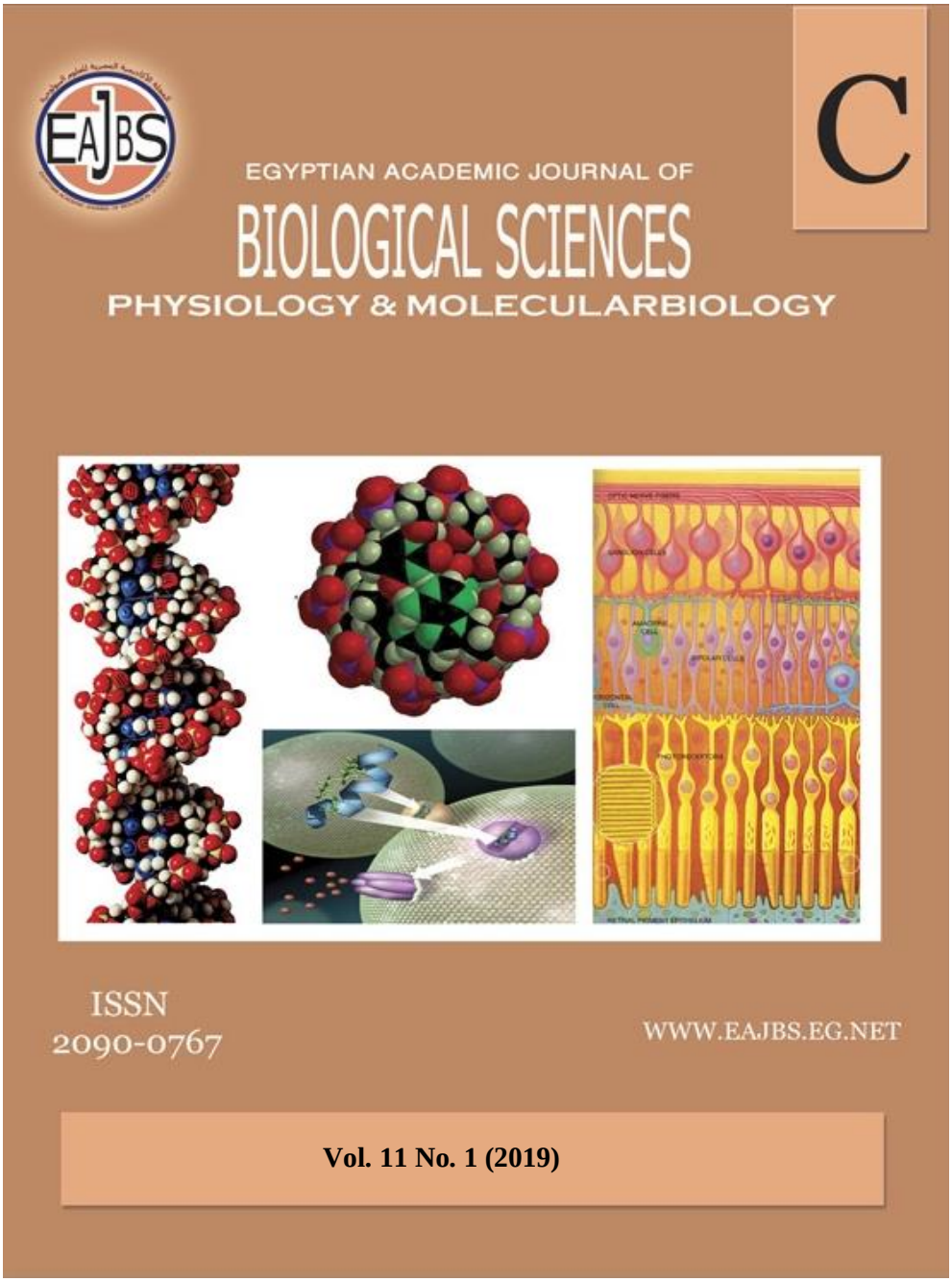

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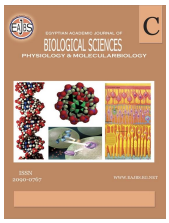

Sex Differential Genetic, Biochemical, Electrolytes and Calcium Values Associated with Chronic Kidney Disease

Samir Abdulkarim Alharbi
Medical Laboratory Department, Applied Medical Sciences College - Alquwayiyah Province, Shaqra University, Riyadh, Kingdom of Saudi Arabia.
E.Mail : saalharbi@su.edu.sa

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ABSTRACT

Background: Several efforts have been made to reduce the burden of CKD through the control of its associated risk factors. The objective of this study was to assess, whether the biochemical, electrolytes and calcium values differ according to the sex of the patients, as well as, the possible association between UMOD gene mutation and certain patterns of biochemical, electrolytes and calcium measures.

Methodology: one hundred patients with CKD were randomly selected from different primary health care centers (PHCs) in the Kingdom of Saudi Arabia (KSA). Biochemical (Creatinine, Urea, Uric acid, and Glucose) and minerals (Na+, K+, Cl− and Ca++) values were estimated, and UMOD gene mutations were investigated. **Results:** About 71.9% of the males were found with high urea values compared to 33.3% of the females, the RR (95% CI) = 2.1563 (1.3253 to 3.5082), P = 0.0020. Low K+ levels were significantly associated with females compared to males, the RR (95% CI) = 2.819 (1.1066 to 5.0773), P = 0.0264. About 60% 80% and 100% of the patients with UMOD gene mutation were found with low Na+ uric acid and Ca++. **Conclusion:** High blood urea is suggested as screening predictor for CKD in males. Low K+ levels were significantly associated with females compared to males. Patients with UMOD gene associated CKD are more susceptible to be with low serum Uric acid, Sodium, and Calcium.

INTRODUCTION

Chronic Kidney disease (CKD) is a common health problem worldwide (Lameire, Jager, Van Biesen, de Bacquer, & Vanholder, 2005; Meguid El Nahas & Bello, 2005). CKD is described by a low estimated glomerular filtration rate (eGFR) and high albuminuria and is associated with adverse outcomes (Fox et al., 2012). Early identification of CKD provides valued prospects for effective interventions that reduce the risk of outcomes particularly renal failure and cardiovascular disease (Ginawi, Elasbali, et al., 2013). Many risk factors have been indicated as risk factors for CKD worldwide, including Type 2 Diabetes mellitus (DM) (Wu, Huang, Chen, & Chen, 2018), hypertension (Shang et al., 2018) and genetic factors (Sjaarda et al., 2018). CKD was reported to be 24% in Saudi Arabia (Ginawi, Elasbali, et al., 2013), variable proportions of different risk factors

included; a family history (FH) of DM representing 72%, followed by family history of hypertension, recurrent urinary tract infection, DM, family history of renal disease, hypertension, and analgesic abuse, constituting 65%, 59%, 26%,26%, 25%, and 22%, respectively (GINAWI, AHMED, et al., 2013).

An Autosomal dominant tubulointerstitial kidney disease caused by mutations in the uromodulin gene (ADTKD-UMOD) is a spectrum of hereditary renal disorders, characterized by early-onset hyperuricemia, gout and progressive nephropathy (Lin et al., 2018).

In Saudi Arabia, there is a wide variation in disease extent between males and females due to several factors including body composition and activities. Consequently, females are more likely to develop many non-communicable diseases due to social settings. Therefore, the factors such as obesity and DM, VitD deficiency are more common among females, which makes them more susceptible for diseases such as CKD (Ahmed, Ginawi, Elasbali, Ashankyty, & Al-Hazimi, 2014; Ginawi et al., 2014). These variations should be considered when applying control and preventive programs. Therefore, the present study aimed to assess, whether the biochemical (Creatinine, Urea, Uric acid and Glucose)and electrolytes (Na+, K+, Cl−) and Ca++ values differ according to the sex of the patients. This in addition to the possible association between UMOD gene mutation and certain patterns of biochemical, electrolytes and calcium measures.

MATERIALS AND METHODS

In the present study, 100 patients with CKD were randomly selected from different primary health care centers (PHCs) in the Kingdom of Saudi Arabia (KSA).CKD was evaluated depending on glomerular infiltration rate GFR calculation using Serum Creatinine equations, using the standardized definition from the Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation (K/DOQI) practice guideline, and particularly focus on the performance of serum-creatinine based equations for GFR estimation. eGFR was estimated using CKD-EPI Creatinine Equation. All individuals with a GFR <60 ml/min/1.73 m^2, were regarded as having CKD (Kopple, 2001). Consequently, only patients with (GFR) less than 60 mL/min/1.73m^2 were included.

Blood samples were collected from each Patient. Biochemical tests were done for Creatinine, Urea, Uric acid, and Glucose. Electrolytes levels were also measured for Sodium, Potassium, Chloride and Calcium. Whole blood was used to obtain DNA.

Blood DNA Extraction:

DNA was extracted from the whole blood as follows: 1.5 ml of blood was added to a centrifuge tube followed by adding of 2 volume of erythrocyte lysis buffer (RS), then the vortex mixed upside down or back and forth, centrifuged at 5000 rpm for 3 minutes. The achieved supernatant was discarded and white or pale red precipitate was collected. Afterward, 200ul digestion buffer (DS) was added, vortex to form a fully uniform suspension, then for the elimination of RNA, 4 ul RNase was added, vortex 5 seconds and incubated at RT 5 minutes. Next, 20ul proteinase Kand 220ul lysate (MS0 was added. Vortex and incubated in a water bath at 65 C for 15 minutes, then 220ul ethanol was added, upside -down mixed until flocculent precipitate arose. Brief centrifugation to remove the inner wall of the tube cap drops, and the solution was transported to the purification column. Centrifuged at 12.000 rpm for 1 minute, and

discarded the filtrate. Then 500 ul of protein solution PS was added, centrifuged at 12.000 rpm for 1 minute, discarded the filtrate. Then 500ul Rinse (PE) was added, centrifuged at 12.000 rpm for 1 minute, discard the filtrate. Then, 500ul Rinse PE was added, centrifuged at 12.000 rpm for 3 minutes, to remove the residual liquid purification column completely. Purification columns placed on a new 1.5 ml centrifuge tube, to the center, dropping 30-100ul eluent TE, incubated at Room Temperature (RT) for 2 minutes. Then centrifuged at 12.000 rpm for 2 minutes, the bottom of the tube was containing high-purity genomic DNA. DNA was stored at -20C for subsequent amplification.

Polymerase Chain Reaction (PCR) amplification of 10 exons contained in the UMOD gene is implemented on the patient's genomic DNA extracted from whole blood (Saeed, Siddiqui, Bajpai, K Srivastava, & Mustafa, 2014). Direct sequencing of amplification products is done in both forward (GAGCGGCTCAGAGAACTTCAG TGG) and reverse (CCCGTGTCCTGTGTTACATTCA TC) directions (Primer Sequence (5´–3´), amplification (529 bp)), using PCR method. PCR amplification of the *UMOD* gene was performed as designated in Table 1and 2. The program used for amplification at the thermal cycler (Eppendorf - Master cycler) from (Beijing Aidlab Biotechnology Co., Ltd.).

The genotyping of the single nucleotide polymorphism (SNP) at the *UMOD* locus was performed. Variant rs12917707 of UMOD gene was genotyped by using the ABI Real-time TaqMan allelic discrimination assay.

Ethical Consent:

The study was approved by the Ethical Review Board, College of Applied Medical Science, University of Shagra, Saudi Arabia. This in addition to the fact that the authors followed the tenants of the Declaration of Helsinki. All participants have consented before inclusion and collection of their blood samples or demographical data.

Statistical Analysis:

For all statistical analyses including frequencies and cross-tabulations, the SPSS statistical software version 16 was used. Pearson chi-square test was used and P. values of 0.05 or less were regarded as statistically significant.

RESULTS

This study investigated 100 Saudi patients with CKD, their ages ranging from 11 to 99 years old with a mean age of 53 years. Out of the 100 patients, 64 were males and 36 were females, giving males' females' ratio of 1.78: 1.00. The majority of patients were at the age range of 55-69 years followed by age groups, 70+, 25-39, 40-54 and <25 years representing 35%, 21%, 18%, 15% and 11%, respectively. Most of the males were found at age group 55-69 years followed by 25-39 and 40-54. Hence, most females were found at age 55-69 years followed 70+ years and <25 years, as shown in Figure 1.

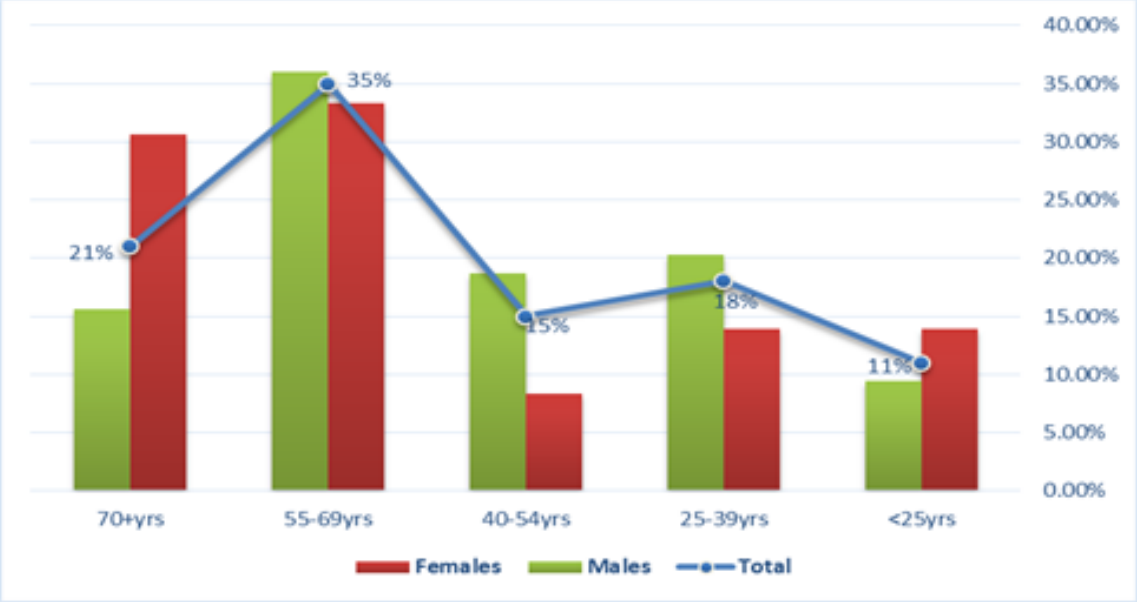

Fig. 1. Description of the study population by age and sex

All patients were with GFR less than 60 mL/min/1.73m^2.

Table 1, summarized the distribution of the patients by sex uromodulin gene mutation and biochemical parameters. Out of the 100 investigated patients, uromodulin gene mutations were found in 10 patients, of whom 5/64(7.8%) were males and 5/36(13.9%) were females. Elevated Creatinine was found among 92% of the patients, of whom 61/64(95.3%) were males and 31/36(86%) females, whereas, low Creatinine was identified only in 2/36(5.6%) females. Risk of high Creatinine associated with male sex and the 95% confidence interval (CI) and relative risk (RR) was RR(95% CI)= 1.1069(0.9603 to 1.2757), P = 0.1611. High levels of urea were detected among 48% of the patients, of whom 46/64(71.9%) were males and 12/36(33.3%) were females. The RR (95% CI) = 2.1563 (1.3253 to 3.5082), P = 0.0020.

High uric acid measures were determined in 12% of the patients of whom, 10/64(15.6%) were males and 4/36(11%) were females. The RR (95% CI) = 1.4063 (0.4750 to 4.1629), P = 0.5381.

High blood glucose levels were detected among 67% of the patients, of whom 45/64(70.3%) were males and 22/36(61%) were females, as shown in Figure 2. The RR(95% CI)= 1.1506 (0.8478 to 1.5615), P = 0.3680.

Table 1. Distribution of the patients by sex and biochemical parameters

Variable	Category	Males	Females	Total
UMOD gene				
	Positive	5	5	10
	Negative	59	31	90
	Total	64	36	100
Creatinine				
	Low	0	1	2
	Normal	3	4	6
	high	61	31	92
Urea				
	Low	3	4	7
	Normal	14	21	35
	high	46	12	48
Uric acid				
	Low	35	25	57
	Normal	19	7	23
	high	10	4	12
Glucose				
	Low	1	1	2
	Normal	18	13	31
	high	45	22	67

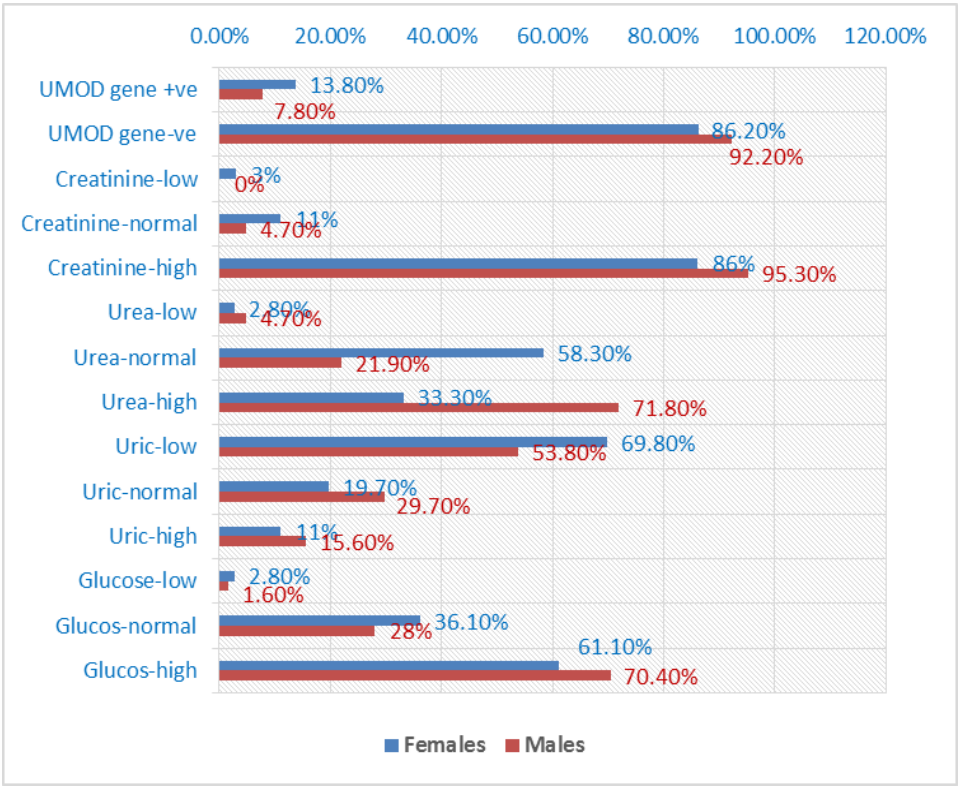

Fig. 2. Description of the patients by sex and biochemical parameters

With regard to the serum minerals estimation, low sodium levels were detected in 58% of the patients, of whom 40/64(62.5%) were males and 18/36(50%) were females. The RR (95% CI) = 1.2500 (0.8567 to 1.8238), P = 0.2470.

Low Potassium levels were detected among 21% of the patients of whom 9/64(14%) were males and 12/36(33.3%) were females. Hence, high potassium levels were found among 17% of the patients among whom, 15/64(23.4%) males and 2/36(5.6%) females. The RR (95% CI) = 2.819 (1.1066 to 5.0773), P = 0.0264.

Low chloride levels were identified in 25% of the patients, of whom 14/64(21.9%) were males and 11/36(30.6%) were females, whereas, high chloride levels were detected among 8% of the patients, of whom 6/64(9.4%) were males and 2/36(5.6%) were females. The risk associated with low chloride; the RR (95% CI) = 1.3968 (0.7105 to 2.7460), P = 0.3325.

All of the patients (100%) were found with low calcium levels, as indicated in Table 2, and Figure 3.

Table 2. Distribution of the patients by sex and minerals levels status

Variable	Category	Males	Females	Total
Sodium				
	low	40	18	58
	normal	24	17	43
	high	0	1	1
	Total	64	36	100
Potassium				
	Low	9	12	21
	Normal	40	22	62
	high	15	2	17
Chloride				
	Low	14	11	25
	Normal	44	23	67
	high	6	2	8
Calcium				
	Low	64	36	100
	Normal	0	0	0
	high	0	0	0

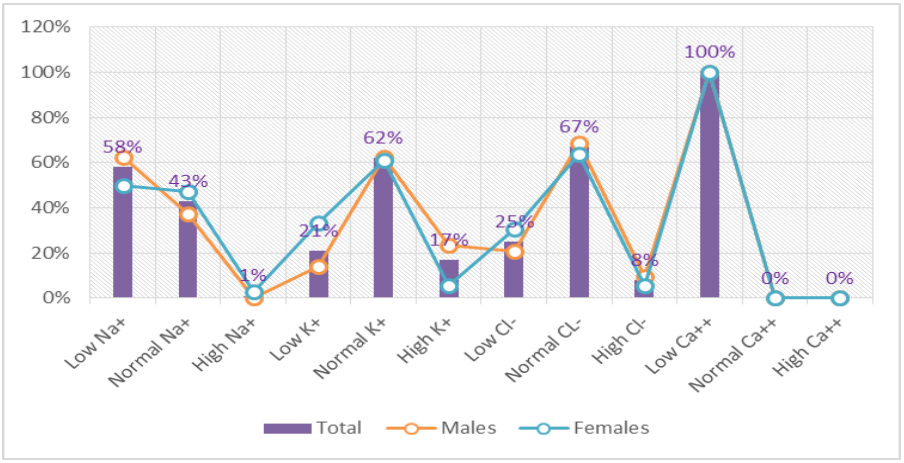

Fig. 3. Description of the patients by sex and minerals levels status

Table 3, summarized the distribution of UMOD gene mutation by age, biochemical and minerals levels status. The majority of uromodulin mutations were detected among age group 70+ years followed by 40-54 years, constituting 4/10(40%) and 3/10(30%) in this order. About 90% of the patients with UMOD gene mutations were found with high Creatinine levels. Around 60% of the patients with the mutation were found with high urea. On the other hand, 80% of the patients with mutations were found with low uric acid levels. About 60%, 40%, 50% and 100% of the patients with UMOD gene mutations were found with low Sodium, Potassium, Chloride and Calcium levels, correspondingly.

Table 3. Distribution of UMOD gene mutation by biochemical and minerals levels status

Variable	Category	Normal	Mutation	Total
Age				
	<25 years	10	1	11
	25-39	17	1	18
	40-54	12	3	15
	55-69	34	1	35
	70+	90	4	21
	Total		10	100
Creatinine				
	low	1	0	1
	normal	6	1	7
	high	83	9	92
Urea				
	Low	6	1	7
	Normal	31	3	34
	high	53	6	59
Uric acid				
	Low	50	8	58
	Normal	22	1	23
	high	11	1	12
Sodium				
	low	51	6	57
	normal	33	4	37
	high	1	0	1
Potassium				
	Low	19	4	23
	Normal	53	5	58
	high	15	1	16
Chloride				
	Low	21	5	26
	Normal	62	4	66
	high	7	1	8
Calcium				
	Low	90	10	100
	Normal	0	0	0
	high	0	0	0

DISCUSSION

Chronic Kidney disease (CKD) represents a major health problem worldwide, as it is a potential risk for the development of renal failure (J. Wang et al., 2018). Several efforts have been made to reduce the burden of kidney failure through the control of CKD and its associated risk factors.

In Saudi Arabia, CKD represents an alarming health problem (GINAWI, AHMED, et al., 2013)[8] due to the progressive uprising of its risk factors, particularly type 2 diabetes mellitus (Ginawi et al., 2014), hypertension (Gadelkarim, Abdelmajeed, & Mohamed, 2014) and obesity (Ahmed et al., 2014).

In the present study, a group of patients with CKD was randomly selected regardless of sex and age and underwent a diverse analysis included biochemical and mineral measures in addition to molecular testing for identification of uromodulin gene mutation. The ultimate goal of the investigation to assess, whether the biochemical (Creatinine, Urea, Uric acid and Glucose)and minerals (Na^+, K^+, Cl^- and Ca^{++}) values differ according to the sex of the patients. This in addition to the possible association between UMOD gene mutation and certain patterns of biochemical and minerals measures.

With regard to the Creatinine values, 95.3% of the males were found with high values compared to 86% of the females, the RR (95% CI) = 1.1069(0.9603 to 1.2757), P = 0.1611. This in line with literature that Creatinine and GFR were higher among males compared to females (Fesler & Mimran, 2011; Hafeez, Idrees, & Akhtar, 2016).

With regard to the urea values, 71.9% of the males were found with high values compared to 33.3% of the females, the RR (95% CI) = 2.1563 (1.3253 to 3.5082), P = 0.0020. These findings show that urea values have statistically significant differences among males compared to females. Although there no sex difference in the reference range of urea, regardless of other factors such as age, but we did not find any study in this context during the review of the literature. In view of these findings, we suggest the significance of blood urea as screening predictor for CKD in males with high risk for developing CKD.

For uric acid, it didn't show any significant value among males compared to females.

Though high blood glucose levels were identified among 70.3% of the males and 61% of the females, there is no significant difference with sex.

Furthermore, low sodium levels were detected in 62.5% of the males and 50% of the females, which didn't show a significant difference. Though both high and low sodium intakes were associated with increased risk for CKD, in people with hypertension (Yoon et al., 2018), the association between low sodium level and renal sex in patients with impaired kidney function remains unclear.

With regard to Potassium levels, we found that low K^+ levels were significantly associated with females compared to males, the RR (95% CI) = 2.819 (1.1066 to 5.0773), P = 0.0264. Prolonged hypokalemia increased intracellular ATP, cell cycle arrest and cell death in renal tubular cells, which might be responsible for mechanisms underlying the development of hypokalemic nephropathy (Fong-Ngern, Ausakunpipat, Singhto, Sueksakit, & Thongboonkerd, 2017; Kieneker et al., 2017). However, few evidence suggests a link between hypokalemia more extent among females compared to males. Some studies have indicated that the risk of hypokalemia in patients with CKD is higher among females compared to males; hence, the risk of hyperkalemia is higher among males compared to females (Hawkins, 2003; Kleinfeld, Borra, Gavani, & Corcoran, 1993;

Korgaonkar et al., 2010; H. H. Wang et al., 2013; Wysowski, Kornegay, Nourjah, & Trontell, 2003).

Although low Cl^- was associated with increased mortality and risk of cardiovascular events in pre-dialysis CKD patients (Mandai et al., 2017), the present study showed no statistical difference between males and females. However, there is a lack of literature in this context.

All of the study subjects in the present study have shown low serum Ca++. Disorders in calcium metabolism are common in patients with CKD, but whether they are associated with subsequent kidney function deterioration is unclear (Janmaat et al., 2018).

Uromodulin belongs to a group of autosomal dominant inherited diseases caused by mutations in the UMOD gene, which codes for uromodulin, a Tamm-Horsfall protein (THP) entirely expressed in renal tubular cells of the ascending limb of the loop of Henle (Satanovskij et al., 2017). In the present study, about 10% of the patients were found with UMOD gene mutation. Previous studies have reported a prevalence of 7% to 20% of patients with CKD (Onoe et al., 2016; Smith et al., 2011).

In the present study, the majority of patients were found with high levels of creatinine and urea, which indicates the deterioration of kidney function associated with the loss of uromodulin. On the other hand, 80% of the patients with mutations were found with low uric acid levels. It was reported that the excretion of uromodulin correlates with clinical, genetic, and urinary factors. The strongest associations were between uric acid, sodium, and uromodulin excretions and are likely linked to the extracellular volume status (Troyanov et al., 2016). The gene SLC2A9 encodes for GLUT9, an important proximal tubule transporter of uric acid. Polymorphisms of the gene have been linked to gout susceptibility and to hereditary hypouricemia. Familial childhood gout with progressive renal impairment attributable to mutations of the uromodulin (UMOD) gene is associated with reduced uromodulin in the proximal tubule cilia (Janmaat et al., 2018).

In this study, about 60% and 100% of the patients with UMOD gene mutation were found with low Na+ and Ca++. Disorders in calcium metabolism are common in patients with CKD, but whether they are related to later kidney function deterioration is uncertain (Janmaat et al., 2018). It was reported that Hypocalcemia, hyperphosphataemia and elevated serum PTH levels are noted in later CKD stages & worsen with disease progression (Rouf et al., 2018). High urinary calcium excretion has been shown to lead to accelerated renal function decline in individuals with renal tubular diseases (Taylor et al., 2017).

The limitation in the present study includes the absence of urine uromodulin estimation.

In conclusion: CKD is a major health problem in Saudi Arabia due to the comprehensive presentation of major risk factors, such as hypertension diabetes, obesity, and genetic susceptibility. High blood urea is suggested as screening predictor for CKD in males with high risk for developing CKD. Low K+ levels were significantly associated with females compared to males. Patients with UMOD gene associated CKD are more susceptible to be with low serum Uric acid, Sodium and Calcium.

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