty healthy subjects of matched age, sex, free of any medical illness.

The patients were recruited from inpatient internal medicine departments and outpatient clinics of the main Alexandria University Hospital; the control subjects were healthy volunteers.

Exclusion criteria include type 1 diabetes mellitus, any primary renal disease and any primary fundal affection.

The study was carried out after taking informed consent from the patients and after approval of the ethical committee of the Faculty of Medicine, Alexandria University.

All patients were subjected to the following criteria:

1. Full clinical examination including thorough history taking, complete physical examination with special emphasis on neurological examination for detection of diabetic neuropathy mainly peripheral neuropathy, and fundus examination for detection of diabetic retinopathy regardless of its stage.

2. Laboratory investigations including: complete blood picture [6] measured by cell counter ADVIA 120 (Siemens Healthcare Diagnostics, USA), fasting blood glucose, renal function tests [7] which include Blood urea nitrogen (BUN), serum creatinine Dimension RXLMax chemistry autoanalyzer (Siemens Healthcare Diagnostics, USA), HB A1C (glycated haemoglobin) estimation [8] total urinary protein in random urine sample [9], microalbuminuria [9], protein/creatinine ratio, albumin/creatinine ratio [9], and detection of the aldose reductase C (-106) T polymorphism gene by genomic DNA extraction from peripheral blood. A fragment of the ALR2 gene promoter region containing the C (-106)T polymorphism will be amplified by PCR technique followed by restriction digestion (RFLP) [10].

TABLE 1
GENOTYPE DISTRIBUTION AMONG DIFFERENT STUDIED GROUPS

	Nephropathy (G I)		Retinopathy (G II)		Neuropathy (G III)		(G IV)		(G V)	
	No.	%	No.	%	No.	%	No.	%	No.	%
PCR RE										
CC	12	40.0	24	81.3	16	53.3	16	53.3	18	60.0
CT	10	33.3	6	18.8	10	33.3	14	46.7	6	20.0
TT	8	26.7	0	0.0	4	13.3	0	0.0	6	20.0
MCp					0.124					
Significance	II*		I*		NS		NS		NS	
p_1			0.024*		0.731		0.170		0.635	
p_2					0.206		0.135		0.240	
p_3							0.473		0.777	
p_4									0.131	

Detection of C (-106) T polymorphism of the aldose reductase gene

DNA extraction was done by the GE Healthcare illustra blood genomicprep using Mini Spin Kit (UK) followed by PCR amplification using PCR-GOLD Master-Mix Beads (BIORON). The reaction mixture was subjected to the following PCR protocol: initial denaturation at 95°C for 2 minutes, followed by 35 cycles of:denaturation at 95°C for 1 minute, annealing at 66°C for 1 minute, and extension at 72°C for 1 minute, then final extension at 72°C for 5 minutes using (Techne thermal cycler TC-312). The extracted DNA was amplified resulting in DNA fragment (amplicon) of 520bp in size. The following sets of primers obtained from Sigma Aldrich were used [11]:

Forward 5' TTC GCT TTC CCA CCA GAT AC'3
Reverse 5 'CGC CGT TGT TGA GCA GGA GAC'3

The amplified PCR products were subjected to enzyme digestion by the restriction enzyme BfaI (FspBI) from Biolabs New England was used to determine the C(-106)T polymorphism by the following regimen:Ten µl of the PCR products, 18 µl of nuclease free water ,2 µl of buffer tango and 1 µl of FspBI were incubated at 37° C for 2 hours in the manufacturer buffer and followed by agarose gel electrophoresis through 3.5% New Sieve agarose gel and visualized by UV transilluminator after staining with ethidium bromide to identify the different polymorphisms. After incubation of the PCR amplicon with the BfaI enzyme for 2 hours at 37°c the different genotypes of the gene appeared on the NewSieve agarose gel (3.5%) and then photographed by the Dolphin gel documentation system where it showed: CC genotype is represented by 2 bands at 234bp and 92bp. CT genotype is represented by 3 bands at 234bp, 175bp, 92bp and a hidden band could not be seen because it is too small to appear on the gel equivalent to (59bp). TT genotype is represented by 2 bands 175bp and 92bp and also a hidden band could not be seen because it is too small to appear on the gel equivalent to (59bp).

Statistical analysis

Data was analyzed using SPSS software package version 18.0 (SPSS, Chicago, IL, USA).

Qualitative data was analyzed using Chi-square test and Fisher's Exact, and Monte Carlo was applied to compare different groups. Qualitative data was expressed in frequency and percent.

Results

Among diabetic patients with microvascular complications there was 50 males (56.5%) and 40 females (43.5%) while the diabetic patients without mivrovascular complications were distributed as 16 males (53.3%) and 14 females (46.7%) with no statistical significance between them with $\chi 2 = 0.047$ at p=0.829.

The age ranged from 21.0 – 67.0 years with a mean and SD of 51.63±10.03 among the diabetic patients with microvascular complications; while the age ranged from 39.0 – 67.0 years with a mean and SD of 55.0±8.74 among the diabetic population without microvascular complications with no statistical significance with Mann Whitney test=1.002 at p=0.316.

ALR Genotype frequency and distribution among different studied group

In group I, the highest prevalence was for the CC genotype 40.0 %(12) while for the CT and TT genotypes the percentage was as follows: 33.3 % (10) and 26.7 % (8) respectively. In group II, no TT genotype was found with highest percentage for the CC genotype 81.3 % (24) while CT was 18.8 % (6). In group III, 53.3 % (16) for the CC genotype, 33.3% (10) for the CT genotype and 13.3% (4) for the TT genotype.

While the control groups (IV and V) demonstrated 53.3% (16), 60.0% (18) for the CC genotype, 0.0%, 20.0% (6) for the TT genotype and finally 46.7% (14), 20.0% (6) for the CT genotype, respectively. The CC genotype among the retinopathy (GII) was statistically significantly higher than the patients with nephropathy (GI) at $p \leq 0.05$

TABLE 2
C-106T GENOTYPING OF ALDOSE REDUCTASE AMONG THE DIABETIC PATIENTS WITH AND WITHOUT MICROVASCULAR COMPLICATIONS

Aldose Reductase Genotypes	Diabetic patients with microvascular complications (n = 90)		Diabetic patients without microvascular complications (n = 30)		χ^2 (p)
	No.	%	No.	%	
CC	54	58.7	16	53.3	
CT	24	28.3	14	46.7	3.182 (0.204)
TT	12	13.0	0	0.0	

TABLE 3
GENOTYPING OF ALDOSE REDUCTASE AMONG DIABETIC AND NON-DIABETIC SUBJECTS

Aldose Reductase Genotypes	Diabetic (n = 120)		Non-diabetic (n = 30)		MCp
	No.	%	No.	%	
CC	40	32.8	6	20.0	
CT	12	9.8	6	20.0	0.410
TT	68	57.4	18	60.0	

while the TT genotype was statistically significantly higher in the nephropathy group (GI) when compared to the retinopathy group (GII) at $p \leq 0.05$ with Monte Carlo test.

Genotype distribution of AR among diabetic patients with and without microvascular complications

The CC genotype was the highest among the diabetic patients with microvascular complications (54 patients; 58.7%) or without microvascular complications (53 patients; 53.3%) with no statistical significance by Chi square for Kruskal Wallis test ($\chi 2=3.182$, $p=0.204$). The CT and TT genotypes were 24 (28.3%) and 12 (13.0%) respectively among diabetic patients with microvascular complications while CT was 14 (46.7%) and no TT genotype was found in diabetic patients without microvascular complications.

Genotype distribution of aldose reductase among diabetic versus non diabetic subjects

TT genotype was the highest among both the diabetic (68 patients; 57.4%) and non-diabetic (18 subjects; 60%) subjects with no statistical significance.

The other two genotypes represented the following frequencies among the diabetic patients: CC (40 patients (32.8%) and CT (12 patients; 9.85%) while among the non-diabetic population the CC and CT each occurred in 6 (20.0%) also no statistical significant difference with Monte Carlo test=0.410.

Validity of Hardy-Weinberg equilibrium for the three genotypes in control group

The CC genotype among the control group (group V) was in 18 subjects while the CT and TT genotype each was in 6 subjects. The observed CC genotype value among all studied group V was 18 while the expected value was 7.350. The observed CT genotype value among all studied group V was 6 while the expected value was 6.300. Finally the observed TT genotype value among the studied group V was 6 while the expected value was 1.350. The group V population was found to be in Hardy-Weinberg equilibrium with Chi-square test=4.12 at $p=0.7$ and no statistical significance.

Discussion

Diabetes mellitus is a metabolic disorder of multiple etiologies which is characterized by chronic hyperglycemia. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy.

The present work aimed at detection of the association of C(-106)T polymorphism of the aldose reductase gene and its relation to the occurrence of diabetic microvascular complications: retinopathy, nephropathy and neuropathy.

In the present study, the gender distribution among the studied groups did not show statistically significant differences where it was homogenously distributed. Comparing subgroups of patients in the present study based on the presence or absence of microvascular complications revealed that among patients with microvascular complications, there were 50 males (56.5%) and 40 females (43.5%), while patients without microvascular complications consisted of 16 males (53.3%) and 14 females (46.7%) with no statistical significant difference. Studying diabetic patients in comparison to the non-diabetic group, it was found that diabetic cohort consisted of 68 males (55.7%) and 52 females (44.3%) while non-diabetic cohort consisted of 20 males (66.7%) and 10 females (33.3%) with no statistical significant difference. These findings were similar to those reported previously

TABLE 4
THE OBSERVED AND EXPECTED VALUES OF THE GENOTYPE FREQUENCIES AMONG GROUPS (I, II, III, IV)

Genotype	Observed	Expected		Difference	p	q	χ^2	Significance
CC	68	p^2n	32.27	1.733				
CT	40	2pqn	23.47	3.467	0.73	0.27	1.31	NS
TT	12	q^2n	4.27	1.733				

TABLE 5
THE OBSERVED AND EXPECTED VALUES OF GENOTYPE FREQUENCIES AMONG GROUP V

Genotype	Observed	Expected		Difference	p	q	χ^2	Significance
CC	18	p^2n	7.350	1.650				
CT	6	2pqn	6.300	-3.300	0.7	0.3	4.12	NS
TT	6	q^2n	1.350	1.650				

[12-16].

Studying diabetic patients in comparison to the non-diabetic group, it was found that diabetic cohort consisted of 68 males (55.7%) and 52 females (44.3%) while non-diabetic cohort consisted of 20 males (66.7%) and 10 females (33.3%) with no statistical significant difference. These findings were similar to those reported previously [12-16].

When studying the population and dividing them into diabetic patients versus non diabetic patients the age ranged among the diabetics from 21.0-67.0 years and ranged from 30.0-58.0 years among the non diabetics with no statistical significance found. Similar findings were observed by other investigators [12-16].

On the other hand, Buraczynska et al. [17] found a statistically significant difference in age between the control group, diabetic patients and patients with diabetic retinopathy ($P<0.05$). This could be attributed to the difference in sample size, ethnic variations and socioeconomic classes.

In the present study it was found that the mean and SD of FBG among the healthy individuals in group V was statistically significantly lower than the other groups (group I, III and IV; $p=0.001$). The retinopathy patients was found to have a statistically significantly lower mean and SD of FBG when compared with the nephropathy patients and uncomplicated diabetic patients ($p=0.01$ and $P=0.05$ respectively). On the other hand our study revealed that in the healthy individuals HbA1C (5.15 ± 0.50%) was statistically significantly lower than in all other groups of I, II, III, IV ($p=0.001$). HbA1C was significantly higher among patients with nephropathy when compared with retinopathy ($p=0.001$) and diabetic patients without microvascular complications ($p=0.05$). On the other hand, the patients with neuropathy had a higher HbA1C than patients with retinopathy ($p=0.01$). This was in accordance with Wang et al. [18] and Shera et al. [15] who found a significant association between the HbA1C and both the neuropathy and the nephropathy patients ($p<0.05$ and $p=0.001$ respectively). This could be explained in the shadow that the diabetic patient with complications frequently encountered uncontrolled diabetes with elevated HbA1C.

On the contrary, others have found difference between the studied groups regarding the mean and SD of HbA1C which included diabetic patients with retinopathy and the uncomplicated diabetic patients [17, 19, 20]. They showed that HbA1C level correlated significantly with daily plasma glucose concentration in patients with type 2 diabetes during the standardized MTT (Meal Tolerance Test) (all $P < 0.001$).

It was found that the correlation between the FBG and HbA1C among the patients with microvascualr complications was significantly proportional at $p\leq0.05$, while no statistical significant difference was found among the uncomplicated diabetic patients. The correlation was also significantly proportional when studied among the diabetic population, while it was inversely proportional among the non-diabetic patients but with no statistical significance. Regarding the HbA1C, the mean and SD of the diabetic patients with microvascular complications (8.5±2.2%) was statistically significantly higher than the diabetic patients without microvascular complications (6.9±2.2%) or the healthy individuals (5.2±0.3%; $p<0.05$). The mean and SD for diabetic patients without microvascular complications was also statistically higher than those of the healthy individuals ($p<0.05$). These results were in accordance with Bonakdaran et al. [21].

In the present study, we found that in the patients with nephropathy the mean and SD level of renal functions (BUN, serum creatinine, proteinuria, microalbuminuria, protein/creatinine ratio and albumin/creatinine ratio) was statistically significantly higher than the other groups including retinopathy, neuropathy, diabetic patients without microvascular complications and the healthy individuals. Diabetic patients without microvascular complications had a higher mean and SD than the healthy individuals regarding the renal function tests. The patients with neuropathy and the uncomplicated diabetic patients showed significantly higher mean and SD of serum creatinine than the healthy individuals. The patients with neuropathy and uncomplicated diabetic patients showed significantly higher

microalbuminuria than those with retinopathy which was in accordance with Wang et al. and Sinha et al. [18, 14]. All diabetic patients with microvascular complications had proteinuria as a complication criteria with a statistical significance ($p<0.001$) between the mean of serum creatinine among patients with retinopathy (1.2 mg/dl) and those without retinopathy (0.8 mg/dl). The albumin/ creatinine ratio in spot urine sample values among patients with nephropathy and retinopathy were statistically significantly higher than those without nephropathy and retinopathy ($p<0.001$). These results were in accordance with Bonakdaran et al. [21] who stated that elevated serum uric acid concentration can be a consequence of kidney dysfunction and corresponded to our results as the mean and SD for serum uric acid was highest among the group I or nephropathy group.

The CC genotype was statistically significantly higher among the retinopathy group when compared with the nephropathy patients at $p<0.05$.whileThe TT genotype was statistically significantly higher among the nephropathy patients when compared with retinopathy subjects at $p<0.05$.

Sivenius et al. [13] found that the allelic distribution of the aldose reductase gene C(-106)T polymorphism among the non diabetic subjects was C164 (65.1%) and T 88 (34.9%) while among the diabetic patients the distribution was C 140 (82.5%) and T 30 (17.6%).

The C allele of the C-106T polymorphism was significantly more common in type 2 diabetic patients than in control subjects. The frequencies of the polymorphisms were in Hardy-Weinberg equilibrium in both groups. The subjects with 106 T allele of the C-106T polymorphism were associated with neuropathy patients than Subjects with the C-106C genotypes.

In the present study although the association between promoter C/T polymorphism and microvascular complications, it was unable to show an association in the whole group between the T allele of the promoter C/T polymorphism and either nephropathy or retinopathy alone, the CT/TT genotype carriers had higher urinary AER than the CC carriers. This association was further strengthened by the association between the T allele and coexistence of nephropathy and retinopathy. This was in accordance with. Zhang X et al, [22]where The aldose reductase gene in promoter region was amplified by polymerase chain reaction and the frequency of genotype of type 2 diabetes were analyzed by restriction enzyme BfaI. The frequencies of genotype CC were significantly higher and the frequencies of genotype TT were much lower in type 2 diabetes patients with microangiopathy than in those without microangiopathy. This finding suggests that (C-106T) single nucleotide polymorphism in the aldose reductase gene promoter region may be associated with diabetic microangiopathy of Chinese type 2 diabetic population .It may be a functional polymorphism and can serve as one of inherited marker for susceptibility of diabetic microangiopathy.

In the present study the mean and SD of HbA1C were higher in the nephropathy patients with the genotype TT, CC than carrying the CT genotype but without any statistical significant difference. These findings were in accordance with Gosek.K et al, [23] who showed that no distortion in the genotype frequency among the study groups was observed. When they stratified the study population by HbA1c they found that patients with HbA1c ≥9% (median) the CT and TT genotypes were more frequent in patients with diabetic nephropathy (proteinuria and microalbuminuria) than those with normoalbuminuria. As a result the C-106T polymorphism in the AR gene is a risk factor for development of diabetic nephropathy in type 2 diabetes in patients with poor glycaemic control.

Watarai et al. [24] stated that the genotype distributions of the C-106T polymorphism of aldose reductase gene showed that the frequencies of the C allele were 82.6% in the diabetic group and 80.7% in the non-diabetic group. The distribution of genotypes was in Hardy–Weinberg equilibrium. There were no significant differences between the two groups because the number of diabetic patients with the TT genotype was only 3.7%, subjects with the CT and TT genotypes were combined for further analyses.

Olmos et al. [25] stated in his study that among 53 diabetics (CC=24; 45.3%), the frequency of the C(-106)T polymorphism of the ALR2 gene was similar to that reported by others. Type-2 diabetics with the CC genotype were more susceptible for developing retinopathy as a result of chronic hyperglycemia than those with the CT or TT genotype. Katakami et al. [26] is likely that the C allele of the polymorphism at position −106 in the promoter of aldose reductase gene, which codes a rate-limiting enzyme of the polyol pathway, is a susceptibility allele for diabetic retinopathy in Japanese type 2 diabetic patients. Demaine et al. [27] stated that the frequencies of C(-106)T genotypes in the patients subgroups as well as the normal control subjects were found to be in Hardy-Weinberg equilibrium.

Conclusion and recommendation

All diabetic patients should undergo frequent micro-albuminuria, fundus examination and neurological examination to detect the non evident microvascular complications among diabetic patients who may be neglected for years without treatment and which may worsen their conditions .Follow-up study after the genotype detection among recently diagnosed diabetic patients we give them aldose reductase inhibitors and observe the incidence of occurrence of any microvascular complications .Studying the microvascular complications in parallel with the occurrence of the macrovascular complications and its relation to the genotype polymorphisms. Finally The study may include multiple gene polymorphisms to make the relation between the gene and the occurrence of these complications more evident.

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