Angiotensin I Converting Enzyme Gene Polymorphism in Type 2 Diabetes Mellitus with Nephropathy in Saudi Population

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ABSTRACT

Background and aim: *Diabetic Nephropathy (DN) is a major microvascular complication in Type 2 diabetes mellitus (T2DM). Several environmental and genetic factors play a pivotal role in the development and pathogenesis of DN. The angiotensin-converting enzyme (ACE) gene insertion (I)/deletion (D) polymorphism is one of the various genetic factors associated with DN. This study was undertaken to assess the frequency and distribution of ACE I/D polymorphism in T2DM with and without nephropathy patients and controls.* ***Materials and methods:*** *We conducted a case-control study. In a population of 61 patients suffering from T2DM without nephropathy and 61 patients T2DM with nephropathy along with the 52 age matched controls in Saudi populations. ACE I/D polymorphism was detected by PCR amplification using allele specific primers and some biochemical and behavioral markers were monitored. Chi-square and multiple logistic regression analyses were done to determine the odds ratio for development of nephropathy.* ***Results:*** *The observed genotype frequencies of II, ID and DD in T2DM with nephropathy were 8.2%, 34.4% and 57.4%, in T2DM without nephropathy 16.4%, 44.2% and 39.4%, in control 30.8%, 48.1%, 21.1% respectively. The DD genotype of the T2DM with nephropathy group was higher than the T2DM without nephropathy patients (OR=2.075, 95% CI: 1.008-4.272, p=0.047).* ***Conclusion:*** *The study illustrated that significant association was observed between the D allele, DD genotype of the ACE gene polymorphism and T2DM with nephropathy in Al-Quwayiyah region of Saudi Arabia. We suggest potential and new multi-property of ACE inhibitors therapy for the prevention or delay of progression of nephropathy.*

Keywords: Type 2 diabetes mellitus, Diabetic nephropathy, Angiotensin converting enzyme, Insertion/gene deletion polymorphism

INTRODUCTION

Type 2 diabetes mellitus (T2DM), considered as the most frequent form of diabetes, and is characterized by modifications in the insulin action or secretion, being generally associated with genetic susceptibility [1]. The prevalence of DM will likely rise from 6% to 10% in the next decade. In 2000, the World Health Organization (WHO) recorded a total of 171 million people for all age groups worldwide (2.8% of the global population) who have DM, and the numbers are excepted to rise in 366 million (4.4% of the global population) by 2030 [2].

DM, it is often associated with macrovascular and microvascular complications, including diabetic nephropathy (DN), a primary cause of end-stage renal disease (ESRD). The kidneys have many tiny blood vessels that filter waste from the blood. High blood glucose and hypertension from DM can diminish the blood vessels and afterwards kidney will not able to work and it completely stops functioning. The condition not only causes disability but is associated with a high mortality rate in diabetic patients as well [3]. Due to the global increase in prevalence of diabetes there has been a concomitant rise in the number of patients with DN indicating a prevalence of 30-40% of the patients with T2DM being affected. Mostly, individuals with long duration of diabetes and poor glycaemic control develop progressive DN [4].

In recent years, there is considerable evidence supporting the theory that genetic susceptibility plays a major role in the pathogenesis of DN. Although DN is traditionally considered a non-immune disease, recent findings indicate a significant role of immune-mediated inflammatory processes in the pathophysiology of DN [5]. Some of studies

suggest that genetic factors may be involved in the aetiology of renal disease in T2DM. Studies have shown that rennin-angiotensin system (RAS) may play an important role in the development of nephropathy in T2DM, and thus the angiotensin converting enzyme (ACE) gene polymorphism may be a potential predictor for development of nephropathy in T2DM [6].

ACE catalyzes production of the vaso active peptide Angiotensin II from its precursors Angiotensin I. Within the diabetic kidney, the effects of Angiotensin II include an increase on intraglomerular pressure and glomerular filtration rate. In addition to its hemodynamic effects Angiotensin II stimulates the production or release of several cytokine mediators of glomerulosclerosis such as osteopontin, platelet derived growth factor, fibronectin and transforming growth factor β [7]. Glomerular hyper filtration leads to progressive destruction of nephrons, with characteristic histologic lesions of focal sclerosis. It also effects on BP regulation and electrolyte balance because of its potency as a vasopressor and an aldosterone-stimulating peptide. Subsequently, high levels of aldosterone cause increased levels of water, sodium reabsorption and extracellular fluid volume [8].

Genetic studies have revealed that the genes of RAS are highly polymorphic; raising the possibility that in addition to environmental factors, the genetic makeup of RAS affects the status of RAS in individuals. The human ACE is located on the chromosome 17q23 and has several variants such as rs464994, rs8066114 and rs4461142. One of the most important ACE polymorphism is a 287 bp Alu determined by the Insertion/Deletion sequence polymorphism based on the presence of (insertion, I) or absence (deletion, D) of an intron 16; it is nonsense, repetitive DNA domain that leads to the generation of three genotypes: DD homozygote, II homozygote and ID heterozygote are found [9]. The polymorphism is responsible for the large proportion of variability of serum and tissue ACE activity, where insertion is associated with lower ACE activity, and deletion is associated with higher ACE activity. Previous investigations have demonstrated that this polymorphism accounts for 47% of the total phenotypic variance of serum ACE, supporting the theory that the ACE gene locus is the major determinant of serum ACE concentration [10].

So many studies have shown that a strong association between ACE I/D gene polymorphism is the risk of DN claiming high frequency of D allele in DN patients [11]. However, some studies have shown that negative association in gene polymorphism in the risk of DN [12]. So many controversial studies have been done in ACE I/D gene polymorphism in DN patients in different populations. Therefore, the aim of the present study was to find out pattern of distribution and frequency of ACE gene polymorphism in T2DM with and without nephropathy and healthy controls and also, we observed the relationship between ID, DD and II polymorphisms in diabetes with and without nephropathy patients in Al-Quwayiyah region of Saudi Arabia.

MATERIALS AND METHODS

Study area and subjects

A case-controlled study was conducted during the period of October 2015 to February 2017. The T2DM with and without nephropathy patients attending the outpatient department (OPD) of medicine and some patients were admitted to medicine and nephrology unit of the Al-Quwayiyah Government General Hospital, Saudi Arabia. This study was carried out in 3 groups of both the sexes: healthy controls (n=52, male 27 and female 25), T2DM without nephropathy (n=61, male 35 and female 26) and T2DM with nephropathy (n=61, male 38 and female 23). The criteria for the diagnosis of T2DM were based on the WHO report [13]. The T2DM with nephropathy was confirmed by the presence of persistent proteinuria, microalbuminuria (>30 mg/day) in T2DM patients in the absence of urinary tract disease and on the basis of clinical history, physical examination, renal biopsy and biochemical investigations. Blood pressure was measured as recommended by the American Diabetic Association. Those patients whose body mass index (BMI) was >30 kg/m^2 were considered as obese. Fifty-two healthy volunteers, both age and sex matched who were non-diabetic subjects without nephropathy were considered as controls. Subjects suffering from hepatic disease, autoimmune disease, strokes, cerebrovascular accidents, absence of heart failure, any chronic or acute inflammatory illness, pregnancy and lactating mothers, smokers, alcoholics, type 1 DM, and patients taking any kind of multivitamin, lipid lowering drugs and ACE-inhibitor therapy were excluded from the study.

Collection of samples and biochemical assays

A total of 4 ml of plain blood and 3 ml of EDTA blood were collected from each subject after an overnight fast. The

serum was carefully separated and transferred to micro tubes and stored at 4°C before analysis. The fasting blood sugar and creatinine levels were measured by using automated biochemistry analyzers [14]. Glycaemic control was assessed by measuring glycated haemoglobin by using the resin-ion exchange method [15]. For collecting urine samples at 24 hours, wide mouthed 3 litre sterile containers were provided to the patients and control subjects, and instructions were given regarding urine collection. The urine volume was measured, and 10 ml of sample was preserved for analysis.

DNA extraction and determination of ACE I/D polymorphism

The Genomic DNA was extracted from peripheral blood specimens, which were in tubes containing EDTA, and DNA was stored at −80°C until use (Bio-Rad DNA extraction kit from USA).

Polymerase chain reaction (PCR) amplification was carried using in a DNA thermocycler. First, PCR was performed using 20 pmoles of each primer: Sense oligo 5'- CTG GAG ACC ACT CCC ATC CTT TCT- 3' and antisense oligo: 5'- GAT GTG GCC ATC ACA TTG GTC AGA T- 3' in a final volume of 25 μL that containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris- Hcl (pH=8.3), 0.2 mM dNTP, 0.8 U Taq polymerase (Bangalore Genei, India) and 0.5 μg genomic DNA. PCR was done with an initial denaturing time at 94°C for 1 min. Then the DNA was amplified for 30 cycles with denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min. This was followed by final extension at 72°C for 8 min. PCR products were directly visualized using ethidium bromide staining after electrophoresis in a 2% agarose gel [16,17]. The amplification product is a 490 bp fragment in the presence of insertion (I) allele and a 190 bp fragment in the presence of the deletion (D) allele. Thus, there were three genotypes after electrophoresis: a 490 bp band (homozygote II), a 190 bp band (homozygote DD), or both 490 and 190 bp bands (heterozygote ID). The ID heterozygote may be missed as DD homozygote's due to the suppressed amplification of the I-fragment in relation to D fragment may occur. In order to correct this problem, a new PCR with specific primers for the I allele amplification was performed in all samples classified as DD genotype. The primers used in this new reaction were 5'- TGG GAC CAC AGC GCC CGC CAC TAC -3' (Sense) and 5'- TCG CCA GCC CTC CCA TGC CCA TAA - 3' (antisense) [18]. This second reaction was performed under the same condition as the first one, except for the annealing temperature of 67°C. The reaction yields a 335-bp amplicon only in the presence of an I allele, and no product for homozygous DD samples.

Ethical approval

The study was conducted with the approval of institutional ethical and human scientific research committee. Written informed consent was also taken from all the patients.

Statistical analysis

Statistical analysis was performed with the statistical package for social sciences (SPSS) for windows, version 21.0 (SPSS, Chicago, IL, USA). Measurement data were summarized by mean ± Standard deviation (SD) and analysed by student's t-test for the significant difference between the groups. Hardy-Weinberg equilibrium was tested for T2DM with and without nephropathy patients and controls. The allelic frequencies were calculated based on the number of different alleles observed and the total number of allele examined. The allele and genotype frequency differences between the two patient groups and control subjects were tested by chi-square analysis. Odds ratios (ORs) and 95% confidence intervals were calculated by multiple logistic regression analysis. Analysis of variance (ANOVA) was used to compare the genotype group differences for various parameters. $p < 0.05$ was considered statistically significant.

RESULTS

The clinical and biochemical characteristic of the two groups of the T2DM patients with and without DN and controls are presented in Table 1. In the present study, the number of patients with BMI, systolic and diastolic blood pressure was significantly higher in T2DM with nephropathy, when compared to T2DM without nephropathy and controls. The biochemical parameters including FBS, HbA1c, creatinine and urine microalbuminuria were increased in T2DM without nephropathy, and further increased in T2DM with nephropathy patients ($p < 0.001$).

Table 1 Baseline clinical characteristics of the controls and patient groups

Clinical Parameters	Controls (n=52)	T2DM without nephropathy (n=61)	T2DM with nephropathy (n=61)	*p-value
Age (yrs)	50.1 ± 19.2	52.3 ± 5.8	53.8 ± 6.3	0.035
Sex (male/female)	27/25	35/26	38/23	0.041
BMI (kg/m²)	25.1 ± 0.7	27.3 ± 1.2	28.2 ± 1.5	0.053
SBP (mmHg)	122.3 ± 3.2	134.8 ± 13.2	140.6 ± 15.8	0.025
DBP (mmHg)	80.9 ± 4.1	89.7 ± 7.3	93.2 ± 8.8	0.031
Duration of DM (yrs)	-	6.6 ± 2.1	9.2 ± 3.3	0.039
FBS (mg/dl)	98.0 ± 9.3	191.0 ± 20.1	224.3 ± 18.3	0.018
Creatinine (mg/dl)	0.7 ± 0.21	1.2 ± 0.32	3.01 ± 1.2	0.02
HbA1c (%)	4.5 ± 0.5	7.9 ± 1.8	8.6 ± 2.0	0.022
Microalbuminuria (mg/24 hrs)	8.6 ± 2.2	18.4 ± 4.0	126.5 ± 32.2	0.01

Data are represented as the mean ±SD, BMI=Body mass index, FBS=Fasting blood glucose and HbA1c=Glycated hemoglobin. Comparisons were made by using students t-test. *p<0.05, indicates statistically significant, between the patient groups and controls

Distribution of ACE genotypes with clinical and biochemical parameters

The ACE gene polymorphism was found to be associated with II, ID and DD genotypes, based on the anthropometric, clinical, and biochemical parameters of the two diseases (T2DM with and without nephropathy). We observed that DD genotypes in combination with II and ID was significantly associated with elevated FBS, creatinine and urine microalbuminuria (p<0.05) as shown in Table 2.

Table 2 Distribution of T2DM with and without nephropathy characteristic according to anthropometric and metabolic parameters in ACE genotypes

Clinical Parameters	II	ID	DD	*p-value
Age (yrs)	52.5 ± 4.2	53.1 ± 5.0	53.8 ± 5.3	0.72
Sex (male/female)	15/13	22/24	25/23	0.322
BMI (kg/m²)	26.2 ± 0.9	26.8 ± 3.4	27.1 ± 2.2	0.512
SBP (mmHg)	137.2 ± 15.8	140.1 ± 16.3	138.5 ± 17.0	0.483
DBP (mmHg)	88.2 ± 10.1	90.3 ± 8.2	91.1 ± 8.7	0.514
Duration of DM (yrs)	7.2 ± 3.21	8.2 ± 4.21	8.8 ± 5.0	0.221
FBS (mg/dl)	201.2 ± 24.1	215.8 ± 24.8	222.9 ± 31.2	0.033
Creatinine (mg/dl)	1.8 ± 1.0	2.5 ± 0.7	2.9 ± 0.1	0.056
HbA1c (%)	7.8 ± 2.0	8.2 ± 2.8	8.5 ± 3.1	0.141
Microalbuminuria (mg/24 hrs)	17.8 ± 10.2	72.2 ± 15.3	111.5 ± 21.2	0.043

Data are represented as the mean ± SD, comparisons were made using students t-test (for continuous variables). D/D=deletion/deletion, I/D=insertion/deletion, I/I=insertion/insertion. *p<0.05 indicates statistically significant difference, between DD and ID/II genotypes.

Molecular analysis

The distribution of ACE I/D polymorphism in the patients of T2DM with and without nephropathy and controls are shown in Table 3. The frequencies of ACE II, ID and DD genotypes in T2DM with nephropathy were 8.2%, 34.4% and 57.4%, in T2DM without nephropathy 16.4%, 44.2% and 39.4%, in control 30.8%, 48.1%, 21.1% respectively. A comparative analysis revealed that there was a significant increase of 36.3% in the frequency of DD genotype in T2DM with nephropathy when compared to controls. Whereas in II genotype percentage was significantly reduced in patients (30.8% in controls vs 8.2% in patients). The frequencies of D allele and I alleles among the two patient groups and controls are also provided in Table 3. The frequency of D allele in the T2DM with nephropathy was 62.3%, and without nephropathy was 44.2% compared to 32.7% in controls. The frequency of D allele in both the patient groups which was statistically significant.

Table 3 Dissemination of ACE gene I/D polymorphism and allele frequencies in T2DM without nephropathy, T2DM with nephropathy and control subjects

Frequency	Controls (n=52) (%)	T2DM without nephropathy (n=61) (%)	T2DM with nephropathy (n=61) (%)	DM vs Controls	DN vs controls	DM vs DN
Genotype						
II	16 (30.8)	10 (16.4)	05 (8.2)	p=0.080	p=0.005	p=0.181
ID	25 (48.1)	27 (44.2)	21 (34.4)	p=0.701	p=0.151	p=0.321
DD	11 (21.1)	24 (39.4)	35 (57.4)	p=0.040	p=0.002	p=0.047
Allele						
I	70 (67.3)	68 (55.7)	46 (37.7)	p=0.077	p=0.002	p=0.005
D	34 (32.7)	54 (44.3)	76 (62.3)	p=0.076	p=0.001	p=0.004

DM=type 2 diabetes mellitus, DN =Diabetic nephropathy. D/D=deletion/deletion, I/D=insertion/deletion, I/I=insertion/insertion. D=deletion, I=insertion. The X2 test was used to distribution and comparison of genotypes and alleles frequency of ACE gene polymorphism. p<0.05 indicates statistically significant difference

We also observed the positive correlation between the DD genotypes of ACE gene polymorphism in T2DM with nephropathy and T2DM without nephropathy OR=2.075 (95% CI: 1.008-4.272, p<0.047). Meanwhile, relationship between the DD genotype in the development of T2DM and controls (95% CI: 1.043-5.604, OR=2.417, p<0.040) respectively, further increase of odds ratio 5.017 was observed in DD genotypes of T2DM with nephropathy compared to controls. The frequencies of D allele in the T2DM with nephropathy group which was statistically significance when compared to controls (OR=3.401, 95% CI: 1.963-5.892, p<0.001). Whereas I allele OR=0.294 (95% CI: 0.169-0.509) respectively as shown in Table 4. These above results indicate that T2DM patients with D allele and DD genotype have increased risk of developing diabetic nephropathy.

Table 4 Odds ratio and 95% CI in the genotype and Allele distribution of ACE gene I/D polymorphism between the groups

ACE gene polymorphism	DM vs Controls Odds ratio (OR) (95% CI)	DN vs Controls Odds ratio (OR) (95% CI)	DM vs DN Odds ratio (OR) (95% CI)
Genotype			
II	0.441	0.2	0.455
	(0.179-1.082)	(0.067-0.596)	(0.145-1.421)
ID	0.857	0.567	0.661
	(0.408-1.802)	(0.265-1.210)	(0.318-1.373)
DD	2.417	5.017	2.075
	(1.043-5.604)	(2.172-11.586)	(1.008-4.272)
Allele			
I	0.611	0.294	0.48
	(0.355-1.053)	(0.169-0.509)	(0.288-0.801)
D	1.634	3.401	2.08
	(0.949-2.815)	(1.963-5.892)	(1.247-3.470)

DM=type 2 diabetes mellitus, DN =Diabetic nephropathy, ACE=angiotensin converting enzyme; CI: confidence interval, D/D=deletion/deletion, I/D=insertion/deletion, I/I=insertion/insertion. D=deletion, I=insertion

DISCUSSION

DN is one of the common complications of T2DM, has leading cause of ESRD in many countries. It is believed that the physiological mechanisms of renal disorder are similar in both types of diabetes. The pathogenesis of clinical course of DN can be monitored by structural and hemodynamic changes. The earliest change is an increase in glomerular filtration rate, also called "hyperfiltration" stage, which is followed by detectable glomerular lesions with normal albumin excretion rate. The next stage is the development of microalbuminuria. Diabetic subjects with persistent microalbuminuria are at increased risk for "over DN" [19].

Why some diabetics develop nephropathy, whereas others do not, despite having long term hyperglycaemia remains an unresolved question. So, some researchers have sought the answer in the genetic background of the host [20]. Within the next decade, the genes that increase risk of developing all forms of diabetes will likely be known. It is

therefore, important that scientists, health professionals and members of population at large consider how to maximize the advantages, and minimizes the disadvantages of predictive genetic testing for diabetes.

The familial clustering of patients with DN and beneficial effects of ACE inhibition has favoured most researchers to investigate genetics of renin-angiotensin system (RAS). It is known that ACE gene is one of the important genes of RAS and plays a major role in the T2DM patients with and without nephropathy [21]. The molecular mechanism of the association between ACE I/D polymorphism and the risk of T2DM with and without nephropathy are relatively unclear. A commonly occurring variant of ACE has I/D polymorphism with a 287 bp Alu repetitive sequence in intron 16 [22]. The first study on ACE I/D gene polymorphism in DN was that Marre, et al. [23] who proved protective role of II genotype against the development of DN. Subsequently a sizable number of studies have investigated the possible role of DD genotype is strongly associated with increased plasma or serum ACE levels, thus predisposing individuals to T2DM and its complications especially DN [24].

In the present study, we observed the diabetic D- allele carriers tended to have a significantly higher fasting blood glucose levels when compared to II genotype subjects. Similar results were seen in Lebanon population [10]. In the urine microalbuminuria, more distribution of DD genotypes was seen when compares to II and ID genotypes of T2DM with and without nephropathy. Interestingly, Solini, et al. also observed that microalbuminuria of T2DM patients with the DD genotype present more severe glomerular lesions than patients with the I allele [25].

ACE I/D gene polymorphism in the pathophysiology of DN, and the most of them have reported association of DD genotype allele has a risk factor. However, there is a lack of consistency among the reported studies and there are so many controversial studies are there regarding, is it ACE I/D gene polymorphism is a risk factor in T2DM with and nephropathy. Data from Turkish study have shown that T2DM patients without nephropathy carried the DD-genotype 1.7 times more frequent than the control subjects [26]. Similar findings were observed by Iranian populations that the frequency of the DD genotype as well as the D-allele was significantly increased in diabetic patient's comparison to control groups [27]. A cross sectional study from Japan population reported that more individuals with DD and ID genotypes were diabetics than compare to II, and that the D-allele is a risk factor for DM and impaired glucose tolerance (IGT) [28].

The observation of our study found that there is a significant increase of DD genotype and D-allele frequencies in T2DM without nephropathy when compared to controls. We also investigate the relationship between the DD genotype in the development of T2DM and controls (95% CI: 1.043-5.604, OR=2.417, p<0.040) in Saudi populations. In contrast some studies reporting that no significance association between the DD genotype nor the alleles were consistently associated with the presence of T2DM in the Lebanese and South Asians populations [10,29]. In a population based cross- sectional study in south London, no significance between I/D polymorphism and impaired glucose metabolism in three different ethnic groups was observed [30].

As inflammation and angiogenesis play a crucial role in the patho-mechanism of T2DM with nephropathy, many studies have investigated the association of genetic polymorphism in these pathways genes with the risk of DN. Although the data from Caucasian studies failed to confirm an increased risk for the development of DN in IDDM and NIDDM being associated with D-allele because cluster in families at risk of T2DM, whereas the odds ratio for a family history was approximately 50% in DD subjects than in II subjects [31]. Haque, et al. [32] found that patients with DD allele of the ACE gene are more likely to have progressive DN with microvascular and macrovascular complications. On the other hand, Grzeszczak, et al. from Poland, Schmidt, et al. from Germany and Liao, et al. from China, did not find any association between ACE allele and genotype frequency in T2DM patients with and without nephropathy [33-35]. In Tunisian population the ACE I/D gene polymorphism are not associated with DN in Type 1 or Type 2 diabetes [36]. Moleda, et al. [37] reported that ACE genotypes were not associated with presence of microvascular complications in T2DM. Meanwhile Yashoda, et al. from Japan [38], Nikzamir, et al. from Iran [39] and Jeffers, et al. from USA [6] found a strong association between ACE-DD genotype and/or allele and the risk for nephropathy in T2DM.

In the present study, we observed the significant higher frequency of DD genotype and D-allele in T2DM with nephropathy compared to T2DM without nephropathy patients. Our study also shows a positive association between the DD genotypes of ACE gene polymorphism in T2DM with nephropathy and T2DM without nephropathy. Relative risk of DD homozygous subjects was OR=2.075 (95% CI: 1.008-4.272, p<0.047). Whereas it is interesting to note that

the odds of developing diabetic nephropathy were increased close to 5.01-fold in the DD polymorphism compared to healthy controls in Saudi population. Our results were consistent with the recent findings of South Indian and Chinese studies [7,40]. Khan, et al. [11] also reported that indicate that T2DM with D-allele have more than two-fold risk of developing nephropathy. El-Bazz, et al. [41] suggested that DD genotype of ACE gene may be associated with development of DN among Egyptian patients. Whereas Ajay kumar, et al. and Prasad et al. found no relationship between ACE gene polymorphism and development of DN in T2DM in North Indian populations [42,43].

The possible mechanism by which ACE DD genotype affects DN explained by the presence of D allele is associated with higher circulating levels of ACE, which is thought to increase the activity of the RAS system. In diabetic individuals who have chronic hyperglycaemia leading to endothelial dysfunction, increased stimulation of RAS system may more likely increase intraglomerular pressure, protein leakage, subsequent destructions of nephrons and increased protein expression of ACE is responsible for high level of angiotensin II. Angiotensin II increases the podocyte injury and loss of podocytes is a hallmark of progressive kidney diseases including DN which is finally leading to ESRD [44].

Some recent studies are observed that beneficial effects of ACE inhibitor therapy for the DN patients. Yuying Wang, et al. [40] revealed that there was a better response to the treatment with valsartan in DN patients with the DD genotype. Similarly, the Losartan therapy had greater influence on the ACE activity and decreased urinary albumin excretion rate in the D- allele patients of DN [45].

There was some limitation in the present study, our sample size was small and it was a hospital based study. So, we can't represent over the entire population. We believe that it is necessary to conduct larger studies on this genetic polymorphism, haplotypes, and other genes that might be related to ACE gene function, post transcriptional and translational interactions, and even ambient factors; for a correct classification of development risk and complications related to T2DM.

CONCLUSION

This will be first report to measure ACE I/D polymorphism in T2DM with and without nephropathy among the Saudi population in Al-Quwayiyah region of Saudi Arabia: there are taking more junk food, animal saturated fat and high cholesterol food all these affect for so many diseases. However, they are not very well aware of their diabetes, kidney disease and cardiovascular disease. The study illustrated that significant association was observed between the D allele, DD genotype of the ACE polymorphism and the risk of T2DM with nephropathy in Saudi population. We suggest potential and new multi- property of ACE inhibitors therapy for the prevention or delay of progression of nephropathy. Identification of different genes involved in the primary mechanisms contributing to onset and progression of diabetic nephropathy is important for developing new therapeutic interventions for the control of this disease, which is considered a worldwide epidemic in this decade.

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