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Assessment of Serum Levels of Total Bile Acids in Chronic Kidney Disease Patients on Hemodialysis with and without Uremic Pruritus

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Abstract

Chronic kidney disease patients undergoing hemodialysis are frequently suffering from uremic pruritus, which can be quite uncomfortable. Uremic pruritus affects over 40% of hemodialysis patients. Bile acids have been implicated in the pathogenesis of pruritus; especially in conditions associated with cholestasis. The purpose of the study is to investigate the serum levels of total bile acids in chronic kidney disease patients on hemodialysis, with and without uremic pruritus. Ninety adults, chronic kidney disease patients who were on chronic hemodialysis in the hemodialysis unit at Al-Fallujah Teaching Hospital/Fallujah City; Iraq were involved. Patients were grouped equally in two groups; according to the presence or absence of pruritus, based on the itching severity scale. Serum levels of total bile acids, urea, creatinine, calcium, phosphorus, and intact parathormone were measured before and after the hemodialysis session. The serum level of total bile acids in the pre-dialysis session samples were non-significantly higher in patients with uremic pruritus compared to those without uremic pruritus [179.89(40.8) µmol/L and 175.28 (34.2) µmol/L; respectively]; P=0.56. Serum levels of total bile acids were not correlated with the patient's age, sex, and serum levels of urea, creatinine, calcium, phosphorous, or intact parathormone; p>0.05. Finally, serum levels of total bile acids were reduced by dialysis, and the serum levels of patients with uremic pruritus were significantly higher than those in patients without uremic pruritus in the post-dialysis session samples [133.99(23.69) µmol/L and 122.60(21.41) µmol/; respectively]; P=0.001. In conclusion, the hemodialysis procedure can be inadequate in the elimination of serum total bile acids and may need to be individualized based on the patient's condition and the initial serum total bile acids levels.

Keywords; Bile acids, Chronic kidney disease, Uremic pruritus, Hemodialysis, Itching.

Introduction

Uremic pruritus (UP), also identified as chronic kidney disease-associated pruritus, is a prevalent, troublesome, and devastating symptom experienced by chronic kidney disease (CKD) or end-stage renal disease (ESRD) patients. A comprehensive international cohort study, the Dialysis Outcomes and Practice Patterns Study (DOPPS), revealed that approximately 70% of adult hemodialysis patients experienced pruritus; the intensity of pruritus was at least moderate in 40% of them[(1)].

CKD has multiple causes, such as hypertension, diabetes mellitus, diabetic nephropathy, focal segmental glomerulosclerosis, and polycystic kidney disease[(2–7)].The prevalence of moderate to severe pruritus was noted to be approximately 25% in non-dialysis CKD patients[(8)], underscoring that this symptom extends beyond individuals undergoing dialysis. Several risk factors of UP in hemodialysis patients have been reported; however, results were inconsistent in different studies[(9,10)].

.thought to benefit greatly from maintenance hemodialysis[(18)].The study aims to investigate serum levels of total bile acids in CKD patients on hemodialysis with and without uremic pruritus.

Generally, itch transmission can be mediated by histaminergic and non-histaminergic pathways[(11)]. Due to the insufficient response to antihistamine treatment in UP, the non-histaminergic pathway is suggested to be important in UP. The pathogenesis of UP is not completely understood. The possible pathogenesis of UP is multifactorial and includes uremic toxins, immune dysregulation, neuropathy, and opioid imbalance[(12,13)].The role of parathyroid hormone, which also impacts the metabolism of bone may be responsible for UP[(14)]. Bile acids, the end products of cholesterol catabolism, are distinguished as immunoregulatory biological molecules with unclear targets[(15)]. Increasing evidence supports that bile acids functionalize beyond a lipid solubilizer and, more importantly, as signaling molecules in maintaining systemic homeostasis and the immune system[(16)]. The pathophysiology of pruritus has been linked to total bile acids, particularly in circumstances related to cholestasis[(17)]. Patients with such diseases are

Materials and Method

Ninety adult CKD patients, of both sexes, attending the hemodialysis unit at Al-Fallujah Teaching Hospital in Fallujah City-Iraq, were enrolled in this cross-sectional study; during the period extending from July to November; 2023. The selected patients were divided into two groups; the first group involved 45 patients with UP. The second group involved 45 patients without UP. The grouping of patients in the two groups was based on the itching intensity scale (ISS). Patients with the following conditions were excluded from the study: Patients with skin rash, primary skin disorders, systemic causes of pruritus including polycythemia Vera, chronic liver disease, thyroid and parathyroid diseases, malignancy, and neuropsychiatric disorders or communication problems, and those on antipruritic therapy were excluded from the study. Pre- and post-dialysis session blood samples (5ml) were collected from each participant to obtain serum. Serum samples were utilized for the measurement of levels of urea, creatinine, calcium, phosphorus, intact parathormone, and total bile acids. The FX CorDiax 80 (Fresenius Medical Care, Germany) was used in the hemodialysis that contains Helixone® high-flux membrane with a surface area of 1.8 square meters. The blood flow rate was 250 ml/min; the dialysate flow was 375 ml/min, and the dialysis session duration was three hours.

Serum total bile acid levels were measured by a colorimetric method, using a ready-made kit purchased from (Elabscince-USA). The assay principle was as follows:

Bile acids are dehydrogenated to 3-ketone steroids catalyzed by 3α-hydroxy steroid dehydrogenase in the presence of S-$NAD^{+,}$ which acts as a hydrogen receptor that is reduced to S-NADH. In the meantime, NADH is used as a hydrogen donor, and 3α-hydroxy steroid dehydrogenase catalyzes the reduction of 3-ketone steroids into bile acids. Through this enzymatic cycle reaction, S-NADH is continuously produced, which shows the maximum absorption peak at 405 nm. The change of absorbance is proportional to the concentration of bile acids.

Serum levels of urea, creatinine, and calcium were measured by colorimetric methods using the corresponding kits purchased from (Linear Chemicals- Spain). Serum phosphorus levels were measured by a colorimetric method, using a ready-made kit purchased from (Elabscince-USA). Finally, serum intact parathormone levels were measured by sandwich-type enzyme-linked immunosorbent assay using a kit purchased from (Cloud-clone Corp – USA).

Statistical Analysis

The statistical analysis was performed using the Statistical Package for Social Science (SPSS), version 26 software. Shapiro-Wilk test was used to check the uniformity of data distribution. The median and interquartile range (IQR) were used to present data on the continuous variables. Mann-Whitney test was used to compare the differences between medians of the two groups, and Wilikson test was used to compare the differences between medians of total bile acids before and after hemodialysis. Categorical variables were expressed as numbers (percent) and the difference was checked by chi-square test. Spearman's correlation was employed to assess the correlation among the studied variables. A P-value less than 0.05 was considered significant.

Results

There was no significant difference between patients in the two study groups about age and sex (P=0.58 and 0.52; respectively). The age range of the enrolled patients in the study was (18-82) years. The median (IQR) of patients with UP was [53 (24)]; while that of patients without UP was [56 (24)]. Out of 45 patients with UP, 24 (53.3%) were male and 21(46.7%) were female; while, 27(60%) of the patients without UP were male, and 18 (40%) were female "Table 1". According to the severity of pruritus, 5(11.1%) of patients in this group had mild pruritus, 19(42.2%) had moderate pruritus and 21(46.7%) had severe pruritus "Table 1".

Table 1. Demographic characteristics of patients

Variable	CKD without UP (n=45)	CKD with UP (n=45)	P-value
Age	[56 (24)]	[53 (24)]	0.580
Sex			
Male	27(60%)	24 (53.3%)	0.523
Female	18 (40%)	21(46.7%)	
Severity of pruritus			
Mild	-----	5(11.1%)	
Moderate	-----	19(42.2%)	
Severe	-----	21(46.7%)	

Where; n=number; CKD=chronic kidney disease; UP=uremic pruritus.

Serum levels of urea, creatinine, phosphorus, calcium, and intact parathormone were significantly reduced by hemodialysis, with P <0.001 for each; as shown in "Table 2". In the pre-dialysis session samples, serum levels of urea, calcium, phosphorus, and intact parathormone were not significantly

different between patients with UP and those without UP; P>0.05. Whereas, serum creatinine levels were significantly higher in patients with UP as compared to those of patients without UP, [9.8(5.5) and 8.15 (3.18); respectively], P=0.02; as shown in "Table 2".

Table 2. Biochemical characteristics of participants before and after the hemodialysis session

Variable	CKD without UP (n=45)	CKD with UP (n=45)	P-value
Urea (mg/dL)			
Pre-dialysis	[127 (50.15)]	[127 (74.75)]	0.75
Post-dialysis	[35.09 (4.45)]	[36 (5.8)]	0.217
P-value	<0.001*	<0.001*	
Creatinine (mg/dL)			
Pre-dialysis	[8.15 (3.18)]	[9.8(5.5)]	0.02*
Post-dialysis	[0.61 (0.24)]	[0.67 0.32)]	0.065
P-value	<0.001*	<0.001*	
Phosphorus (mmol/L)			
Pre-dialysis	[5.3 (1.65)]	[5.8 (3.3)]	0.18
Post-dialysis	[3.56 (0.44)]	[3.78 (0.36)]	0.015*
P-value	<0.001*	<0.001*	
Calcium (mg/dL)			
Pre-dialysis	[9 (0.8)]	[9.5 (1.4)]	0.08
Post-dialysis	[6.67 (1.24)]	[6.88 (0.92)]	0.771
P-value	<0.001*	<0.001*	
Intact parathormone (pg/ml)			
Pre-dialysis	[15.85 (9.8)]	[17.78 (17.8)]	0.39
Post-dialysis	[4.14 (2.95)]	[3.8 (2.45)]	0.455
P-value	<0.001*	<0.001*	

* Significant difference .Where n=number; CKD=chronic kidney disease; UP=uremic pruritus

Serum total bile acids levels of patients with and without UP were significantly reduced by hemodialysis, P<0.001; "Table 3". Serum total bile acids levels of patients with UP were higher than that of patients without UP; in both pre-and post-dialysis samples. However, the difference was significant in the post-dialysis samples only.

In the pre-dialysis session samples, serum total bile acids level was 179.89(40.76) umol/L in patients with UP, while the level was 175.28 (34.17) umol/L in patients without UP, P=0.56. In the post-dialysis, serum total bile acids level was 133.99(23.69) umol/L in patients with UP, while the level was 122.60 (21.41) umol/L in patients without UP, P=0.01 "Table 3".

Table 3. Serum levels of total bile acids pre- and post-hemodialysis session samples

	Serum total bile acids (umol/L)		P-value
	CKD without UP (n=45)	CKD with UP (n=45)	
Pre-dialysis	175.28 (34.17)	179.89(40.76)	0.56
Post-dialysis	122.60 (21.41)	133.99(23.69)	0.01*
P-value	<0.001*	<0.001*	

* Significant difference.Where n=number; CKD=chronic kidney disease; UP=uremic pruritus.

The pre-dialysis session serum total bile acids levels did not show any significant correlation with the measured variables, P >0.05 for each "Table 4".

Table 4. Correlation of pre-hemodialysis session serum total bile acids with the studied variables

Variable	ρ -value	P-value
Age	-0.107	0.315
Gender	-0.17	0.11
Urea	0.14	0.12
Creatinine	-0.32	0.52
Phosphorus	0.31	0.71
Calcium	-0.13	0.219
Intact parathormone	.016	0.879

Where: ρ = Spearman's correlation coefficient.

The post-dialysis serum total bile acids levels were positively correlated with serum urea, creatinine, and phosphorus levels, P<0.001 for each; "Table 5".

Table 5. Correlation of post-dialysis serum total bile acids with the studied variables

Variable	ρ -value	P-value
Age	-0.017	0.31
Gender	-0.17	0.71
Urea	0.236	0.02*
Creatinine	0.41	<0.001*
Phosphorus	0.81	<0.001*
Calcium	-0.13	0.87
Intact parathormone	0.16	0.16

*Significant difference.Where: ρ = Spearman's correlation coefficient.

Discussion

Several factors have been investigated as potential contributors to UP in dialysis patients with inconsistent findings[19,20].The exact pathogenesis of UP is not completely understood and several mechanisms have been proposed[12,13]. The impact of age and sex on the development of UP in CKD patients on chronic dialysis was inconsistent in the literature. Older age and male sex were reported to be risk factors for UP in some studies[21,22]. in contrast, others showed that younger age and female sex are associated with UP in dialysis patients[9,23].Yet, several studies reported no association between sex and the development of UP among patients of CKD[24,25]. Blood cholesterol level and subsequently its degradation products, bile acids, tend to increase with age. In men the increase in cholesterol level continues until the age of 45 to 55 years, then decreases; while in women it continues to increase until the age 55-56 years and then decreases[26]. In the present study patients with and without UP were of comparable age and sex, which eliminated the effect of these two variables on serum total bile acid levels in the development of UP in participants. The severity of pruritus in CKD patients can be mild, moderate, or severe. In an Indian cross-sectional study carried out on 120 eligible participants, 67 (55.83) of them had UP; the majority (73.1%) had mild pruritus, while moderate and severe pruritus was reported in (19.4%) and (7.5%); respectively[27]. Another cross-sectional study that was conducted in China

on 148 CKD patients on hemodialysis showed that 60 (40.54%) patients had UP; half of the patients with UP had moderate pruritus; while mild and severe pruritus was reported in (36.7%), and (13.3%) of the patients; respectively[28]. Furthermore, a cross-sectional study that was conducted in Pakistan on 173 male patients on hemodialysis observed that out of 85 (49.1%) of the patients had UP; (55.3%) of patients with UP had mild pruritus; while moderate and severe pruritus were reported in (34.1%), and (10.6%) of the patients; respectively[29]. In a single center, cross-sectional study performed in Iraq, out of 103 CKD patients on hemodialysis that were included in the study, 79 (76.7%) had UP of whom, 27 (34.1%) had mild pruritus, 30 (38%) had moderate and 22 (27.9%) had severe pruritus[30]. In the present study, a larger percentage (46.2%) of patients with UP had moderate pruritus, followed by severe (42.2%), and mild (11.1%) pruritus. The difference in serum levels of urea, creatinine, phosphorus, calcium, and intact parathormone among CKD patients with and without UP was the subject of several studies, with very variable findings. Hu *et al.* reported higher levels of all of those analytes in patients with UP as compared to those without UP[31]. Makhlough *et al.* reported elevated serum levels of blood urea nitrogen and intact parathormone only[32]; while

Tajbakhsh *et al.* reported abnormal serum calcium levels only[25]. The studies in this regard evaluated the post-dialysis session serum samples. In the present study, serum creatinine levels of the pre-dialysis session samples and serum phosphorus levels of the post-dialysis session samples were significantly higher in patients with UP. The disparity of findings regarding prevalence, severity of UP, and biochemical findings in CKD patients with and without UP among different studies may be attributed to the differences in the inclusion and exclusion criteria, the number of participants, the categorizing criteria of severity of pruritus, duration since the onset of hemodialysis, and the type of dialysate and the semipermeable membranes used in hemodialysis; in different studies. The pathogenesis of UP is not fully elucidated and several hypotheses have been proposed in this regard[31]. Bile acids have often been suggested as key players in developing pruritus in liver disease. Meixiong *et al.* demonstrated that bile acids can trigger itch by binding to Mas-related G protein-coupled receptors (Mrgprs) in the sensory neurons' afferent terminals and dorsal root ganglia[33]. The itching experienced in liver failure and that in kidney failure share a common feature; the build-up of waste products in the blood has been implicated in both scenarios [34]. While the liver and intestine primarily eliminate bile acids, the kidneys also play a role through glomerular filtration and tubular secretion[35]. Consequently, bile acid levels in the blood rise in kidney failure, albeit to a lesser extent than in liver failure[36,37]. Research on the correlation between total bile acids levels and UP has reported inconsistent findings[36–38].In the present study, serum total bile acids levels were higher in patients with UP compared to those in patients without UP; however, the difference was significant in the post-dialysis session samples only and not in the pre-hemodialysis session samples. The lack of significant difference in serum total bile acids of the pre-dialysis samples between the study groups can be attributed to the build-up of the bile acids in blood due to overproduction or impairment of intestinal secretion, in both of the study groups. These two factors were not assessed as they were beyond the scope of the present study. After the hemodialysis session serum total bile acids were dependent on the efficiency of the dialysis components and procedure. The significant difference in serum total bile acids levels between the study groups post the hemodialysis session, along with the reported positive correlation of the serum levels of the total bile acids of the patients, may refer to the inadequacy or inefficiency of the hemodialysis procedure. Whether the relatively low number of patients in the two-study group, which is one limitation of the present study, has masked the association of total bile acids with UP at high concentrations needs to be verified. Other limitations of the study include the cross-sectional nature of the study which does not permit testing the causal relationship between total bile acids and UP. Furthermore, the effect of diet on the biochemical findings, and the residual renal function difference among participants were not considered because of the lack of cooperation of patients. The strength points of the present study include the controlling for clinical parameters and the measurement of the serum levels of the biochemical parameters pre- and post-the hemodialysis session that enables the examination of the accumulation of those biochemicals, as well as, the efficiency and adequacy of the hemodialysis procedure.

Conclusions

Serum total bile acid levels in CKD patients after the hemodialysis session are higher in patients with UP. The hemodialysis procedure can be inadequate in eliminating serum total bile acids and may need to be individualized based on the patient's condition and the initial serum total bile acids levels.

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Conflicts of Interest

None.

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Ethics statements

Ethical approval for the study was granted by the Ethics Committee of the College of Pharmacy, University of Baghdad, (RECAUBCP2572623 on 25/7/2023). All participants were informed about the aim and the expected benefits of the study; verbal consent was obtained from participants before being enrolled in the study.

Author contribution

The authors contributed equally to this work.

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تقييم مستويات احماض الصفراء الكلية في المصل لمرضى الكلى المزمن الذين يخضعون للديلزة الدموية المصابين بالحكة اليوريمية او غير المصابين

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[2]فرع العلوم المختبرية السريرية، كلية الصيدلة، جامعة بغداد، بغداد، العراق

الخلاصة

غالبا ما يعاني مرضى فشل الكلى المزمن الذين يخضعون لغسيل الكلى بشكل متكرر من الحكة اليوريمية، والتي تكون مزعجة للغاية. تؤثر هذه الحكة على أكثر من ٤٠٪ من المرضى الخاضعين للديلزة. لقد ارتبطت أحماض الصفراء بالمساهمة في ظهور الحكة؛ خاصةً في الحالات المرتبطة بالاستسقاء الصفراوي. تهدف الدراسة الى التحقق من مستويات أحماض الصفراء الكلية في مصل مرضى مرض الكلى المزمن الخاضعين للديلزة الدموية، المصابين بالحكة اليوريمية وغير المصابين. تم إشراك تسعين مريضا بالغًا من مرضى مرض الكلى المزمن الخاضعين للديلزة الدموية بشكل روتيني متكرر في وحدة الديلزة بمستشفى الفلوجة التعليمي/ مدينة الفلوجة؛ العراق. قسم المرضى بالتساوي إلى مجموعتين؛ وفقًا لوجود أو غياب الحكة، اعتمادًا على مقياس شدة الحكة. تم قياس مستويات أحماض الصفراء الكلية واليوريا والكرياتينين والكالسيوم والفوسفور وهرمون الغدة الجاردرقية التام في المصل قبل وبعد جلسة الديلزة. أظهرت النتائج ان مستوى أحماض الصفراء الكلية في عينات ما قبل الديلزة أعلى ولكن بشكل غير معنوي احصائيا لدى المرضى الذين يعانون من الحكة اليوريمية مقارنةً بأولئك الذين لا يعانون منها [١٧٩,٨٩(٤٠,٨) ميكرو مول/لتر و١٧٥,٢٨ (٣٤,٢) ميكرو مول/لتر؛ على التوالي]؛ قيمة الاحتمال = ٠,٥٦. ولم تكن هناك علاقة بين مستويات أحماض الصفراء الكلية في المصل

وعمر المريض وجنسه ومستويات اليوريا والكرياتينين والكالسيوم والفوسفور هرمون الغدة الجاردرقية التام؛ قيمة الاحتمال <٠,٠٥. أخيرًا، انخفضت مستويات أحماض الصفراء الكلية في المصل عن طريق الديلزة، وكانت مستويات المرضى الذين يعانون من الحكة اليوريمية أعلى بشكل ملحوظ من تلك في المرضى الذين لا يعانون من الحكة في عينات ما بعد الديلزة [١٣٣,٩٩(٢٣,٦٩) ميكرو مول / لتر و١٢٢,٦٠ (٢١,٤١) ميكرو مول/لتر؛ على التوالي]؛ قيمة الاحتمال= ٠,٠٠١. خلصت الدراسة الى ان إجراءات عملية الديلزة الدموية المتبعة قد تكون غير كافية في التخلص من احماض الصفراء الكلية وقد تحتاج إلى التخصيص بناءً على حالة المرضى ومستويات أحماض الصفراء الكلية قبل عملية الديلزة.

الكلمات المفتاحية: احماض الصفراء، مرض الكلى المزمن، الحكة اليوريمية، الديلزة الدموية، الحكة.