

The extent of *UMOD* gene polymorphism and its level in type 2 diabetes patients

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Abstract

Background & objectives: Uromodulin is a protein produced only in cells of the kidney tubular and get out with urine. Some the mutations which lead to uromodulin is folding leads to retention in the kidney, and that promote the immune response to the system to cause infection of the kidney disease such as diabetic nephropathy (DN), that is one of the kidney diseases occurring as a result of diabetes. Our study found accompanied by rs4293393 variation of the UMOD gene as well as the susceptibility to renal illness in patients experiencing Type-2 diabetes mellitus.

Methods: 100 samples are including 50 with diabetes and 50 healthy controls, are genotyped for UMOD variant rs4293393T>C via RT-PCR. ELISA tested the uromodulin concentration. In addition, the uric acid, creatinine and urea concentration were performed via auto analyser A15 Biosystems instrument.

Results: The results show significantly higher levels of creatinine (mg/dl), urea (mg/dl) and uric acid (mg/dl) in DN patients than in those from the control group, while serum UMOD was highly significant in group of controls relative to the group of patients. Also, the T allele is more common in controls than in patients, while the C allele is more common in patients than in controls. A considerable change was identified in comparisons between type-2 diabetes patients and kidney diseases and control groups in the T allele. Comparing genotype to UMOD serum level, there was no statistical difference (P more than 0.05) between the control group and TC or CC genotypes, and significant differences (P less than 0.05) in control group UMOD levels compared with TT genotypes.

Conclusions: The frequency related to C allele and UMOD rs4293393 variant was markedly more than in individuals with DN.

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Introduction

The UMOD gene encodes a glycoprotein containing a 640 amino acid named 'uromodulin', which is extensively detected in human urine. Uromodulin is expressed in the luminal membrane then released through proteolytic cleavage into the urine (Vylet' al *et al.*, 2006)(Williams *et al.*, 2009). For those reasons, any mutations in UMOD coding regions could elongation of the maturation rate of the protein (Tinschert *et al.*, 2004). Many studies have identified more than sixty mutations of UMOD that help to the occurrence of ADTKD pathogenesis (Williams *et al.*, 2009), and most are small in-frame deletions, mutations (Nakayama *et al.*, 2012).

While the incidence of both type-1 and type-2 diabetes is increasing, the most marked rise is seen among individuals with T2DM1. The most serious complications of diabetes are retinopathy, neuropathy and nephropathy (Harb *et al.*, 2019). The aim of this work is to studying accompanied by rs4293393 variation of the UMOD gene as well as the susceptibility to renal illness in patients experiencing Type-2 diabetes mellitus.

Materials and methods

Sample collection and analysis

Blood samples from 50 patients suffering from diabetic nephropathy (DN) were collected at Al-Yarmook teaching hospital in Baghdad. These samples came from 25 males and 25 females aged between 35 and 85. In addition, samples from 50 healthy subjects were collected, and all participants gave written informed consent. ELISA was used for estimating human UMOD (uromodulin) utilizing ELISA kit based on the manufacturer's instructions (MyBioSource, USA). Also, DNA has been extracted from blood through utilizing ZYMO Quick-DNA Miniprep Kit (Cat# D3025), depending on company directions. Genotyping of one common polymorphism (rs4293393) of UMOD gene was conducted using TaqMan® SNP Genotyping Assays (Cat# 4331349) using the RT-PCR instrument.

Statistical analysis

Representing the data is mean \pm standard deviation. In addition, the differences between groups are put to test utilizing t-test and Student Q-square test, while the allelic as well as genotype association related to SNP are estimated utilizing odds ratio (OR), Pearson chi-square test, and (95%) of the intervals of confidence are specified. To compare more than 2 groups, one-way ANOVA is utilized. Two-tailed (P less than 0.05) was considerable. All analyses tests were achieved via SPSS 16.0 software.

Results The average ages of the DN and control groups were 57 ± 7.76 and 36.12 ± 2.25 , respectively. In addition, the number of males that show the DN compare to the control was 31/25, while the number of females that shows the DN compare to the control group was 19/25. The average of BMI (kg/m^2) in the DN and control group was (29.4 ± 2 and 23.6 ± 3.09), respectively, as seen in the table below:

Table 1: Properties of study groups

Characteristic	DN mean±SD	Control mean±SD
Age(years)	57±7.76	36.12±2.25
Gender (male/female)	31/19	25/25
BMI(kg/m ²)	29.4±2	23.6±3.09

Among a total of 50 patients and 50 controls, the results for diabetic patients with kidney disease shows significantly higher levels of creatinine (mg/dl), uric acid (mg/dl) and urea (mg/dl) than for those in the control group, as presented in Figure 1.

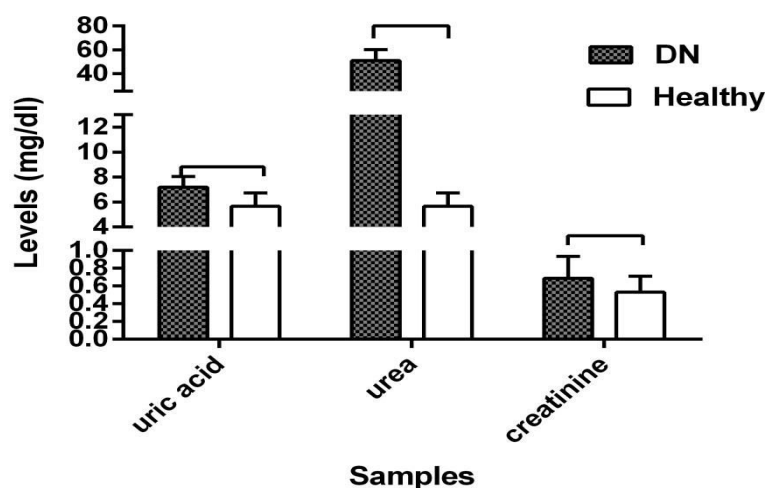


Figure 1: Levels of creatinine, urea and uric acid (mg/dl) of renal disease patients with 2DM and control representing healthy subjects. Values represent mean ±SD.

The values of serum UMOD are illustrated in Figure 2 and are highly significant in the control group in comparison to those in the group of patients (DN).

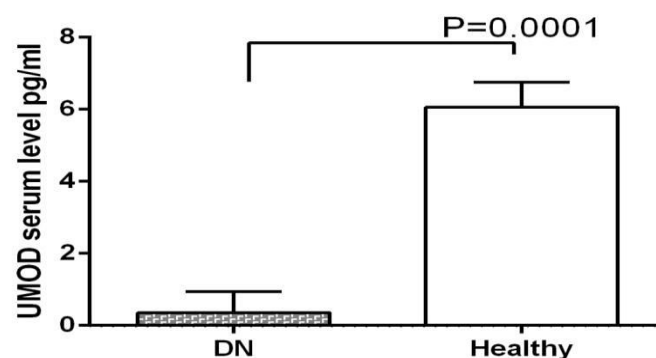


Figure 2: Levels of serum UMOD of renal disease patients with 2DM and control representing healthy subjects. Values represent mean \pm SD.

RT-PCR was performed in order to reveal the genotype of rs42993393 T > C, each genotype T or C was targeted by a specific probe labelled with a different dye. After completion, each probe was detected according to its colour dye: T appeared in Fam (green) and C appeared in Hex (blue), the amplification curves were shown in Figure 3.

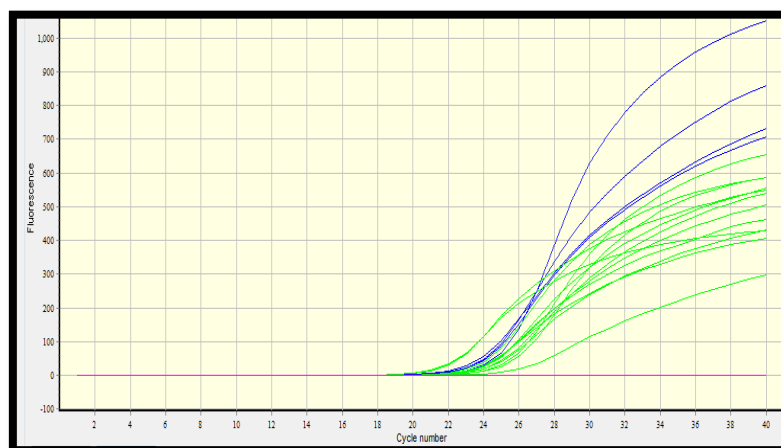


Figure 3: The qPCR amplification plot representing the different genotypes of the UMOD gene. The Fam plot (green) shows the amplification of rs42993393 T, whereas the Hex plot (blue) shows the amplification of rs42993393 C.

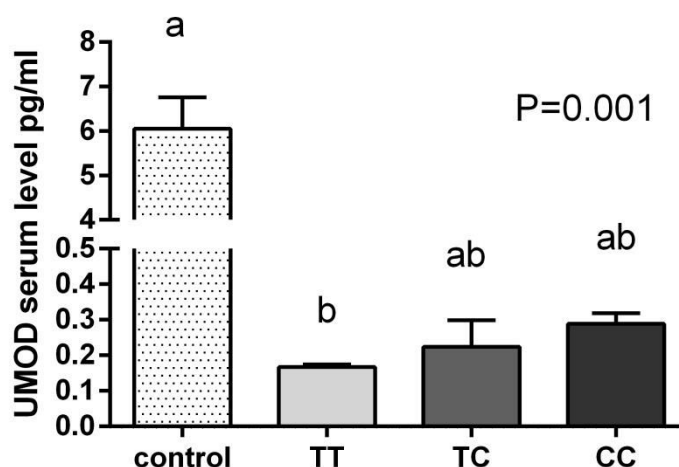
Table 2 illustrates the genotype distribution of the UMOD gene in healthy controls and patients. A TT allele (homozygous) genotype is more likely to be found in controls than in patients. The T allele is more common in controls than in patients, while the C allele is more in the group of patients compared to the group of controls. Considerable changes in T allele were identified in the comparison between kidney disease patients with 2DM and control group. The odds ratio in comparing controls with patients for TT subjects was 0.53 (95% CI, 0.31–1.08), while for TC subjects it was 1.56 (95% CI, 0.81–2.99) and for CC subjects it was 2.04 (95% CI, 0.51–8.43).

Table 2: Genotype distribution of the UMOD gene in healthy controls and patients

Genotype	Controls	Patients	OR	P-value	
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					CI 95%
TT	38	33	0.53	0.117	0.31 to 1.08
TC	10	14	1.56	0.246	0.81 to 2.99
CC	2	3	2.04	0.498	0.51 to 8.43
Allele frequency					
T	87	80	0.63	0.045	0.35 to 1.02
C	13	20	1.67	0.07	0.98 to 2.86

The results show, when comparing between genotype and serum UMOD level, that there were no statistical differences (P more than 0.05) among the group of controls, TC and CC genotypes, and a significant difference (P less than 0.05) in the level of UMOD in the group of controls in comparison to TT genotype, as can be seen in Figure 4.



*different letters mean significantly different.

Figure 4: Correlation between genotype and serum UMOD level.

Discussion

Nephropathy can be defined as the major cause of end-stage renal disease (ESRD) despite recent advancements in the diabetes management. Endothelial dysfunction and inflammation have a role in DN's development. Also, the uric acid was inflammatory and have a role in endothelial dysfunction (Williams *et al.*, 2009; Rampoldi *et al.*, 2003). In addition, such results led us to investigate the uric acid role in initiation and developments of the diabetic nephropathy. In type-2 diabetic nephropathy, we evaluated the uric acid level. Chambers *et al.* (2010) found the uric acid have related to diabetic nephropathy and the 2MD patients showed that the uric acid developed to nephropathy, that similar to our study.

The easiest approach for monitoring the kidney function was to test the blood for urea and creatinine (Molitoris *et al.*, 2007). The typical creatinine level is between 0.8 and 1.4 mg/dL. Usually, females have low creatinine (0.6-1.2 mg/dL) compared to males as they are typically having low muscle mass (Molitoris *et al.*, 2007). The creatinine level depended on the muscle mass. Thus, the concentration of

plasma creatinine was extremely stable and a direct reflection related to skeletal muscle mass (Martin *et al.*,2003). Interestingly, another study found that low creatinine in serum has occurred with an increased risk of 2DM, that lead to a decrease in the volume of skeletal muscle (Harita *et al.*,2009). The voluntary muscle is the main target via the insulin, and a low muscle volume indicates fewer target of insulin (insulin resistance) and that development of 2DM. the pathogenesis of 2MD associated with decreasing of the creatinine as (Dabla *et al.*,2010).

This work attempted on linking kidney disease and UMOD gene variant rs42993393 in Iraqi individuals experiencing 2-diabetes, such SNP, which is located 550bp upstream of the uromodulin site of transcription, was associated to kidney diseases in various researches (Trojanov *et al.*,2016; Köttgen *et al.*,2010; Gudbjartsson *et al.*,2010). Also, the frequency related to C allele as well as TC+CC genotype is different in the general populations of the persons experiencing diabetes in comparison to the group of controls, while the frequency related to C allele is high in DN patients compared to the group of controls, in accordance with the presented work, the results of this study indicating that C allele and C allele genotype might show a risk of kidney disease in person experiencing diabetes.

A study conducted by Gudbjartsson *et al.*(2010) indicated that patients experiencing type-II diabetes which carries the T allele had high levels of the serum creatinine after the age of fifty, in comparison with the one with no such variants. A study which has been conducted by Köttgen *et al.*(2010) examined functional links between such SNP and uromodulin secretion, the researchers identified that more uromodulin's secretion precedes CKD development. In addition, the allelic frequency and genotype distribution regarding such SNP in the population of this work is different from past researches (Trojanov *et al.*,2016; Köttgen *et al.*,2010; Gudbjartsson *et al.*,2010). Yet, other researches indicated that either no differences in the frequency related to rs-42993393 genotype/allele in the patients experiencing urinary tract infections in multicentric cohort research (van der Starre *et al.*,2015) or protection against kidney stones (Gudbjartsson *et al.*,2010).

In the presented work, it has been indicated that the serum uromodulin levels in persons with DN were reduced in comparison to the group of controls, this is different from a work carried out via Kumar indicating increased serum uromodulin levels in persons with DN (Kumar *et al.*, 2017). Yet, the level wasn't impacted via the distributions of rs-4293393 genotype. Furthermore, a previous work in nondiabetic persons assumed that lower urinary as well as higher uromodulin serum levels are related to kidney diseases (Prajczera *et al.*, 2010).

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Author contributions

The experiment was designed as follows: Dr Israa A. Alwan performed the experiments (ELISA). Dr Rasha Al-khalidi performed the real-time PCR experiment. Dr Hadeel A.A. Razaak analysed the data. All authors shared the writing and proofreading of the manuscript.

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