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Comparison of Allele Frequency of Uromodulin Gene rs13333226 and rs13333144 in a Sample of Iraqi Patients on Dialysis

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Abstract

Chronic kidney disease (CKD) is a major public health concern around the world. UMOD gene variants are linked to a higher incidence of hypertension and CKD in the general population. This study aimed to investigate the role of uromodulin rs13333226 and rs13333144 genes association with chronic kidney disease.The study samples were divided into two groups. The first group included 100 patient samples and 70 chosen among them were under the dialysis and had kidney failure aged between 18-88 years old. The second group included 30 samples from healthy individuals who were used as a control. One of the ways used to identify the genotype is the tetra-primers amplification refractory mutation system–polymerase chain reaction (ARMS–PCR). Regarding the results of SNP (rs13333226 and rs13333144), the genotypes GG (OR=150.3), and AA (OR=0.01) for rs13333226. The genotypes GG (OR=0.02) and TT(OR=140.4) for rs13333144 when comparing between patients and control (P≤ 0.01), they were observed when the odds ratio is less than 1 means they are preventive factors, while OR more than 1 means the risk increase (P≤ 0.01). It was concluded that there was an association between SNP (rs13333226 and rs13333144) and uromodulin in kidney diseases. Where genotype GG(rs13333226) and TT (rs13333144) represents a risk factor and AA rs13333226 and GG rs13333144 represents a protective factor.

Keyword: UMOD gene, rs13333226, rs13333144**,** Kidney disease

مقارنة لتردد الانماط الجينية ل rs13333226 و rs13333144 والخاص بـ Uromodulin في عينة من المرضى العراقيين تحت غسيل الكلى

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الخلاصة

مرض الكلى المزمن هو مصدر قلق كبير للصحة العامة في جميع أنحاء العالم. ترتبط المتغيرات لجين ال UMOD بارتفاع معدل الإصابة بارتفاع ضغط الدم و مرض الكلى المزمن في عموم البشر. هدفت هذه الدراسة إلى التحقق من دور ارتباط جينات rs13333226 و rs13333144 والخاص بـ uromodulin بأمراض الكلى المزمنة . وتم تقسيم عينات الدراسة إلى مجموعتين ضمت 100 عينة, تم اختيار المجموعة الأولى 70 عينة وهم تحت غسيل الكلى وكانوا مصابين بفشل كلوي تتراوح أعمارهم بين 18–88 سنة. ضمت المجموعة

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الثانية 30 عينة من أفراد أصحاء تم استخدامهم كعنصر سيطرة. إحدى الطرق المستخدمة لتحديد النمط الجيني هي نظام (ARMS–PCR). فيما يتعلق بنتائج الأنماط الجينية rs13333226 و rs13333144 ، (GG (OR = 150.3 و (OR= 0.01) AA لـ rs13333226. الأنماط الجينية (OR = 0.02) GG و (TT (OR = 140.4 لـ rs13333144 عند المقارنة بين المرضى والمجموعة السيطرة (P≤ 0.01) . عندما تكون نسبة الأرجحية أقل من 1 تعني أنها عوامل وقائية ، اما اذا كان أكثر من 1 يمثل عامل مرضي (P≤ 0.01). تم استنتاج أن هناك علاقة بين SNP rs13333226 و rs13333144 لل uromodulin في أمراض الكلى. حيث يمثل النمط الجيني (rs13333226) GG و (rs13333144) TT عامل خطر ويمثل (rs13333226) AA و (rs13333144) GGعاملاً وقائيًا.

Introduction

Chronic kidney disease (CKD) is a major public health concern around the world. Following the global trend, the prevalence of CKD is rising in developing countries Furthermore, CKD is a significant source of morbidity and mortality, as it is an independent risk factor for cardiovascular disease and mortality [1]. Uromodulin is a glycoprotein that according to researchers, is likely to be locked inside the protection of tubular cells against ascending urinary tract infections such as chronic pyelonephritis and urolithiasis [2]. It is made in the tubular cells of the thick ascending limb and the early distal tubule and then released into the tubular lumen where it forms a coating on the tubular cell surface. There is a lot of uromodulin in urine. It is also released into the interstitium by tubular cells, even though the physiological role it plays there is unclear [3]. Uromodulin concentrations in the urine and serum are lower in persons with interstitial fibrosis or tubular degradation as a result of chronic kidney disease. The greater uromodulin concentrations in persons without CKD were suggested to be attributable to the fact that there is no tubular work avoidance component in contrast to the glomerular filtration [4].

Common UMOD gene variants, which are linked to a higher incidence of hypertension and CKD in the general population, increase UMOD expression and uromodulin excretion in the urine, hence, resulting in salt-sensitive hypertension and renal lesions [5]. A common variant of the UMOD gene was linked with prevalent chronic kidney disease (CKD) in large genomics consortia. One community-based study found that urine concentrations of the uromodulin protein forecast risk of incident CKD [6] . The UMOD gene is found on chromosome 16 and codes for a glycoprotein called UMOD, which is generated solely in the thick ascending loop of Henle and distal convoluted tubules of the kidney before being released in the urine [7]. Several genes have been discovered as a consequence of recent breakthroughs in genome-wide association (GWA) investigations of renal disease [8]. UMOD, which has been identified as one of the top loci related with renal function measures in numerous cohorts, takes a prominent place among them. rs12917707 and numerous additional SNPs in significant linkage disequilibrium (LD) were found in a region upstream from the UMOD gene in multiple GWA investigations (LD) [9]. In perfect LD with rs12917707, rs4293393 and rs13333226, were associated with lower urinary uromodulin levels[10], and the minor alleles of SNPs in perfect LD with rs13333144, rs4293393, and rs13333226, were associated with lower urinary uromodulin levels[11]. Our research aimed to know the role of uromodulin gene rs13332878 association with chronic kidney disease in different age groups

Material and Methods

Sample Collection and Ethics Consent

Samples were collected from Al-Karama Hospital (Al-Hayat Dialysis Center). The study samples were divided into two groups. There were than 100 patient samples out of which 70 chosen were under the dialysis and had kidney failure, aged between 18-88 years old. The second group included 30 samples from healthy individuals who were used as a control. The

average age of the controls was 35 years. Random samples of the general population were taken as control. This study was approved by Ethical Review Board, College of Science of Biology in University of Baghdad on October 15, 2020 Ref.: CSEC/1020/0056. The DNA was extracted from samples using the Korean company's DNA extraction kit. Genotyping was performed by Tetra ARMS–PCR. Electrophoresis was used to identify the result of PCR in the presence of standard DNA in order to differentiate the bands size of the PCR result on an agarose gel. PCR reaction for SNPs (rs13333226 and rs13333144) [12] in Tables 1 and 2 were at a final volume of 25µl consisting of Taq PCR Pre Mix5µl (Bioneer/Korea), outer primer 0.5µl , reverse primer 0.5µl, inner primer 0.5µl , reverse primer 0.5µl-, DNA 1.5 µl and distilled water 16.5 µl. The thermocycling PCR conditions included an initial enzyme activation step at 95℃ for 10 mins and 50 cycles of denaturation at 95℃ for 15 s and annealing and extension at 60℃ for 60 s.

Table 1: Primer product, (rs13333226)

Primer	Sequence	Product size
Forward OUT Primer	5'-CTCCTCAGGATTATGTCCAA - 3'	138
Reverse	5'-CAGAACTGGTGAGTAGTGTT - 3'	
Forward INN Primer	5'-AGAGGTAGCACAGCTGTAGGA - 3'	78
Reverse	5'-TTGGGAAGAGGAGTCAATATC - 3’	

Table 2: Primer product, (rs13333144)

Primer	Sequence	Product size
Forward OUT Primer	5'-TTGTCCAACTGCAGTCTCAC - 3'	629
Reverse	5'-ATGTAGGTGAGACAGAGAAA - 3'	
Forward INN Primer	5'-GGGCTCCATCCTCCTGGGG - 3'	293
Reverse	5'-GTTCTTTGGCCTGCCCACT - 3’	

Statistical Analysis:

The results were analysed by SAS program (Statistical Analysis System 2012) to investigate the differences in the parameters in the current study where it used ANOVA test (Analysis of Variation-ANOVA) and Chi-square test for comparison among the groups. Michael H. Court calculator online (2005 - 2008) was applied for the studied SNPs to investigate Hardy-Weinberg equilibrium, if the p-value > 0.05, for the population coordinates with HWE. Odds ratio (OR) was calculated by the statistical software epidemiological (WINPEPI version 11.65) to detect the risk of genotypes. It was applied by chi-square, and Fisher's exact probability. The results were considered significant where the statistical probability is less than 0.05 and 0.01

Result and Discussion

SNP (rs13333226)

Regarding the results SNP (rs13333226) appears in Figures 1and 2, Figure 3 for the patients samples and Figure 4 for the control samples, the genotype GG shown in the size 78bp, the genotype AG shown in the size 78+138bp, whereas the genotype AA shown in the size 138bp.

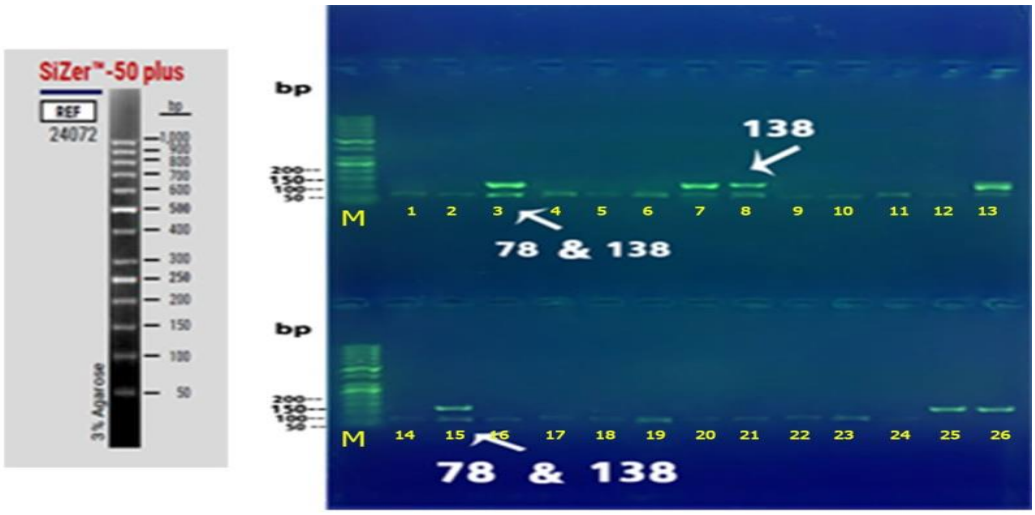

Figure 1: The output of the PCR for UMOD gene (rs13333226) of patients (1- 26 samples) represents the reaction results in 138-bp and 78-bp size in agarose gel at a concentration of 2%.

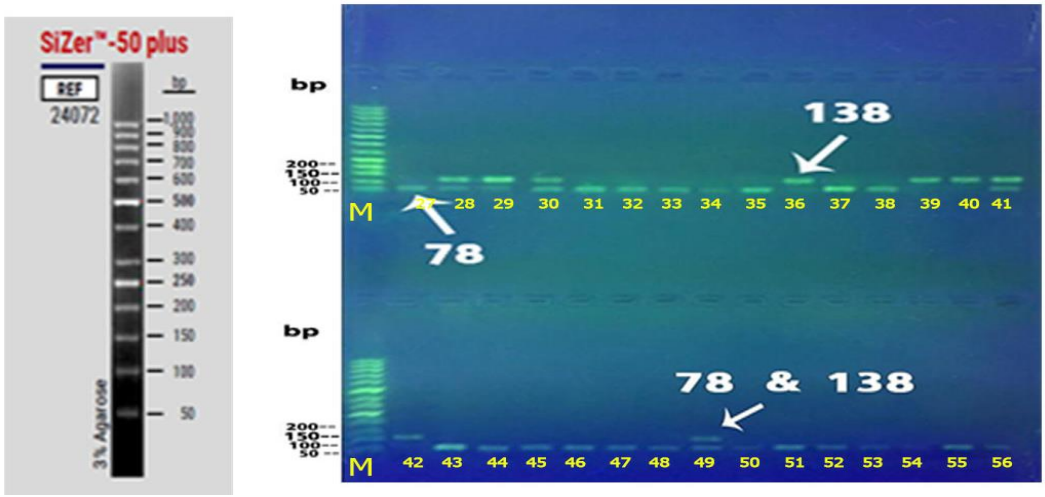

Figure 2: The output of the PCR for UMOD gene (rs13333226)of patients (27-56 samples) represents the reaction results in 138 bp and 78 bp size in agarose gel at a concentration of 2%.

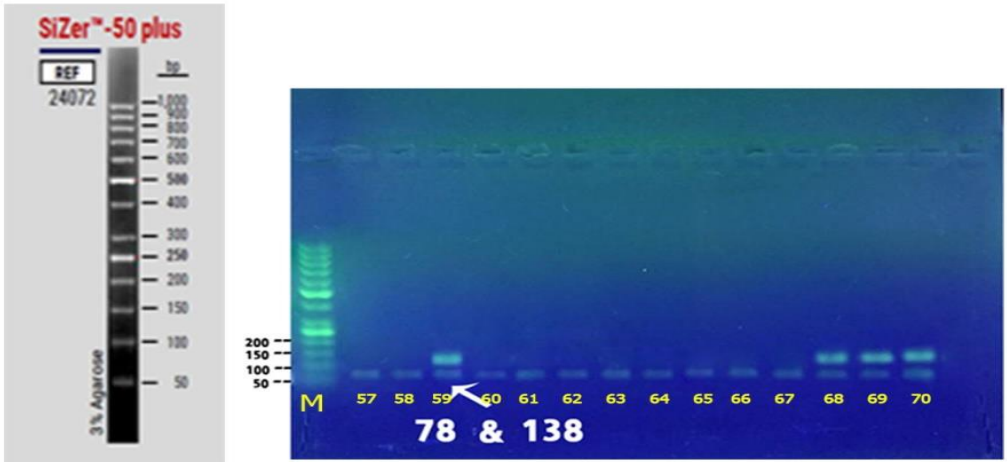

Figure 3: The output of the PCR for UMOD gene (rs13333226)of patients (57-70 samples) represents the reaction results in 138 bp and 78 bp size in agarose gel at a concentration of 2%.

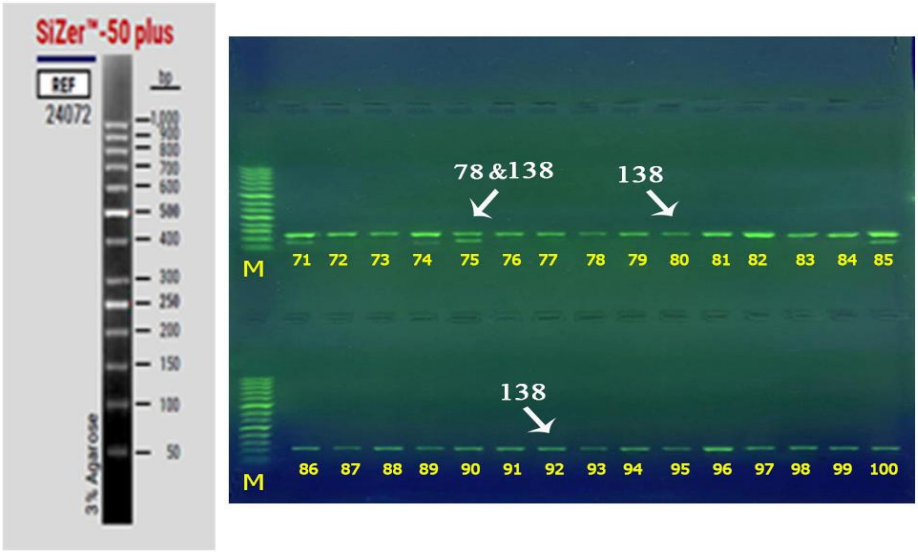

Figure 4: The output of the PCR for UMOD gene (rs13333226) of control (71-100 samples) represents the reaction results in 138 bp and 78 bp size in agarose gel at a concentration of 2%.

Table 3 shows the genotypes distribution for rs13333226 compatible to Hardy-Weinberg equilibrium in healthy women while in it patients were not compatible. The results of Hardy–Weinberg equilibrium should be no less than <0.05 because of the heterogeneous population. Where the characteristic of the population in Hardy–Weinberg equilibrium is the large sample size, random mating, no migration [13, 14] .The results of frequency genotypes appeared for GG (50%), AG (20%), and AA (6%) in patients. Whereas in control the frequency of genotypes was GG (0%), AG (13.33%), and AA (86%). There were significant differences for the genotypes GG(OR=150.3), and AA(OR=0.01) when comparing patients and control (P≤ 0.01). It has been observed when the odds ratio is less than 1 meaning that the genotypes are considered as a preventive factor, while or/more than 1 means increase in the risk [15]. While, there were no significant differences for the genotypes AG were found when compared between patients and control (P> 0.01) as shown in Tables 3 & 6.

Table 3: The genotype frequency and Hardy-Weinberg Equilibrium (HWE) for rs-13333226 in the studied groups

Groups			Genotype frequency			
			AA	AG	GG	HWE P ≥0.05
Controls (N=30)	Observed	N	26	4	0	**0.7**
		%	86.7	13.3	0	
	Expected	N	26.1	3.7	0.1	
		%	87	12.3	0.3	
Patients (N= 70)	Observed	N	6	14	50	**0.005**
		%	8.57	20	71.4	
	Expected	N	2.4	21.2	46.4	
		%	3.43	30.3	66.3	

Table 4: Genotype and Allele frequency of UMOD gene(rs13333226) SNP in patients and control groups.

SNP:rs13333226 Genotype	Patients No. (%)	Control No. (%)	Odd ratio (CI:95%)	P-value	Chi-Square (χ^2)
AA	6 (8.6%)	26 (86.7%)	0.01(0.00-0.05)	0.0001	14.98 **
AG	14 (20 %)	4 (13.33%)	1.6(0.50- 5.33)	0.173	1.462 NS
GG	50 (71.4%)	0 (0 %)	150.3(9.18-2460.85)	0.0001 **	13.45 **
	Allele Frequency				
A	0.19	0.93		--	--
G	0.81	0.07		--	--
** (P≤0.01).					

The current study contradicts with some other studies. [16] illustrated in their study that there was no association between SNP (rs13333226) and chronic renal disease in the African population. Also, some researchers demonstrated the SNPs for UMOD gene (rs13335818, rs4293393 and rs13333226) were not related with renal chronic disease in Caucasians patients [16]. Whereas, the current study concorded with other studies which pointed that the SNPs (rs13333226, rs4293393, rs6497476) for UMOD gene were regarding to pathogenesis of chronic renal disease and the risk mortality in Chinese population [17]. There were different common SNPs for UMOD gene that exist in the promotor region, such as rs13333226, rs4293393, rs6497476, and rs12917707 which have been related significantly with chronic renal disease in the studies of genome-wide associations for the population of European ancestry [18, 19]. Another study reported the role rs13333226 genotypes in chronic renal disease. The genotypes AA showed a short time of kidney survival in patients with the chronic renal disease than patients with the rs13333226 AG+GG [12]. On the other hand, in the mice transgenic, it observed a risk in variants of UMOD gene which raised the UMOD protein expression. The overexpression of uromodulin in mice led to the increase of hypertension, which due to salts sensitivity and the existence of kidney lesions was related to age [20].

SNP(rs13333144)

Regarding the results SNP (rs13333144) that appears in Figures 5, 6 and 7) for the patients samples and in Figure 8 for the control samples, the genotype GG shown in the size 293bp, the genotype GT shown in the size 293+629bp, whereas the genotype TT shown in the size 629bp.

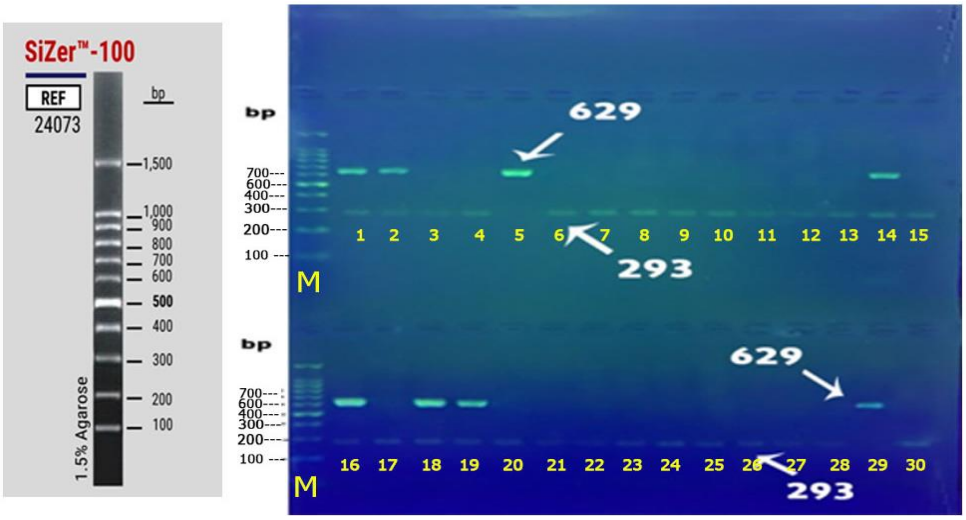

Figure 5: The output of the PCR for UMOD gene (rs13333144) of patients (1-30 samples) represents the reaction results in 293bp and 629 bp size in agarose gel at a concentration of 2%.

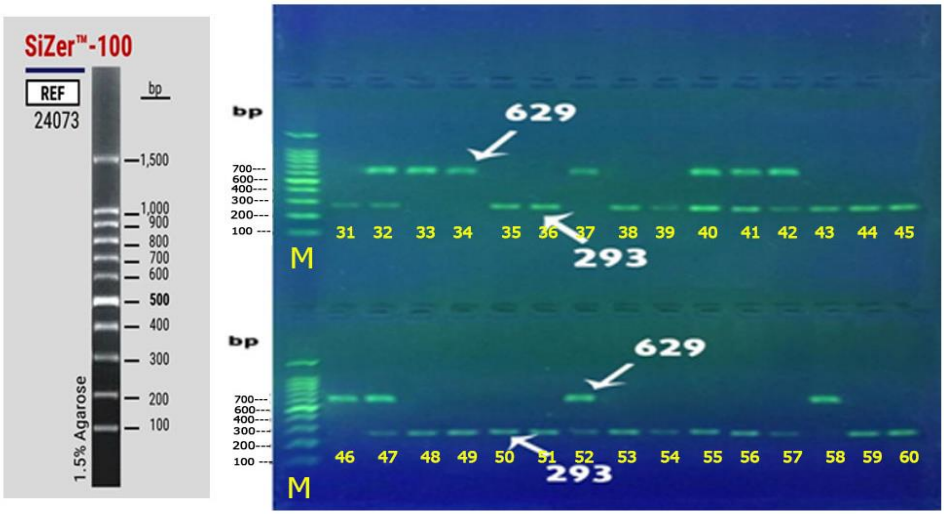

Figure 6 : The output of the PCR for UMOD gene (rs13333144) of patients (31-60 samples) represents the reaction results in 293bp and 629 bp size in agarose gel at a concentration of 2%.

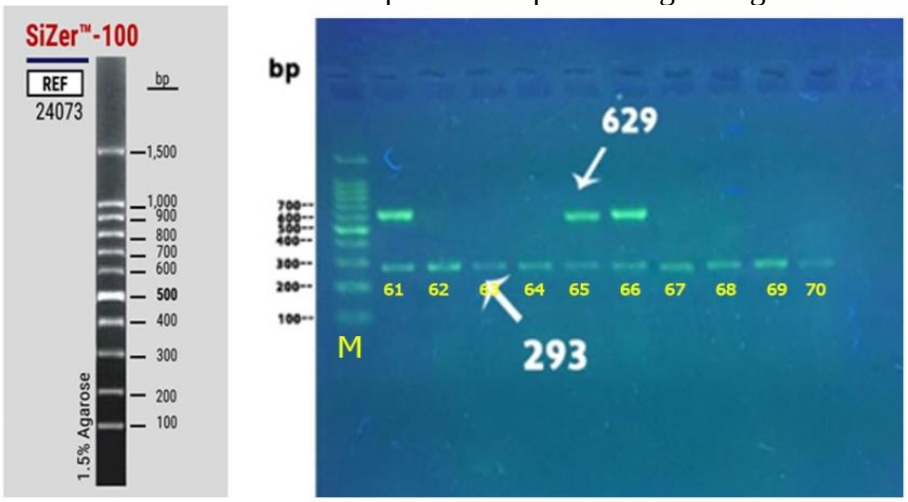

Figure 7: The output of the PCR for UMOD gene (rs13333144) of patients (61-70 samples) represents the reaction results in 293bp and 629 bp size in agarose gel at a concentration of 2%.

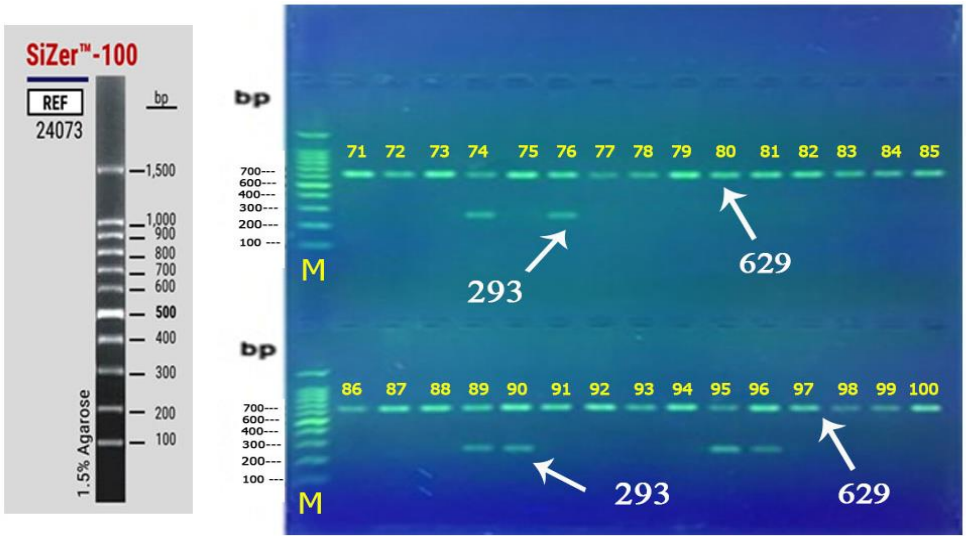

Figure 8: The output of the PCR for UMOD gene (rs13333144) of patients (71-100 samples) represents the reaction results in 293bp and 629 bp size in agarose gel at a concentration of 2%.

Table 5 shows the genotypes distribution for rs13333144 compatible with Hardy-Weinberg equilibrium in healthy women while in patients it was not compatible. The results of Hardy–Weinberg equilibrium should be no less than <0.05 because of the heterogeneous population, where the characteristic of the population in Hardy–Weinberg equilibrium is the large sample size, random mating, no migration [21]. The results of frequency genotypes appeared for GG (8.57%), GT (21.43%), and TT (70%) in patients. Whereas, in control the frequency of genotypes were GG (80%), GT (20%), and TT (0%). There were significant differences in the genotypes GG(OR=0.02) and TT(OR=140.4) when comparing the patients and control (P≤ 0.01). It has been observed that less than 1 odds ratio meaning that the genotypes are considered to be a preventive factor, whereas more than 1 means an increase in the risk [10]. While there were no significant differences in the genotypes GT when comparing patients and control (P> 0.01) as shown in Table 6.

Table 5: The genotype frequency and Hardy-Weinberg Equilibrium (HWE) for rs13333144 in the studied groups.

Groups			Genotype frequency			
			GG	GT	TT	HWE P ≥0.05
Controls (N=30)	Observed	N	24	6	0	**0.5**
		%	80	20	0	
	Expected	N	24.3	5.4	0.3	
		%	81	17	1	
Patients (N= 70)	Observed	N	6	15	49	**0.009**
		%	8.57	21.4	70	
	Expected	N	2.6	21.8	45.6	
		%	3.7	31.1	65.1	

Table 6: Genotype and Allele frequency of UMOD gene rs13333144 SNP in patients and control groups.

SNP: rs13333144	Patients No. (%)	Control No. (%)	Odd ratio (IC: 95%)	P-value	Chi-Square (χ^2)
Genotype					
GG	6 (8.57%)	24 (80 %)	0.02(0.01-0.08)	0.0001	14.26 **
GT	15(21.43%)	6 (20 %)	1.1(0.38 -3.11)	0.896	0.251 NS
TT	49(70.00%)	0 (0 %)	140.4(8.6- 2297.02)	0.0001	12.75 **
	Allele Frequency				
G	0.19	0.90		--	--
T	0.81	0.10		--	--
	** (P≤0.01).				

Concerning SNP of (rs13333144), there is a lack in related studies apart from one previous study conducted about the association of chronic renal disease with SNPs in UMOD gene promoter (rs13332873, rs13332923, rs13332898, rs13333144, rs13333292, and rs13333226), where these SNPs showed no relation with chronic renal disease, except rs13333226, that has showed a significant association with chronic renal disease [11].

Conclusions

It was concluded that there was an association between SNP (rs13333226 and rs13333144) and uromodulin in kidney diseases, where the genotype GG((rs13333226) and TT (rs13333144) represents a risk factor and AA rs13333226 and GG rs13333144 represents a protective factor.

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