Involvement of *Insulin Like Growth Factor-I* Gene Polymorphism with Diabetes and Diabetic Nephropathy in Individuals with Type 2 Diabetes in Babylon Province

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Abstract

Concerning type 2 diabetes mellitus (T2DM), diabetic nephropathy (DN) is a chiefmicrovascular complication. Preceding studies have proposed that insulin-like growth factor-1(IGF-1) signaling might do a significant role in DN. The existing study was implemented to explorethe allianceof single nucleotide polymorphism (SNP) rs6214 of the *IGF-1*gene with T2DM and DN. The study consists of three groups, the first oneincludes 50 subjects, those were apparently healthy were considered as controls. The second oneinvolves 69 patients with T2DM without nephropathy and the third oneencompasses 89 patients who suffer from T2DM with DN. The analysis of results elucidated that thegenotype CT was decided to significantly increase the hazard of T2DM by about 2 folds and the genotype TT was established to significantly increase the risk of DN by about 3 folds with respect to those of the genotype CC. This study concludes that theSNPrs6214 of the*IGF-1* gene associated with T2DM and DN.

Keywords*: insulin like growth factor-1,type 2 diabetes mellitus, diabetic nephropathy, polymorphism.*

Introduction

Diabetic kidney disease is the real upshot of diabetes mellitus (DM). It is a cumulative disease[(1)].Even though long-term DM and unfortunate glycaemic management are hazardous factors for diabetic nephropathy (DN), the predisposition to such comorbidities is too expanded by ethnic variability in the genotype of the person[(2)]. There is strong proof that genetic tendency does a great role in DN progress[(3,4)].

In the expansion of DM vascular problems, growth factors are assumed to play a decisive role[(5,6)]. It was revealed that insulin-like growth factor-1 (IGF-1) be connected to complications of delayed microvascular DM, comprising DN[(7,8)].The IGF-1 level in circulation may not accurately describe IGF-1 bioactivity,in sickness situations, in particular, because of the IGF-1 binding proteins intervention, hence genetic-dependent researches can be a substitute approximation[(9)].

Thegene (*IGF-1*) is placed on the chromosome 12q22-24.1, and the encoded protein which is (IGF-1) exists as a single chain polypeptide comprising seventy amino acids[(10)].The structural similarity both for IGF-1 and its particular receptor (IGF-IR) with pro-insulin and insulin receptor is powerful.Accordingly, adding to their mitogenic influences, they do profound impacts on the metabolism of protein and glucose,this contributes to the key routes of exacerbation and/or progression of microangiopathic problems of DM[(11,12)].

The*IGF-1* gene rs6214 SNP is viewed as a functional polymorphism owing to its locality in three prime untranslated region (3'-UTR) of the gene. The non-coding sequence (3'-UTR) encompasses regulatory motifs that are important for the expression of the gene, translation, the stability of mRNA and mRNA cellular location, or microRNA binding, and thus, 3′-UTR variants can do a significant job in diverse genetic diseases[(13,14)].

Since DN is the chief reason for end-stage kidney disease and the role of IGF-1 in the DM microangiopathic triad has been proven[(15)],this study aimed to assess the association of *IGF-1* gene single nucleotide

polymorphism (SNP) with T2DM and DN.

Materials and Methods

The study includes three groups from Arab descent. Group1(controls) comprises 50 subjects who were apparently healthy. Any individual who underwent any other health problems were omitted from the study. Group2 contains 69 patients with T2DM without nephropathy. Group3 involves 89 patients who had T2DM with DN. The patients were diagnosed by specialist physicians and selected from Merjanhospital in Babylon Province after their agreement and after taking agreementfromResearch Uniteof Center for Training and Human Development of Babylon Health Directorate and Merjan hospital.

The serum levels of glucose,alkaline phosphatase (ALP) activity,and creatinine,and urine creatinineand total urinary protein were determinedby spectrophotometric method. Serum IGF-1 level was estimated by sandwich ELISA technique.

The DNA extracted from blood according to method[16]. Genotyping of rs6214 polymorphism of *IGF-1* gene was done via polymerase chain reaction (PCR). The PCR was used for amplification of DNA by using particular primers after designing them [the forward primer (5'- GGCAGTGCATCTTTCAGCTT-3') and the reverse primer (5'- TTGCCCTCTGCCTGTTTTCC-3')].

For the determination of the SNP, the thermal cycler was utilized as follows: initial denaturation for 5 minutes (min.) at 94°C, followed by 35 cycles under the next conditions: denaturation for 30 seconds (sec.) at 94°C, annealing for 30 sec. at 60°C, elongation for 30 sec. at 72°C and then final elongation for 5 min. at 72°C.

The products of PCR were analyzed on agarose gel (2%) electrophoresis. The PCR product fragment is279bp.After the digestion of the PCR product by a restriction enzyme(HpySE526 I) and following the analysis of the digestion restricts on agarose gel (2%) electrophoresis, there are three possible forms of genotype for each DNA sample:the fragment with 279bp indicate to the existence of the genotype (TT), while the fragments with (117bp) and (162bp) indicate to the existence of the genotype (CC), while (279bp), (162bp) and (117bp) indicate to the existence of the genotype (CT).

The mean±SD,Student's t-test and ANOVAwere utilized for the assessment of general characteristics and phenotypes data, while odds ratio (OR) and confidence interval (CI) 95% were utilized for the assessment of genotype data. A statistically significant level was deliberated once the P value lower than 0.05.

Results

The features of the groups were revealed in table (1).

Table (1): Features of All Groups

Features	Groups		Mean ± SD
Number	Group1	50	
	Group2	69	
	Group3	89	
Sex Male/female	Group1	28/22	
	Group2	30/39	
	Group3	60/29	
Sex Male% / female%	Group1	56 % / 44 %	
	Group2	43.48%/ 56.52%	
	Group3	67.42 % / 32.58 %	

Cont... **Table (1): Features of All Groups**

Age (year)	Group1	26.72 ± 9.50
	Group2	50.56 ±14.40[***]
	Group3	[*] [**]57.75 ± 11.99
BMI	Group1	24.59 ± 2.79
	Group2	28.40 ± 6.29[***]
	Group3	25.69 ± 5.88 [**]
Duration of DM (year)	Group1	
	Group2	5.32 ± 3.41
	Group3	14.14 ± 6.15[**]

[*] Means significant difference between group3 and 1 at (P<0.05).

[**]Mean significant difference between group3 and 2 at (P<0.05).

[***] Mean significant difference between group2 and 1 at (P<0.05).

A significant decrease in mean serumIGF-1 level present in group2 compared to group1. Conversely,a significant increase in mean level of glucose in group2 compared to group1. Also, a significant increase in mean levels of serum glucose, ALP,and creatinine, andaverage urinary protein to creatinine ratio (UPCR) in group3 when compared to group1 and group2. Too, there is a significant increase in mean serum level of IGF-1 in group3 compared to group2 as exhibited in table (2).

Table (2): Biochemical results of All Groups

Parameters	Groups	Mean ± SD
IGF-1(pg/mL)	Group1	478.50 ± 402.68
	Group2	125.39 ± 105.71[***]
	Group3	551.35 ± 244.24[**]
Glucose (mmol/L)	Group1	4.49 ± 0.46
	Group2	7.91± 0.50[***]
	Group3	9.51±2.38[*][**]
ALP (U/L)	Group1	69.91 ± 15.10
	Group2	71.91 ± 15.67
	Group3	85.03 ±24.26[*][**]
Creatinine (mmol/L)	Group1	84.25 ± 13.17
	Group2	80.12 ± 11.21
	Group3	311.87 ±226.51[*][**]
UPCR mg/g	Group1	132.96 ± 21.32
	Group2	134.85 ± 21.45
	Group3	831.77 ± 483.51[*][**]

[*], [**], [***]offer the similar indications as in table (2)

Genotyping frequencies of this gene were consistent with Hardy Weinbergs equilibrium (HWE) in controls group. While, they were deviated from HWE in all diabetic patients group. (x^2 = 0.101, P = 0.749 and x^2 = 7.413, P = 0.006) in control and all diabetic patients groups respectively.

Genotyping frequencies of this gene were consistent with HWE in diabetics without nephropathy group. However, they were deviated from HWE in DN patients group. (x^2 = 2.614, P = 0.105 and x^2 = 5.951, P = 0.014) in DM patients without nephropathy and DM patients having nephropathy groups respectively.

With regard to rapport of *IGF-1* rs6214 polymorphism with T2DM,for normal control group and all diabetic patients, the frequencies and distributions of genotypes and allelesof *IGF-1* geners6214 SNPweredisplayed in table (3). The CTgenotype was found to be significantly increase the hazard of T2DM by about 2 doublingswith respect to those of the wild genotype CC. The TTgenotype was found to be non-significantly increase the jeopardy of T2DM.The minor allele T was non-significantly higher in DM patients when compared with that of the controls group.

Table (3): Frequencies andDistributions of Genotypes and Alleles of*IGF-1*rs6214 SNPin the Diabetic Patients and the Controls Groups

Genotypes		Controls	Patients	OR	CI 95%	P-value
CC	No.	21	40	Reference	Reference	Reference
	%	42%	25.31%			
CT	No.	22	95	2.267	1.122-4.578	0.022
	%	44%	60.13%			
TT	No.	7	23	1.725	0.636-4.676	0.284
	%	14%	14.56%			
Total	No.	50	158			
	%	100%	100%			
Alleles		Controls	Patients	OR	95% CI	P-value
C	No.	64	175	Reference	Reference	Reference
	%	64%	55.38%			
T	No.	36	141	1.432	0.900-2.279	0.129
	%	36%	44.62%			
Total	No.	100	316			
	%	100%	100%			

Concerning the linkage of *IGF-1* rs6214 polymorphism with DN, the genotypes and allelesdistributions and frequenciesbelong to*IGF-1* gene rs6214 SNPfor diabetics with DN and those without DN weredemonstrated in the table (4). The CTgenotype was found to be non-significantly increase the hazard of DN with respect to those of the wild CC genotype. The genotype TT was found to be significantly increase

the jeopardy of DN by about 3 folds with respect to those of the wild genotype CC. The minor allele T in DN and DM without nephropathy patients was not significantly higher in DN patients when compared with that of the DM without nephropathy group.

Table (4): Frequencies and Distributions of Genotypes and Alleles of *IGF-1*rs6214 SNPin the Diabetic Patients without Nephropathy and Diabetic Nephropathy Groups

Genotypes		**Diabetics without nephropathy**	**Diabetic nephropathy patients**	**OR**	**CI 95%**	**P-value**
CC	No.	23	17	Reference	Reference	Reference
	%	33.33%	19.10%			
CT	No.	39	56	1.942	0.919-4.106	0.082
	%	56.52%	62.92%			
TT	No.	7	16	3.092	1.042-9.171	0.041
	%	10.15%	17.98%			
Total	No.	69	89			
	%	100%	100%			
Alleles		**Diabetics without nephropathy**	**Diabetics nephropathy patients**	**OR**	**95% CI**	**P-value**
C	No.	85	90	Reference	Reference	Reference
	%	61.59%	50.56%			
T	No.	53	88	1.568	0.998-2.463	0.0509
	%	38.41%	49.44%			
Total	No.	138	178			
	%	100%	100%			

There is no association of phenotypic parameters with genotypes of *IGF-1*rs6214 SNP in persons with T2DM without nephropathy except in ALP in persons having CT genotype when compared to CC genotype as appeared in table (5).

Table (5): Association of parameters with genotypes of *IGF-1*rs6214 SNP in Patients with Diabetes Mellitus without Nephropathy

Parameters	Genotypes	Mean ± SD	P value	
IGF-1	CC	144.794 ± 126.947		
	CT	116.206 ± 97.651	CT, CC P=0.323	
	TT	112.872 ± 70.714	TT,CC P=0.533	TT, CT P=0.931
Glucose	CC	7.874 ± 0.547		
	CT	7.953 ± 0.513	CT, CC P=0.569	
	TT	7.581 ± 0.324	TT,CC P=0.917	TT, CT 0.616
ALP	CC	64.535 ± 15.696		
	CT	76.369 ±12.801[*]	CT, CC P=0.002	
	TT	71.295 ± 22.497	TT,CC P=0.375	TT, CT P=0.398
Creatinine	CC	79.930 ± 9.507		
	CT	80.052 ± 11.860	CT, CC P=0.966	
	TT	81.188 ± 14.220	TT,CC P=0.787	TT, CT P=0.821
UPCR	CC	133.464 ± 22.458		
	CT	133.078 ± 18.580	CT, CC P=0.941	
	TT	149.347 ± 30.136	TT,CC P=0.141	TT, CT P=0.060

[*]Connotes significance at ($P < 0.05$) in comparison to CC

Concerning the association of phenotype parameters with genotypes of *IGF-1*rs6214SNP in patients with DN**,**the average serum values of IGF-1, ALP, and creatinine, and the mean of UPCR exhibited association in DN individuals possessing TT in comparison to CC and CT. Though, no significant differences among the genotypes were found in glucose as explained in table (6).

Table (6):Association of parameters with genotypes of *IGF-1*rs6214 SNP in Patients with Diabetic Nephropathy

Parameters	Genotype	Mean ± SD	P value	
IGF-1	CC	506.188 ± 219.338		
	CT	527.896 ± 244.261	CT, CC P=0.743	
	TT	681.426 ± 239.780 [*] [**]	TT,CC P=0.035	TT, CT P=0.029
Glucose	CC	9.526 ± 2.286		
	CT	9.279 ± 2.387	CT, CC P=0.707	
	TT	10.312 ± 2.432	TT,CC P=0.345	TT, CT P=0.132
ALP	CC	80.116 ± 20.256		
	CT	81.075 ± 22.078	CT, CC P=0.873	
	TT	104.119 ± 27.489[*] [**]	TT,CC P=0.007	TT, CT P=0.0008
Creatinine	CC	262.717 ± 191.752		
	CT	280.852 ± 207.785	CT, CC P=0.749	
	TT	472.668 ± 263.746 [*] [**]	TT,CC P=0.013	TT, CT P=0.003
UPCR	CC	705.993 ± 463.296		
	CT	755.565 ± 466.275	CT, CC P=0.701	
	TT	1232.148 ± 367.212 [*] [**]	TT,CC P=0.001	TT, CT P=0.0003

[*]Connotes significance at ($P < 0.05$) in comparison to CC

[**]Connotes significance at ($P < 0.05$) as compared with CT

Discussion

Numerous genetic and environmental elements do a key starring role in the pathogenesis of DN[17]. In the enlargement of DM vascular problems, growth factors are assumed to do aninfluentialrole[5,6]. In renal fibrosis and apoptosis, IGF-1 signaling might play a key role[18].

The group1 is younger than other groups to avoid to greater extent age related nephropathy and to get very healthier control persons. The mean period of DM in patients with nephropathy is lengthier than that of DM patients without nephropathy which can give a clue that lengthier period of DM can influence DN development.

There is insignificant difference in mean serum level of creatinine, and ALP, and the mean of UPCR between group2 and group1, this because group2 patients were in well stabilized condition and didn't haverenal disease. But there is significant increase in mean glucose level in group2 when compared with group1 because of DM.

There is significant decrease in mean IGF-1 level in group2 than group1,the outcome of the study is consistent with the outcomes of OmarN., *et al*.[19], Mancuso E., *et al*.[20]andTeppala S., *et al*.[21].Such results might be owing to the chronic hyperinsulinemia. Towering levels of insulin may indirectly rising the bioavailability of IGF-1 by putting down the generation of insulin like growth factor binding protein-1(IGFBP-1). In turn, raised IGF-1 bioavailability can have reverse feedback impact on GH leading to a drop in GH secretion and lower liver manufacturing of IGF-1 and IGFBP-3[22].

There is significant increase in mean serum level of creatinineand ALP, and UPCR average in group3when compared to group2 and group1, this because group3had renal disease. Also there is significant increase in glucose level in group3 when compared to group2 and 1,this may be another risk factor for DN in group3.Proteinuria defined as UPCR>200 mg/g, and normal as <200 mg/g[23]. Accordingly, group3 patients had abnormal proteinuria. In DM persons, raised levels of creatininehappen when the kidney isimpaired or does not function properly[24] which was present in group3. Increased ALP levels can be linked to chronic inflammation, dyslipidemia, bad regulation of glycemia, fatty liver, resistance to insulin and increased oxidative stress[25,26] which might existed in group3.

There is insignificant increase in IGF-1 level in group3 when compared to group1.

There is significant increase in IGF-1 level in group3 when compared to group2, which is in agreement with OmarN., *et al*. study[19].These findings might be attributed to elevated insulin levels that could increase hepatic GH receptor number and activity. This impact can bring about a spike in GH-regulated output of liver IGF-1 and IGFBP-3, with a higher rise in levels of IGF-1in circulation[27].Also IGF-1 is among the proteins impacted by renal dysfunction[28,29].

The deviations from HWE may be attributed to size of the sample which is relatively small[30],hence a larger number of participants is requisite.

The outcomes of the study illustrate that the genotype CT was associatedwith T2DM, and the genotype TT was found to be non-significantly increase the hazard of T2DM.The minor allele T was not significantly higher in T2DM persons when compared with that of the control group.This is in disagreement with the outcomes of Mohammed A., *et al.*[31].

The CTgenotype was found to be non-significantly increase the risk of DN. The genotype TT was found to be associated with DN.The minor allele T was non-significantly higher in DN patients when compared with that of the DM without nephropathy group.This is in disagreement with the outcomes of Mohammed A., *et al.*[31].Our result could be because of the *IGF-1* eminent genetic role in the evolvement of DN as submitted by Ewens*et al*.[32]. In dissimilarity to such evidence that infers a genetic role for *IGF-1* in diabetic complications, Bazza*et al*.[33]established no association between the DN growthand polymorphisms in *IGF-1* gene.

The mean serum value of ALP presented genotype-dependent variation in T2DM patients without nephropathy possessing CT genotype in comparison to CC genotype. Conversely, other parameters did not display any significant variances among the three genotypes.

The average serum values of IGF-1, ALP, and creatinine and the mean of UPCR exhibited genotype-dependent variation in DN patients possessing TT in comparison to CC and CT genotypes. These results were convenient with poor renal function. However, no significant differences among the genotypes have existed in glucose.

The IGF-1 mediates DN histological features. It binds glomerular mesangial cells to induce their increase in numbers, leading to enhanced kidney blood flow plus glomerular filtration rate, speeding up the advancement of DN[34,35].

Conclusion

It was detected that CT and TTgenotypes of *IGF-1* gene polymorphism of patients with T2DM may

modify the vulnerability and/or development of DM and DN respectively. Hence,it was concluded that the recognition of these genetic variants at a biomarker level could permit the revealing of those persons at greatthreat for DM and DN.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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