ApoE Polymorphisms Rs(429358) and its association with ApoE enzyme and some Biochemical parameters in some of patients with Diabetic kidney disease

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Abstract

The current study was designed to investigate the relationship of *ApoE* gene and its association with some lipids profile parameters in a sample of patients with Diabetic kidney disease.This study was conducted on (110) blood samples of people with Diabetic kidney disease, with (42) samples from healthy people as a control group. The results showed a non-significant increase in the level of enzyme (ApoE) (283.56 ± 14.98) in patients. when compared with healthy subjects. the results of the Real-Time PCR technique for the ApoE (rs 429358) gene in patients with diabetic nephropathy and healthy subjects were the presence of two alleles (T, C) and three genotypes (TT, TC, CC) and the frequency of the TT genotype was (0.527) and the value of (OR = 0.44 , 95% CI = 0.27 - 0.93) .The TC genotype is (0.3) and the CC genotype is (0.172). While the frequency of the two alleles C and T was (0. 66) for the T allele and the value of (OR = 0.647 , 95% CI = 0.69 - 1.01) the C allele is (0.33) and the value of (OR = 1.97 , 95% CI = 1.8 - 4.37) .

Introduction

A common complication of diabetes is Diabetic kidney disease (DKD), which is the leading cause of end-stage renal disease (ESRD) associated with increased mortality rates in patients with diabetes (Nichols et al., 2018; Salinero- Fort et al., 2016).

The first stage consists of glomerulomegaly and hyperfiltration, indicated by an increase in kidney volume and glomerular filtration rate (GFR) followed by a moderate increase in albuminuria formerly called microalbuminuria with urinary albumin excretion rate (UAER) urinary albumin excretion rate from 30 to 300 mg/day, after which severely increased albuminuria or macroalbuminuria with a UAER of more than 300 mg/day (Tang and Sharma, 2019). SNPs are the most common form of DNA variation, and an updated SNP database of more than 500 million source or reference SNPs (rs) with allele frequency data has provided essential information for genetic studies of complex diseases including diabetic renal failure and genetic studies in Diabetic kidney disease has previously involved unexpected biological pathways but it was later demonstrated to know and understand the genetic basis of the disease. For most of the common traits that have been studied in Diabetic kidney disease however the genes and SNPs specific to the disease have been identified human DNA variations are only a part of the underlying susceptibility to diabetic kidney failure (Kato and Natarajan, 2014; Allis and Jenuwein, 2016). genetic polymorphism of the ApoE gene is mainly attributed to three alleles ε2, ε3, and ε4

the purpose of separating blood components and obtaining serum and storing it at (-20 ° C) for later use in biochemical tests.

Quantitative detection of human ACE concentration in serum

Using the Sandwich ELISA assay, the accurate quantitative detection of human ACE proteins in blood serum

DNA Extraction: A blood sample (4 ml) for each individual with diabetic nephropathy and healthy individuals is used to extract DNA in the laboratory by means of the prepared DNA extraction kit consisting of the materials shown in Table (3-4).

Table (3-4) Components of the DNA extraction kit

Quick-DNA™ Blood MiniPrep kit	D3025(200 preps)
Genomic Lysis Buffer*	2 x 100 ml
DNA Pre-Wash Buffer**	50 ml
g-DNA Wash Buffer	100 ml
DNA Elution Buffer	2 x 10 ml
Zymo-Spin™ IICR Columns	200
Collection Tubes	400
Instruction Manual	1

was obtained by reading the optical density (OD) at 260 and 280 nm by dividing the first value of the optical density by the second value of the optical density. The acceptable percentage for DNA purity ranges between (1.8-2.0) while its low indicates contamination of DNA with proteins and RNA.

according to meta-analysis and the frequencies of alleles of the ApoE gene differ in different ethnic groups (Shi et al., 2018).

Method: This study was conducted on(152) was (110) blood samples of people with Diabetic kidney disease with (42) samples from healthy people as a control group collected from the artificial kidney unit in Balad General Hospital and Tikrit General Hospital, with ages ranging from 40-70 years. Collected 4 ml of each individual's blood was placed in a tube containing EDTA for the purpose of DNA extraction and use in molecular tests and Collected serum for biochemical tests.

biochemical tests: 6 ml of blood for each individual was placed in a special tube containing a gel tube and placed in a centrifuge at 3000 (rpm) for 10 minutes for

Estimation of DNA concentration and purity

A Nano Spectrophotometer was used to estimate the concentration and purity of DNA where 2 µL of DNA extracted from each sample was used for this purpose and according to the manufacturer's instructions an estimate of DNA purity

method .which contains nucleotide units marked with radioactive particles which allows monitoring after the pairing process between the probe and its complementary nucleic acid. This probe is a dye designed to increase the specificity of the reaction. q-PCR This method was first demonstrated by researcher Kary Mullis in 1991 (Holland et al., 1991).

In this study the ready-made TaqMan probe method was used to detect polymorphisms of the ApoE gene loci rs (4343) as shown in Table (3-5).

Real Time Polymerase Chain reaction (RT-PCR)

The real-time polymerase chain reaction technique was used to detect the two gene ApoE and alleles in samples of (110) patients with Diabetic kidney disease and (42) healthy subjects. This important technique is used in many areas of molecular biology research. The name of this technique is abbreviated as q-PCR and it is also called real time quantitative polymerase chain reaction (q-PCR) (Bustin et al., 2009).

In this research the specialized probe method was used It was named TaqMan

Table (3-5) showing the location of SNP for the ApoE gene.

SNP	Catalog	Assay ID	Sequence
rs (429358)	4351379	C_3084793_20	GCTGGGCGCGGACATGGAGGACGTG[C/T]GCGGCCGCCTGGTGCAGTACCGCGG

kidney disease compared to healthy people as their values amounted to (185.94 ± (5.23 mg/dl) (mg/dl 189.52 ± 6.08) 71.72 ± 2.26 (mg/dl) and (mg/dl 37.90 ± 1.21) respectively for diabetic renal failure, while it was (mg/dl 141.31 ± 6.32). (mg/dl 140.86 ± 5.90) (61.81 ± 2.07) mg/dl) (mg/dl 28.17 ± 1.18) respectively in healthy subjects.

Table (3-4) Values of total fats, triglycerides, high-density lipoprotein and low-density lipoprotein.

Results

Table (4-3) The results showed that there was a slight non-significant increase in the concentration of ApoE enzyme between patients and healthy subjects, as its concentration reached (U/L 283.56 ±14.98) in patients and (ng/ml 271.74 ±13.61) in healthy subjects and there is a significant increase in the values of total fats, triglycerides (TG) high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in patients with Diabetic

Parameters	**The group**		***p-value***
	Control	**Patient**	
	Mean ± SE	**Mean ± SE**	

	(42)	(110)	
ApoE(ng/ml)	271.74 ±13.61 a	283.56 ±14.98 a	0.0821
Total lipids (mg/dl)	141.31 ± 6.32 b	185.94 ± 5.23 a	0.001*
Triglycerides (mg/dl)	140.86 ± 5.90 b	189.52 ± 6.08 a	0.001*
HDL (mg/dl)	61.81 ± 2.07 b	71.72 ± 2.26 a	0.001*
LDL (mg/dl)	28.17 ± 1.18 b	37.90 ± 1.21 a	0.001*

frequency (0.527) and found a value of (OR = 0.44, 95% CI = 0.27 - 0.93) while the TC genotype had a recurrence value of (0.3), and the value was (OR = 1.33, 95% CI = 0.57 - 0.94) while the recurrence of the CC genotype was (0.172) and the value was (OR = 2.9, 95% CI = 1. 13 - 10.27) in patients with renal failure. On the contrary the TT genotype had a recurrence of (0.333) the genotype had a recurrence of (0.476) and the CC genotype had a recurrence of (0.190).

Table (4-8) Frequencies of ApoE genotypes in patients with Diabetic kidney disease and healthy subjects

2-Results Molecular study

The results of the ApoE gene showed that there are three genotypes (TT, TC, and CC). The genotype (TT) represents the normal homozygous genotype the (TC) genotype represents the asymmetric genotype while the genotype (CC) represents the homozygous mutant genotype.

Table (4-8) shows the frequencies of the three genotypes (TT, TC, CC) observed and located in patients with diabetic kidney disease and healthy subjects. The TT genotype recorded the highest

Genotypes		**Groups**		
		TT	**TC**	**CC**
Patient (*n*=110)	**Observed(Frequency)**	58(0.527)	33(0.3)	19(0.172)
	Expected (Frequency)	52 (0.472)	49 (0.445)	9 (0.081)
Control (*n*=42)	**Observed(Frequency)**	14(0.3)	20(0.476)	8(0.190)
	Expected (Frequency)	16 (0.380)	22 (0.523)	4 (0.095)
***p*-value**		0.31	0.61	0.29
Chi-Square (X^2)		5.826	0.369	4.369
OR		0.44	1.33	2.9
95% CI		0.27 – 0.93	0.57 – 0.94	1.13 – 10.27

the frequency of the C allele (0.30) in healthy subjects and a decrease of (0.33) in patients. The value was (OR = 1.97, 95% CI = 1.8 - 4.37).

Table (4-9) Allelic frequency of the ApoE gene in patients with Diabetic kidney disease and healthy subjects

association of polymorphisms of the ApoE gene with the risk of Diabetic kidney disease and this is indicated by (Shi et al., 2020).

The allele (T) of the ApoE gene may act as a catalyst for Diabetic kidney disease in type 2 diabetes but the allele (C) is not associated with the risk of Diabetic kidney disease and this is indicated by (Li et al., 2011). This is consistent with the results of the current study. But the results of the current study differ with what was obtained from studies from France and Turkey in diabetic patients as the results showed that there were no differences in the distribution of genotypes of the ApoE gene between the group of diabetics and the healthy group moreover it was found that the incidence of Diabetic kidney disease in people carrying the allele (T) of the ApoE gene was significantly lower than in carriers of the C allele. The results showed a slight non-significant increase in patients with diabetic renal failure when compared with the healthy group. This enzyme is a multifunctional protein, the most important of which is lipid metabolism. It is primarily synthesized within the liver. However it can also be synthesized in many other tissues and cell types, including brain, kidney, adipocytes, and macrophage cells (Getz and Reardon,

Table (4-9) shows the frequency of the C and T alleles in patients with diabetic kidney disease and healthy subjects, as it was observed that the frequency of the T allele increased (0.66) in patients while it reached (0.69) in healthy subjects and the value of (OR = 0.647, 95% CI = 0.69 - 1.01) The table also shows a decrease in

rs429358			
Allele Frequency		T	C
Patient (*n*=110)	Number (Frequency)	146 (0.66)	74 (0.33)
Control (*n*=42)	Number (Frequency)	58 (0.69)	26(0.30)
Chi-Square (X^2)		4.086	
***p*-value**		0.079	
OR		0.647	1.97
95% CI		0.69-1.01	1.8 – 4.37

Discussion

The results show that there is a relationship between the TT genotype of the ApoE gene and the incidence of Diabetic kidney disease in addition to the presence of the T allele conferring sensitivity to the disease.These results are consistent with the findings of (Yong et al., 2017) when it was studied on a groupof patients with Diabetic kidney disease in China, that the TT genotype of the ApoE gene in the Diabetic kidney disease group was significantly higher than that of the control group in T2DM. The allele T and patterns The inclusion genotypes (TT, TC) may indicate the

patients with T2DM (Mooyaart et al., 2014; Yang et al., 2010).

The level of total fats and triglycerides may be affected by gender and age (Haddy *et al*.,2002).It is also consistent with what was found (El-baz et al., 2018) which indicated an increase in total fat and triglycerides and a decrease in the level of high-density lipoprotein and low-density lipoprotein in people with Diabetic kidney disease. (Al-Badry, 2014) noted that patients with Diabetic kidney disease in the population of Thi-Qar governorate had a significant increase in the levels of total fats, triglycerides and low-density lipoprotein compared to the control group while its results showed a significant decrease in the level of high-density lipoprotein.

(Heerra, 2019) also indicated that there was an increase in the levels of total fats and triglycerides and a decrease in the level of high-density lipoprotein and low-density lipoprotein for patients with Diabetic kidney disease in India compared to the control group.

Conclusion

At study supports data showing the possibility of using an ApoE polymorphism as a genetic risk factor for Diabetic kidney disease and elevated level of ApoE enzyme and some biochemical parameters in cases with Diabetic kidney disease. As this study is limited with fewer cases and controls the observed genetic and allelic differences may not represent a true association .Therefore additional studies regarding gene-gene-environment interactions should be performed to estimate the overall risk of the ApoE gene in causing Diabetic kidney disease.

2009). (Haddy et al., 2002) showed that the concentration of ApoE enzyme is affected by age and sex, and that its concentration in males after the age of 45 begins to gradually decline, while in females it remains relatively high. The reason for the rise in females may be female sex hormones (Wang et al., 2011). An increase in the concentration of ApoE in the blood is an indication of kidney disease, including nephrotic syndrome (NS) and chronic kidney disease that requires dialysis. Therefore this enzyme plays a role in kidney pathology. (Tao et al., 2010) maintaining a normal level of ApoE enzyme is very important for neurological functions as it transports essential cholesterol to the brain and also interacts with many intracellular viral pathways that affect viral cell entry and infection progression (Tudorache et al., 2017). Any decrease in the level of the enzyme ApoE is associated with problems in blood vessels especially the incidence of atherosclerosis (Meir and Leitersdorf, 2004).

Based on the above results they speculated that low-density lipoprotein (LDL) may play a major role in the development of Diabetic kidney disease and suggested that the (T) allele reduces Risk of developing diabetic renal failure due to low plasma total and cholesterol levels (Boize et al., 1998; Tien et al., 2011). Most of the studies on the relationship between Diabetic kidney disease and the ApoE gene and its alleles showed that the (T) allele may be a risk factor for diabetic kidney failure and the frequency of the (T) allele was significantly higher in patients with Diabetic kidney disease compared to

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