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THE IMPACT OF ANGIOTENSIN CONVERTING ENZYME INSERTION/DELETION GENE POLYMORPHISM ON DIABETIC KIDNEY DISEASE RISK AMONG EGYPTIAN CHILDREN WITH TYPE 1 DIABETES

Zahraa Abdelmoneum[1], Ayman Hammad[1], Nanees Abdel-Badie Salem[1], Mona El Wassefy [2], Mohammad Al Haggar[1]

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Abstract

Background : Renin-angiotensin-aldosterone system has been implicated in etiopathogenesis and progression of diabetic kidney disease (DKD). Strong evidence exists for the genetic predisposition in the development of DKD. The role of angiotensin converting enzyme (ACE) gene polymorphism in the susceptibility to nephropathy in type 1 diabetes (T1D) still debatable.

Aim: We aimed to explore the potential association between ACE gene polymorphism and the risk of DKD in a cohort of Egyptian children with T1D.

Research Design and Methods : Cross-sectional study included 102 children with T1D (54 males; aged 12-18 years) who were divided into normoalbuminuric-group (n=62) and albuminuric-group (n=40). ACE insertion/deletion (I/D) gene polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique of the extracted genomic DNA from participants. Odds ratios (ORs) and 95% confidence intervals (CIs) were applied to explore the association between ACE gene polymorphism and the risk of DKD.

Results: 'D' allele was significantly higher in albuminuric-group (risk-allele) (OR=7.4; CI=3.7-15; p<0.001), while 'I' allele was significantly higher in normoalbuminuicgroup (protective-allele). Binary logistic regression revealed that diabetic child with 'D' allele has 7.4-times higher odds to exhibit nephropathy compared to those with 'I' allele. D/D genotype was significantly higher in albuminuric-group (OR= 72.8; CI=11-482; p<0.001). Binary logistic regression revealed that diabetic children with D/D and I/D genotypes have 72.8-times and 4.7-times higher odds to exhibit nephropathy compared to those with I/I genotype, respectively.

Conclusion: ACE D allele/DD genotype could be linked to increased risk for DKD, while ACE I allele/II genotype might be a protective factor for DKD among Egyptian children with T1D.

KEYWORDS: Diabetic nephropathy, Angiotensin converting enzyme gene, Polymorphism, Type 1 diabetes.

[1] Department of Pediatrics, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[2]Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

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Introduction

Diabetic kidney disease (DKD) is a common drastic microvascular complication in patients with diabetes and is considered a major contributor to morbidity and premature mortality in young people with childhood-onset T1DM [1, 2]. DKD can progress eventually to end-stage renal disease (ESRD) many years later and may require dialysis or kidney transplantation [3].

In clinical practice, elevated albuminuria (formerly microalbuminuria) has classically been considered the earliest clinical manifestation of DKD, it corresponds to diabetic nephropathy stage-3. Albuminuria of values ≥30 mg/g (3 mg/mmol) is now recommended for the diagnosis of DKD [4].

In addition, regular monitoring of estimated glomerular filtration rate (eGFR) is important for evaluation of kidney function in children with T1D to detect both declining kidney function (eGFR <90ml/min/1.73 m^2) and also hyperfiltration, a potentially important risk factor early in the disease course [5].

The recent International Society of Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Guidelines (2022) recommended that screening for albuminuria and screening of eGFR in T1D should start at puberty or from age 11 years whichever is earlier, with 2-5 years diabetes duration, and repeated annually thereafter [5].

Poor glycemic control, longer duration of diabetes, older age, puberty, and smoking are well known risk factors for diabetes-related vascular complications [6, 7]. Moreover, a clustering of cardiometabolic risk factors including overweight/obesity, hypertension and dyslipidemia in children and adolescents with T1D was associated with high rates of multiple vascular complications [8].

Unfortunately, the detailed pathogenesis of DKD is still not fully understood, but available data suggests major pathogenic mechanisms that contribute to the development and progression of DKD such as hemodynamic alterations, metabolic abnormalities, inflammation, oxidative stress and various growth factors [9, 10].

Moreover, strong evidence exists for the role of genetic predisposition in the onset and progression of DKD [11, 12]. based on differential disease risk in DKD with familial clustering of DKD, as well as differences in the prevalence of DKD between ethnic groups [13, 14]. In addition, not all patients with poor glycemic control eventually develop DKD; on the contrary, some DM patients with well-controlled blood glucose can still develop this disease [15].

In experimental and human DKD, systemic and glomerular hypertension play a role in the initiation and progression of nephropathy. These hemodynamic changes may be explained in part by alterations in renin-angiotensin-aldosterone system (RAAS) [16].

Angiotensin-I converting enzyme (ACE), an endothelial ectoenzyme secreted in plasma, possesses a crucial role in RAAS cascade by activating Angiotensin-I into the Angiotensin-II (Ang-II) which plays a key role in regulation of blood pressure as well as electrolytes and fluid balance [17]. Ang-II shows increased activity under high glucose conditions. It has a strong pressor effect on arteriolar smooth muscles, thereby resulting in increased systemic and glomerular blood pressure [18]. It also induces inflammation, apoptosis, migration and differentiation, in addition, it stimulates mesangial cell proliferation and hypertrophy of various renal cells [19].

The ACE gene is located on 17q23 and composed of 26 exons and 25 introns, with a length of 21 kb. Although intraindividual plasma ACE activity is very stable, its interindividual variability in plasma or cellular levels is quite high and under the influence of ACE gene polymorphism [20].

There are >160 ACE gene polymorphisms that are currently known;

most of them are single nucleotide polymorphisms (SNPs). Rigat et al first reported that the ACE gene insertion/deletion (I/D) polymorphism is caused by the insertion or deletion of a 287 bp Alu repeat sequence within intron 16, which results in three genotypes (DD, II, ID) [(21)].

The I/D polymorphism determine most of ACE individual variance, whereas the level of ACE in plasma is in direct correlation with the genotype, and plays a critical role in determining intrarenal angiotensin concentrations. Subjects who are homozygote for the deletion (DD) display the highest values and those who are homozygote for the insertion (II) display the lowest, with heterozygotes displaying intermediate values [(22)].

The impact of ACE I/D polymorphism on DKD still a debatable issue [(23)]. The pooled results were controversial and inconsistent [(24-26)].

In this study, we further explore the potential impact of ACE I/D gene polymorphism on DKD risk in a cohort of Egyptian children with T1D.

Subjects and methods

This is a cross sectional study done among 102 Egyptian children diagnosed with T1DM enrolled from the Endocrinology outpatient clinic, Mansoura University Children's Hospital. The study was carried out after taking a fully informed consent from the parents of each child and approval of our institutional research board (IRB) (Code No. " MD.19.09.232"), faculty of medicine, University of Mansoura, Egypt.

Children with T1DM were annually screened for DN according to ISPAD Guidelines, the screening start for children of 11 years of age, or with onset of puberty if earlier, with diabetes duration of 2–5 years. Of the 102 Egyptian children diagnosed with T1DM we found 40 children with DN and 62 without DN according to urinary albumin/creatinine ratio (ACR) in early morning urine sample, microalbuminuria is considered if UACR is more than 30 in 2 of 3 urine samples over 3- 6 months.

Diabetic children with duration less than 5 years, type 2 diabetic patients, other causes of albuminuria as UTI, fever, heavy exercise or menstruation, other associated kidney disease either congenital or acquired by US or urine analysis, history of nephrotoxic drugs administration for 4 weeks or diabetic Ketoacidosis (DKA) during 4 weeks before enrollment in the study were excluded from our study.

Sample Size calculation:

The calculated sample size of the study based on pilot study of 20 cases and controls was 36 participants for each group at 5% level of significance and 95 % power of the study, using G*Power 3 sample size calculator [(27)].

Proportion of DD ACE gene polymorphism among DKD group was (45%). Proportion of DD ACE gene polymorphism among non DKD group was (10%)

The sample size was increased to 40 participants in T1DM with nephropathy and 62 in T1DM without nephropathy to compensate for incomplete data and to increase the study power.

Clinical evaluation

All included children were submitted to full medical history and physical examination. The following data were collected: gender, age, and the duration of diabetes and average value of glycated hemoglobin (HbA1c) from patients' records in the preceding year. Height and weight were measured by standard techniques, and

BMI was calculated as weight (kg)/height (m2). BMI Z-scores were calculated for gender and age using the Egyptian growth charts [(28)]. Systolic blood pressure (SBP) and diastolic BP (DBP) were evaluated by standard technique by conventional mercury manometer and plotted on standard blood pressure curves.

Biochemical evaluation

The average of HbA1c (%) records over the last year, serum creatinine, lipid profile (cholesterol, TG, HDL, LDL, non

HDL) and UACR were extracted from patient's medical files. HbA1c values were represented as a percentage (%).Serum creatinine used in calculation of estimated glomerular filtration rate (eGFR, ml/min/1.73 m2) was calculated by the updated Schwartz formula eGFR = 0.413*height (cm)/serum creatinine (mg/dl). Urinary albumin to creatinine ratio (UACR) (mg/g) was determined on fresh first morning urine sample after exclusion of other causes of albuminuria as mentioned above.

ACE gene polymorphisms analysis

Sample collection and DNA extraction 2ml of peripheral blood samples were collected in EDTA containing tubes. Total genomic DNA was isolated and purified from peripheral blood lymphocytes following the manufacturer's instructions by using DNA Extraction kit (Gene JET Whole Blood Genomic DNA Purification Mini Kit). The quantity and quality of DNA samples were measured by NanoPhotometer. Purified DNA samples were used for genotyping of the rs1799752 (I/D) polymorphism in the ACE gene.

PCR amplifications and genetic typing assay

ACE (I/D) polymorphism was genotyped by polymerase chain reaction restriction fragment length polymorphism technique (PCR- RFLP) by using the primer ACE (I/D) primers: 5'CTGGAGACCACTCCCATCCTTTCT-3' (forward primer) and 5'-GATGTGGCCATCACATTCGTCAGAT-3' (reverse primer), ACE I/D polymorphism region was amplified using 0.5 ul genomic DNA as template. PCR was performed in a total volume of 25µl reaction mixture containing 0.05 µl of forward primer, 0.05 µl of reverse primer, 10 ul of 2x Taq PCR blue master mix and 15 µl of nuclease free water. PCR was performed according to the following conditions: initial denaturation step at 95 °C for 5min, 35 cycles of denaturation at 94 °C for 30sec, annealing at 58 °C for 30sec, extension at 72 °C for 30sec and a final step of 10min at

72 °C was carried out on SimpliAmpà™ Thermal Cycler (applied biosystemsby Thermo Fisher Scientific). The amplified PCR products were digested with 10 U/µl of FastDigest HpyF31restriction enzymes (Thermo Fisher Scientific) at 65 °C for 3h.

The restricted products were analyzed by agarose gel electrophoresis (Biometra, an analytik Jena Company). Expected fragment lengths ACE (II) genotype: 490bp fragment, ACE (DD) genotype: 180bp fragment, ACE (ID) genotype: 180bp and 490bp fragments (figure 1). All experiments were performed in immunology lab in clinical pathology department, Faculty of medicine, University of Mansoura.

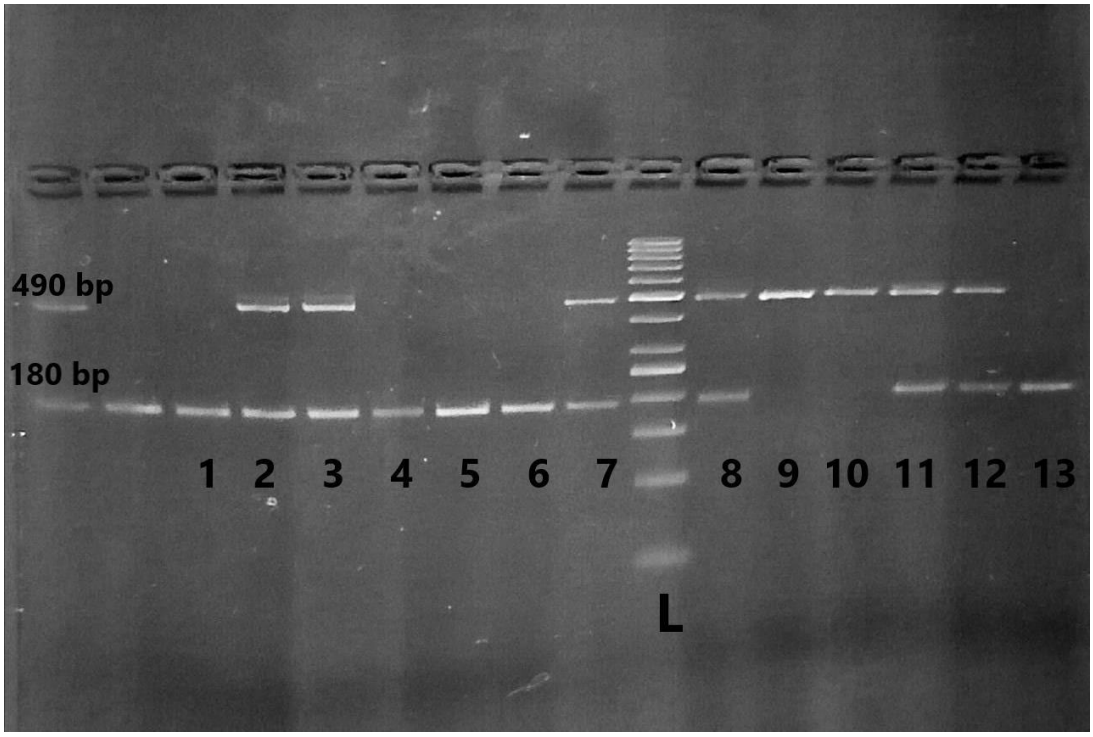

FIGURE1 Agarose gel electrophoresis of ACE gene I/D polymorphism products. Lanes 1, 4, 5, 6, 13: DD genotype; Lanes 9, 10: II genotype, Lanes 2, 3, 7, 8, 11 and 12: ID genotype; Lane: DNA ladder (100 bp).

Statistical analysis

Data were entered and analyzed using IBM-SPSS software (IBM Corp.
Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).

Qualitative data were expressed as N (%).Quantitative data were expressed as median and interquartile range (Q1 or 25th percentile – Q3 or 75th percentile).

Comparison of qualitative data between groups was done using Chi-square test and comparison of quantitative data between two groups was done using MannWhitney U-test and between three groups was done using Kruskal-Wallis H-test.

Binary Logistic regression analysis was used for prediction of the association of ACE gene polymorphism and the risk of having DN.

SNP statistical analysis was performed using online SNP Stats software (http://www.snpstats.net/start.htm)
analysis. For SNP it was used to evaluate allele frequencies, genotype frequencies and Hardy–Weinberg equilibrium (HWE).

For any of the used tests, results were considered as statistically significant if p value ≤ 0.050.

Results

This is a cross-sectional study among T1DM children. They were divided into two groups based on UACR results; T1DM with nephropathy (n = 40), and T1DM without nephropathy (n = 62).The age of children in this study ranged from 12 to 18 years with mean value of 15years. The children were 54 males (52.9%) and 48 females (47.1%). Two groups were age and sex matched.

All participants had normal SBP/DBP and eGFR-Cr values were within normal ranges for gender and age based on the guidelines by the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) for children and adolescents [(29)]. Also it was also observed that none of the diabetic nephropathy children had retinopathy. Comparison between the T1DM with DN and T1DM without DN groups showed that

BMI was statistically significant higher in the DN group p value 0.023, SBP and DBP were statistically significant higher in the DN group p value <0.001. Also lipid profile (Cholesterol, TG, HDL, LDL, NON- HDL) was statistically significant higher in DN group p value 0.028, <0.001 respectively. UACR was statistically significant higher in DN group p value <0.001. HbA1C was statistically significant higher in DN group p value 0.011. While there was no statistically significant difference between both groups as regard duration of DM and eGFR as shown in table (1).

Table (1): Clinical and biochemical characteristics of the study groups:

Variable	T1DM with DN (n = 40)	T1DM without DN (n = 62)	P value
Age (years)	16 (14-17)	15 (14-16)	0.113
Sex			0.823
Male	22(55%)	32(52%)	
Female	18(45%)	30(48%)	
Duration of DM(years)	6 (6-8)	6.5 (6-11.75)	0.272
BMI Z score	1 (0.9 -1.2)	1 (0.8-1)	0.039
SBP (mmHg)	120 (110-130)	110 (100-110)	<0.001
DBP (mmHg)	80 (70-90)	70 (60-70)	<0.001
eGFR ml/min/m2	124.9±38.9	126.9±39	0.866
Cholesterol (mg/dl)	161.5 (140.25-190)	131 (91-182)	0.028
TG (mg/dl)	143.5 (101.25-185)	83 (68.25-100.75)	<0.001
HDL (mg/dl)	44.5 (42.25-45)	45 (45-50)	<0.001
LDL (mg/dl)	121 (110.25132.75)	98 (80-115)	<0.001
NON- HDL (mg/dl)	127.5 (120-142.25)	92.5 (87-114.25)	<0.001
HbA1C %	9.5±1.7	8.5±1.9	0.011
UACR mg/g	52.5 (43.25-69)	11 (9-20.75)	<0.001

IQR=interquartile range, n=number, DM=diabtesmilletus, SBP= systolic blood pressure, DBP= diastolic blood pressure, TG= triglycerides, HDL=high density lipoprotein, LDL= low density lipoprotein, eGFR= estimated glomerular filtration rate, UACR= urinary albumin creatinine ratio. Data are presented as median and IQR, frequency (%) or mean ± SD.

Genotypes distribution in both groups follow Hardy-Weinberg equilibrium (HWE) as shown in table (2).

Table (2): Hardy-Weinberg equilibrium (HWE)

Group	DD		ID		II		X^2	p-value
	Observed	Expected	Observed	Expected	Observed	Expected		
All	25	21.5	44	50.9	33	29.5	2.42	0.10
T1DM without DN	2	3.3	30	27.4	30	31.3	1.02	0.28
T1DM with DN	23	22.5	14	15	3	2.5	0.178	0.45

Allele distributions of ACE gene I/D polymorphism and risk of DN:

Table (3) shows a statistically significant association between allele frequency and nephropathy in diabetic children. This association is of medium strength (φ= 0.463). 'D' allele was statistically significantly higher in the T1DM with DN group (risk allele) while 'I' allele was statistically significantly higher in the T1DM without DN group (protective allele). Binary logistic regression revealed that diabetic child with 'D' allele has 7.4 times higher odds to exhibit nephropathy compared to those with 'I' allele.

Table (3): ACE gene Allele frequencies

Allele	T1DM with DN N=40	T1DM without DN N=62	χ^2	p-value	φ	OR (95% CI)
'D' allele	77 (75.5%)	29 (28.5%)	34.273	**<0.001**	0.463	7.4 (3.7 – 15)
'I' allele	25 (24.5%)	73 (71.5%)				r(1)

Notes: Data is N (%). Test of significance is chi-square (χ^2) test. Phi (φ) is a measure of the strength of association. OR = odds ratio (by binary logistic regression). CI = confidence interval. r(1) = reference category.

Genotypes distributions of ACE gene I/D polymorphism and risk of DN:

Table (4) shows a statistically significant association between genotype frequency and nephropathy in diabetic children. This association is of large strength (Cramer's V = 0.613). D/D genotype was statistically significantly higher in the T1DM with DN group while I/I genotype was statistically significantly higher in T1DM without DN group. Binary logistic regression revealed that diabetic children with I/D and D/D genotypes have 4.7, and 72.8-times higher odds to exhibit nephropathy compared to those with I/I genotype, respectively.

Table (4): ACE gene genotypes frequencies

Genotype	T1DM with DN N=40	T1DM without DN N=62	χ^2	pvalue	Cramer's V	OR(95% CI)
I/I	3 (7.5%)	30 (48%)	30.034	**<0.001**	0.613	r(1)
I/D	14 (35%)	30 (48%)				4.7 (1.2-18.9)
D/D	23 (57.5%)	2 (4%)				72.8 (11-482)

Notes: Data is N (%). Test of significance is chi-square (χ^2) test. Cramer's V is a measure of the strength of association. OR = odds ratio (by binary logistic regression). CI = confidence interval. r(1) = reference category. Multiple z-tests are presented as letters (similar letters = insignificant difference, different letters = significant difference).

As regard ACE gene genotypes the diabetic children were classified into three subgroups DD (25 children), ID (44children) and II (33 children) genotypes. There was a statistically significant difference between them as regard SBP, DBP, lipid profile (Cholesterol, TG, HDL, LDL, NON- HDL). UACR was statistically significant higher in both DD, ID than II while no statistically significant difference was found between DD and ID. This shows that II genotype may be a protective factor against nephropathy. While no statistically significant difference between these subgroups was found as regard BMI, eGFR and HbA1C as shown in table (5).

Table (5): Comparison between the ACE gene polymorphism subgroups as:

Variables	DD(n = 25)		ID (n=44)		II (n=33)		P value
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	
BMI Z score	1 (1-1.3) [b]		1 (0.9-1.2) [b]		0.9 (0.8-1)		0.005[c]
SBP (mmHg)	120 (100-130) [ab]		110 (105-120)		100 (100-120)		0.024[c]
DBP (mmHg)	80 (65-90) [b]		70 (65-80)		65 (60-80)		0.029[c]
eGFR ml/min/m2	118.4±36.7		132.6±44.4		124.6±31.4		0.506
Cholesterol (mg/dl)	167 (150-210) [b]		162 (142-189) [b]		92 (77.5-111)		<0.001
TG (mg/dl)	142 (102-186) [ab]		96 (67.5-138.5)		83.5 (70-107.5)		0.008[c]
HDL (mg/dl)	45 (42.5-45) [b]		45 (44-46.5) [b]		45.5 (45-50)		0.013[c]
LDL(mg/dl)	123 (115-136) [ab]		110 (89-120)		100 (86-116.25)		<0.001[c]
NON-HDL(mg/dl)	130 (120-143) [ab]		115 (88-127.5) [b]		92.5 (88.75-114)		<0.001[c]
HbA1C %	9.4±1.9		9±1.6		8.6±1.9		0.308
UAC	50 (40-60) [b]		23 (10-68) [b]		12.5 (9.75-22)		<0.001[c]

[a] Statistically significant compared to ID group.
[b] Statistically significant compared to II group.
[c] Statistical significant difference.

IQR= interquartile range, n==number, BMI=body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, TG= triglyceride, HDL=high density lipoprotein, LDL= low density lipoprotein, eGFR= estimated glomerular filtration rate, UACR= urinary albumin creatinine ratio. Data are presented as median and IQR or mean ± SD.

Discussion

Diabetes mellitus (DM) is a worldwide disease commonly increasing among all ethnic groups. Diabetic nephropathy is considered the most important cause of end-stage renal disease (ESRD) in diabetic patients [(30)].

Knowing the risk factors and the pathogenesis of DKD, effective screening and treatment protocols enabling us to prevent DKD progression**.**

There are several pathogenic factors of DKD include hyperglycemia, hypertension, obesity, endothelial dysfunction, mitochondrial injury and genetic factors. Genetic element still the most vague and unexplained factor in DN pathogenesis. Also genetic heterogeneity and overlaps between genes make the susceptibility greatly differs between patients with type 1 DM to develop nephropathy. Up to now, the I/D polymorphism of ACE gene is the most marker believed to be a risk factor for developing DKD and progression to ESRD [(31)].

Our study showed that 'D' allele frequency was statistically significant higher in DN children compared to the control group, OR = 7.4, 95% CI: 3.7 – 15, P<0.001, consequently D/D genotype frequency was statistically significant higher in DKD children compared to the control group, OR= 72.8, 95% CI: 11-482, P<0.001. This finding was consistent with 41 studies done among Asian diabetic adult patients (both type 1 and 2 DM) that showed a significant correlation between ACE D allele/DD genotype frequencies and DKD risk (D allele vs I allele: OR = 1.513), (DD genotype vs ID + II genotype: OR = 1.819, II genotype vs DD + ID genotype: OR = 0.678) [(23)].

In another Chinese work done among diabetic adult patients (both type 1 and 2 DM) 27 studies showed that there was a significant correlation between the ACE D allele/DD genotype and DN risk (D allele

vs I allele: OR = 1.552, DD genotype vs ID + II genotype: OR = 1.929, II genotype vs DD + ID genotype: OR = 0.650) [(23)].

Moreover in agreement with our work 50 studies done among adult patients with type 2 diabetes exploring the correlation between ACE I/D gene polymorphism and DN susceptibility showed that ACE D gene allele/DD genotype might increase the risk of nephropathy in type 2 diabetic subjects (D allele vs I allele: OR = 1.361, DD genotype vs ID + II genotype: OR = 1.503, II genotype vs DD + ID genotype: OR = 0.738) [(23)].

In contrary to our study 20 trials done among Caucasian diabetic adult patients (both type 1 and 2 DM) evaluating the impact of ACE I/D gene polymorphism on DN susceptibility showed that there was no significant correlation between ACE I/D gene polymorphism and DN (D allele vs I allele: OR = 1.058, DD genotype vs ID + II genotype: OR = 1.023, II genotype vs DD + ID genotype: OR = 0.858, ID genotype vs DD + II genotype: OR = 1.075) [(23)].

Also opposite to our work, studies done in type 1 diabetic patients demonstrated that there was no significant correlation between ACE I/D gene polymorphism and DKD (D allele vs I allele: OR = 1.139, DD genotype vs ID + II genotype: OR = 1.103, II genotype vs DD + ID genotype: OR = 0.803, ID genotype vs DD + II genotype: OR = 1.048) [(23)].

Another study done among 140 children with type 1 DM showing that ACE gene I/D polymorphism did not affect the risk of nephropathy [(32)]. The explanation of these results is that diabetic children with D allele or D/D genotype may develop DN in the future.

Renin angiotensin aldosterone system activation has an important role in the pathogenesis of DKD as it is the main regulator of renal arterial blood pressure by angiotensin II. However, conversion of low activity angiotensin I to high activity angiotensin II is depending on ACE. In spite of that ACE I/D gene polymorphism occurs in the noncoding gene region, the base insertion or deletion itself can alter the splicing process of the ACE precursor mRNA, then affects the stability of ACE mRNA, and ultimately affect the expression or stabilization of ACE. In situ hybridization for ACE mRNA on renal biopsy studies have found that the expression of ACE mRNA was elevated in those individuals with the ACE DD genotype [(33)].

ACE I/D polymorphism is considered as a functional polymorphism as ACE
D allele carriers had a higher ACE level and activity both in serum and kidney tissue **(22)**.

The impact of ACE I/D gene polymorphism on DN susceptibility varies in different types of diabetes and races. Causes of different results in different races can be explained by firstly, different life style, environmental exposure, and different socioeconomic status. Secondly, different genetic backgrounds in different racial subjects can affect the genetic phenotypes [(25)].

Analyzing the level of blood pressure among children with TIDM with DN group and without DN group revealed that DN children group had higher BP figure compared to the other group, median BP120/80, 110/70 respectively with p value <0.001.

In agreement with our study, study was carried out in 109 South Indian type 2 diabetic adult patients showed that DN patients had significantly higher systolic and diastolic blood pressure values wit p value <0.001 [(34)].

Although hypertension co-exists with micro albuminuria or overt nephropathy in diabetic patients, it can be seen even before renal disease [(35)]. Hypertension in diabetics can be caused by increased inflammation and vascular dysfunction associated with the imbalance between angiotensin II-angiotensin I. So hypertension is one of the most important factors that influence the onset of DN in T1DM or T2DM patients. So that treatment

with ACE inhibitor can be able to reduce the progression of kidney disease in either patients with T1DM or T2DM [(36)].

Analyzing the level of blood pressure among ACE gene genotype subgroups revealed that SBP and DBP were statistically significant higher in children with DD genotype in comparison to those with ID or II genotypes 120/80, 110/70, 100/65 with p value 0.032, 0.037 respectively and this can be explained by the higher serum ACE level and activity in DD genotype.

In our study none of DN children have diabetic retinopathy. The lack of a correlation between the ACE polymorphism and diabetic retinopathy is certainly definitive. All studies have failed to confirm any correlation. The cause of the discrepancy between the impact of the ACE polymorphism on DN and retinopathy is not fully understood. It cannot be argued that the intra-ocular RAAS is not important but the local intrarenal RAAS plays a more vital role than the intraocular RAS in controlling hemodynamics and cell growth [(37)].

Our study showed that both body mass index and lipid profile were higher in TIDM children with DN than in those without nephropathy and higher in DD and ID genotypes than II genotype. This copes with study done among one hundred forty-two children (70 with simple obesity and 72 controls) which showed that DD genotype and D-allele of the ACE gene polymorphism were associated with obesity and hyperlipidemia in Egyptian children [(38)].

It is suggested that RAAS affects the adipose tissue and the satiety centers. A decline in renin-angiotensin system activity usually results in weight loss. Moreover, obesity could be prevented by providing angiotensin-converting enzyme inhibitors, according to the results of an animal study. Also fat deposition is mediated by angiotensin II. So RAAS can have a major role in control of blood pressure, obesity and metabolic syndrome [(39)].

Limitation and strength

This study has several potential limitations. First, small sample size. Second, our study focused on ACE I/D genetic polymorphism, but previous studies have indicated that gene polymorphism in many other genes including Interleukin-6 174G/C and angiotensinogen T174M gene were correlated with DN susceptibility. However, only few studies done among diabetic children with T1DM with DN, our study would confirm the role of ACE gene I/D polymorphism in DN development in Egyptian children with T1DM. This may help in the future in development of personalized approach to reduce the burden of DN. Furthermore, it underlines the importance of ethnicity, which should be considered in all genetic studies.

Conclusion

The results of this study demonstrated that diabetic children with ID and DD genotypes have 4.7 times higher odds with (95% CI(1.2-18.9)) and 72.8-times higher odds with (95% CI(11-482)) to develop DN compared to those with II genotype, respectively. And also revealed that diabetic children with 'D' allele has 7.4 times higher odds with (95% CI (3.7 – 15)) to develop DN compared to those with 'I' allele. So II genotype appears to be a protective factors against the development of nephropathy in children with type 1 DM. Also presence of D allele may carry a high risk to develop nephropathy.

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