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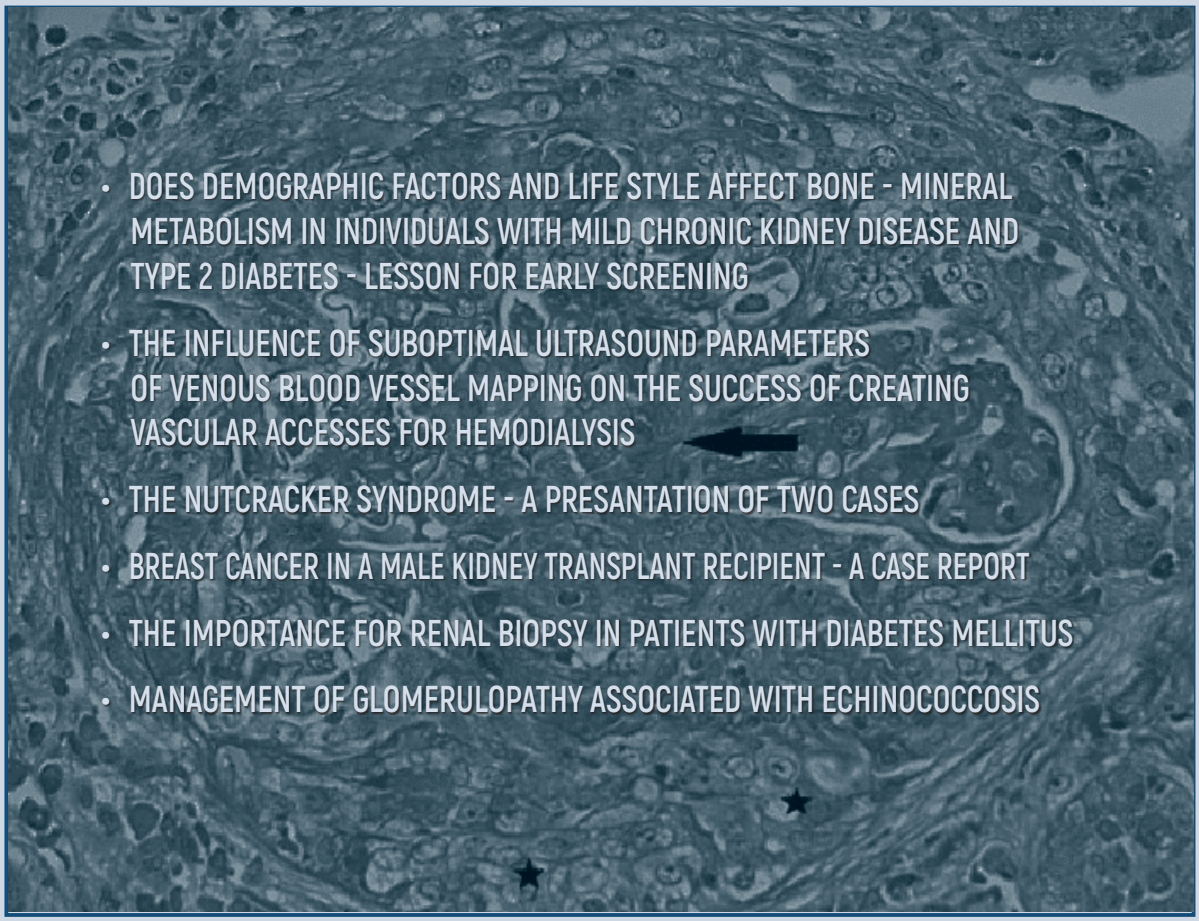

- DOES DEMOGRAPHIC FACTORS AND LIFE STYLE AFFECT BONE - MINERAL METABOLISM IN INDIVIDUALS WITH MILD CHRONIC KIDNEY DISEASE AND TYPE 2 DIABETES - LESSON FOR EARLY SCREENING
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Original article

Does Demographic Factors and Life Style Affect Bone - Mineral Metabolism in Individuals with Mild Chronic Kidney Disease and Type 2 Diabetes - Lesson for an Early Screening

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Abstract

Introduction. Type 2 diabetes mellitus (T2DM) is a pandemic disease and the main cause of development of chronic kidney disease (CKD). The question of whether poor bone mineral metabolism (BMM) affects morbidity in individuals with T2DM and early CKD is a paradigm in science. There is a conflicting information regarding how lifestyle and demographic factors (DF) affect BMM.

Methods. We have conducted a clinical prospective study on 31 patients with T2DM and CKD with eGFR= 45-70ml/min/1.73m2. The patients were examined for specific demographic data (gender, age, Body mass index (BMI), T2DM duration and smoking status) and BMM-specific characteristics (vitamin D, parathormone, phosphorus, calcium, and alkaline phosphatase).

Results. Analysis of T2DM and CKD patients with eGFR 45-70 ml/min/1.73m2 according to the selected bone mineral parameters indicated significantly higher vitamin D values in patients aged ≤65 years compared to >65 years (p=0.010). Borderline but non-significantly higher phosphorus values were determined in patients with T2DM >10 years. A significantly positive moderate correlation was found between DMT2 duration and phosphorus level (R=0.471; p=0.007). Although not significant, duration of DMT2 was negatively associated with the serum calcium levels (R=-0.107; p=0.564).

Conclusion. These data indicate that the amount of phosphorus in the blood varies with the length of T2DM. It is verified that the concentration of vitamin D falls with the advancing age. Although, there were no other significant correlations, a larger research on a greater cohort of patients with more precise criteria is required for further analysis on the topic.

Keywords: Diabetes mellitus, Bone Mineral Metabolism, phosphorus, calcium, vitamin D

Introduction

T2DM is a long-term, multisystemic metabolic disease. The WHO reports that this condition is more common worldwide in low- and middle-developed nations, indicating the impact of lifestyle [1]. Even with the most advanced treatment, cardiovascular events account for death in over 65% of diabetic patients. Two-thirds will get diabetic retinopathy and/or CKD [2,3].

T2DM illness accounts for 30-50% of all cases of CKD and is the primary cause of both its onset and development. There is a substantial overlap between T2DM and CKD; around 40% of people with DMT2 will also have CKD, and vice versa. This further raises the cumulative incidence of morbidity and death from cardiovascular disease [2,4].

Determining if these two entities share any pathophysiological processes for the progression and/or consequences of illness is becoming a more and more intriguing task. In recent years, the scientific community has placed a strong emphasis on BMM. Disturbed homeostasis of calcium and phosphorus, their regulators parathormone (PTH), FGF23, Klotho, alkaline phosphatase (AF), vitamin D, disturbed bone remodeling and structure and the appearance of soft tissue calcifications and/or vascular calcifications are some of the affected parameters of BMM in patients with CKD [5,6]. Disordered BMM associated with CKD (CKD-MBD) as an entity that unites these parameters, imposes the need to study whether these biomarkers can be used as predictors for the development of chronic complications in some stages of the diseases or are themselves a consequence of T2DM and/or CKD [7,8].

There is currently little information consistent about how T2DM duration, gender, age, body weight, and smoking affect BMM.

The aim of our study was to identify any potential variations or interactions between the parameters of relevance for BMM, demographic factors and a few general parameters in individuals with T2DM and mild CKD.

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Material and methods

Our research represents a clinical prospective study that was conducted in the period from 2022-2023 in the diabetes center at the General City Hospital (GCH) 8th of September in Skopje. The sample consisted of patients with T2DM and early stage CKD with an eGFR value between 45-70 ml/min/1.73m2. They were chosen using a straightforward random selection process.
T2DM was diagnosed based on the following criteria: random glycaemia >11.1 mmol/l and/or HbA1C ≥6.5%, glycaemia in the second hour of OGTT with 75g of glucose above 11.1 mmol/l, and/or fasting plasma glucose ≥7.0 mmol/l. The CKD-EPI creatinine equation (2021) was used to calculate eGFR (https://www.mdcalc.com/calc/3939/ckd-epi-equations-glomerular-filtration-rate-gfr).
Patients were examined in relation to selected general factors (gender, age, BMI, T2DM duration, and smoking status) and the following parameters of importance for BMM (alkaline phosphatase, calcium, phosphorus, PTH, and vitamin D). After a 12-hour fast, blood and urine samples were collected, and laboratory analyses for bone mineral parameters were carried out at the central laboratory using the established methods.
The research was voluntary with previously signed informed consent of the participants, after prior approval by the Ethics Committee of the hospital.

Statistical analysis

The data obtained with the research were processed in SPSS software package, version 22.0 for Windows. Quantitative series were analyzed with relationship coefficients, proportions and rates, and quantitative series with measures of central tendency (average, median, minimum and maximum values), as well as measures of dispersion (standard deviation). To ascertain if the frequency distribution of the variables under study was normal, the Shapiro-Wilk W test was employed. The Wilcoxon Signed Ranks Test was used to parameters that had an irregular frequency distribution. To ascertain the connection with a statistically significant difference between two or more independent quantitative parameters having an irregular frequency distribution, the Kruskal-Wallis test: H test and the Mann-Whitney U test were utilized. Spearman rank correlation was used to determine the association between numerical variables with irregular frequency distributions. A two-sided analysis with a significance level of $p<0.05$ was used to determine statistical significance.

Results

A total of 31 patients were processed in the study of which 16 (51.6%) were female with a gender ratio of 1,1:1. ($p>0.05$). The average age of the subjects in the sample was 65.8±7.9 with a range of 38-76 years. The female and male respondents had an average age of 68.2±5.5 with a range of 59-76 years vs. 63.3±9.4 with a range of 38-72 years.
Of all respondents, 11 (35.5%) had a positive smoking status. The average duration of T2DM was 12.3±8.4 years, with a range of duration from 1-40 years and 50% of subjects with duration of T2DM ≤12.3 years. The mean BMI was 28.8±3.6 kg/m2, with 50% of subjects having BMI ≤28.5 kg/m2.
A significant difference in vitamin D levels was found only between patients under 65 and those over 65, when the selected bone mineral parameters were compared based on demographic factors. Patients under 65 also had considerably higher values of this parameter. A borderline non-significant difference was observed between different durations of T2DM and phosphorus values in addition to significantly higher values of this parameter in patients with T2DM >10 years (Table 1a-1b).

Table 1a. Comparison of selected bone mineral parameters according to demographic parameters

Parameters	N	Mean± SD	Min/Max	Median (IQR)	p
Vitamin D					
Female	16	43.6±23.2	14.0/93.9	41.2(27.0-51.9)	Z=-0.949; p=0.3428
Male	15	52.8±25.3	24.6/104.6	43.8(36.6-61.8)	
Age - ≤65	12	62.4±26.6	34.6/104.6	51.4(41.2-90.4)	Z=-2.572; p=0.0100*
Age >65	19	38.9±18.0	14.0/93.9	37.6(26.8-50.3)	
Smoking - No	20	45.6±26.0	14.0/104.6	42,9(27,0-51,2)	Z=-0.928; p=0.3529
Smoking - Yes	11	52.4±21.1	29.3/91.5	43.8(36.5-61.8)	
T2DM: <5 years	10	35.5±14.7	14.0/61.8	37.0(24.6-43.8)	
T2DM: 5-10 years	6	57.8±28.8	20.0/93.9	51.4(41.0-89.4)	X^2=4.00; df=2; p=0.1353
T2DM: >10 years	15	52.5±25.6	26.8/104.6	44.9(34.6-54.3)	
Parathormone					
Female	16	67.3±31.6	19.4/116	67.8(43.5-97.8)	Z=-0.178; p=0.8588
Male	15	69.5±25.1	28.6/113	73.3(46.6-94.2)	
Age - ≤65	12	62.6±18.5	28.6/19.4	61.8(48.0-76.8)	Z=0.872; p=0.3832
Age > 65	19	72.1±32.9	19.4/116.4	73.4(41.4-98.3)	
Smoking - No	20	70.7±32.8	19.4/116.0	76.8(36.3-98.2)	Z=0.888; p=0.3747
Smoking - Yes	11	64.2±17.7	42.6/94.9	61.4(49.4-73.3)	
T2DM: <5 years	10	74.2±25.9	42.7/114.0	74.3(46.6-94.9)	X^2=0.800; df=2; p=0.6703

T2DM: 5-10 years	6	61.7±37.4	19.4/113.0	55.4(28.6-98.2)	
T2DM: >10 years	15	67.2±17,0	21.1/116.0	73.4(41.4-77.9)	
Phosphorus					
Female	16	1.2±0.2	1.1/1.9	1.2(1.1-1.3)	Z=-0.336; p=0.7369
Male	15	1.2±0.3	0.6/1.9	1.2 (1.1-1.4)	
Age - ≤65	12	1.2±0.2	0.6/1.4	1.2(1.1-1.3)	Z=-0.507; p=0.6122
Age > 65	19	1.3±0.3	0.9/1.9	1.2(1.1-1.3)	
Smoking - No	20	1.2±0.3	0.6/1.1	1.2(1.1-1.3)	Z=-0.639; p=0.5222
Smoking - Yes	11	1.2±0.1	0.9/1.4	1.1(1.1-1.3)	
T2DM: <5 years	10	1.1±0.1	0.9/1.3	1.1(1.1-1.2)	
T2DM: 5-10 years	6	1.1±0.3	0.6/1.4	1.1 (1.0-1.3)	X^2=5.448; df=2; p=0.0656
T2DM: >10 years	15	1.3±0.3	1.1/1.9	1.2(1.2-1.4)	

Z=Mann-Whitney U Test, X^2=Kruskal-Wallis test: H test**,** *significant for p<0.,05

Table 1b. Comparison of selected bone mineral parameters according to demographic parameters

Parameters	N	Mean±SD	Min/Max	Median (IQR)	p
Calcium					
Female	16	2.3±0.1	2.1/2.5	2.4(2.5-2.4)	Z=-0.277; p=0.7820
Male	15	2.3±0.1	2.1/ 2.5	2.4(2.3-2.4)	
Age - ≤65	12	2.4±0.1	2.1/2.4	2.4(2.3-2.4)	Z=-0.791; p=0.4290
Age >65	19	2.3±0.2	2.1/2.5	2.3(2.2-2.4)	
Smoking - No	20	2.2±0.1	2.1/2.5	2.3(2.2-2.4)	Z=-1.445; p=0.1484
Smoking - Yes	11	2.4±0.1	2.3/2.5	2.4(2.3-2.4)	
T2DM: <5 years	10	2.3±0.1	2.2/2.5	2.4(2.3-2.4)	
T2DM: 5-10 years	6	2.3±0.1	2.1/2.4	2.4(2.3-2.4)	X^2=0.336; df=2; p=0.8452
T2DM: >10 years	15	2.3±0.1	2.1/2.5	2.3(2.2-2.4)	
Alkaline phosphatase					
Female	16	68.6±20.2	39/131	67(57-75.5)	Z=-1.385; p=0.1665
Male	15	76.7±27.6	32/151	78(60-86)	
Age - ≤65	12	71.7±10.9	57/89	75.5(60-80.5)	Z=-0.506; p=0.6122
Age >65	19	73.1±29.8	32/151	69.0(54-85)	
Smoking - No	20	70.3±23.5	39/151	67(57.5-79)	Z=-0.805; p=0.4208
Smoking - Yes	11	76.5±25.6	32/131	75(57-85)	
T2DM: <5 years	10	73.0±25.8	32/131	71.5(57-82)	X^2=1.1627; df=2; p=0.5591
T2DM: 5-10 years	6	64.2±16.6	42/89	63(54-74)	
T2DM: >10 years	15	75.6±25.9	39/151	75(60-82)	

Z=Mann-Whitney U Test**,** X^2=Kruskal-Wallis test: H test, *significant for p<0.05

The five chosen bone mineral parameters - vitamin D, PTH, phosphorus, calcium, and alkaline phosphatase, and the five demographic parameters - sex, age, BMI, duration of T2DM, and smoking status, were analyzed for their interrelationships (correlations) (Table 2 and Figure 1).

Table 2. Correlation between selected demographic and bone-mineral parameters

Bone-mineral parameters	Demographic parameters				
	Age (years)	Smoking status	Gender	Duration of T2DM	BMI
Vitamin D	R(31)=-0.248; p=0.1777	R(31)=0.173; p=0.3509	R(31)=0.173; p=0.3514	R(31)=0.234; p=0.2047	R(31)=-0.040; p=0.8294
Parathormone	R(31)=0.101; p=0.5891	R(31)=-0.166; p=0.3726	R(31)=0.031; p=0.8623	R(31)=0.020; p=0.9143	R(31)=-0.042; p=0.8214
Phosphorus	R(31)=0.0048; p=0.9845	R(31)=0.121; p=0.5178	R(31)=0.061; p=0.7429	R(31)=0.471; p=0.0075*	R(31)=-0,053; p=0.0777
Calcium	R(31)=-0.104; p=0.5759	R(31)=0.268; p=0.1445	R(31)=0.051; p=0.7867	R(31)=-0.107; p=0.5647	R(31)=-0.147; p=0.4277
Alkaline phosphatase	R(31)=-0.074; p=0.6905	R(31)=0.151; p=0.4179	R(31)=0.253; p=0.1701	R(31)=-0.003; p=0.9849	R(31)=-0.132; p=0.4800

Smoking status: no/yes; Gender: female/male, R=Spearman Rank order coreallations, *significant for p<0.05

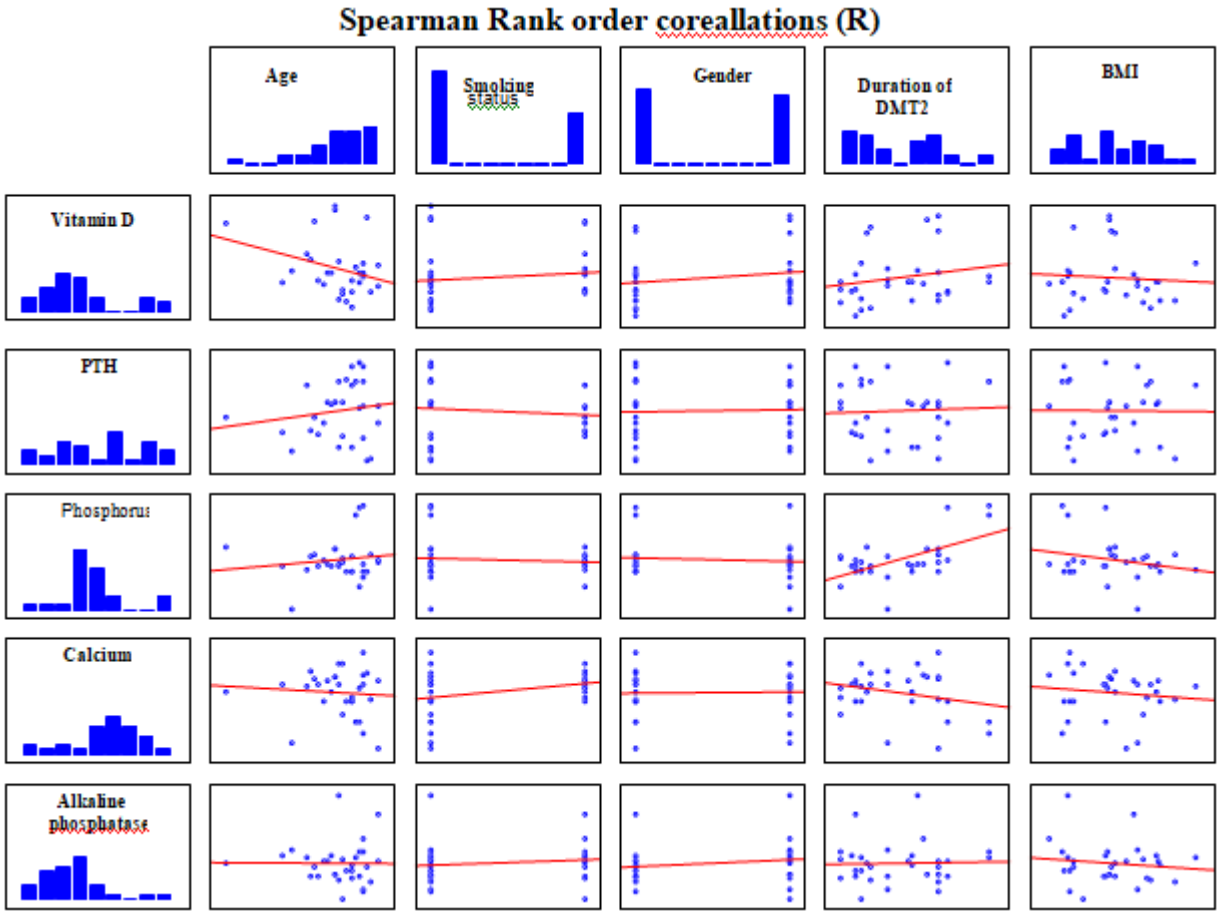

Fig. 1. Correlation between selected demographic and bone-mineral parameters

Age was found to have a non-significant, weak-linear negative association with three of the chosen bone mineral markers: vitamin D, calcium, and alkaline phosphatase. These values also showed a non-significant decline as age increased. Age and parathormone and phosphorus showed a non-significant linear positive association for parathormone vs. phosphorus; namely, these two parameters increased as age increased (Table 2 and Figure 1).

An insignificant, weak, linear positive correlation was found between the smoking status and four of the chosen bone mineral parameters: vitamin D, phosphorus, calcium, and alkaline phosphatase. The positive smoking status marginally increased the values of these parameters. There was a non-significant negative linear association between the parathormone and the positive smoking status; that is, the parathormone value fell non-significantly with the positive smoking status (Table 2 and Figure 1).

A non-significantly positive linear correlation was observed between the gender of the respondents and the value of the bone mineral parameters with a tendency to increase each of these 5 parameters in males (Table 2 and Figure 1).

The length of T2DM and the amount of phosphorus showed a strong positive moderate association; when DMT2 duration increases, phosphorus levels likewise rise noticeably. T2DM length did not significantly correlate with the other four bone-mineral metrics.

A non-significant linear negative connection was also found between BMI and each of the five bone mineral parameters under analysis, and these parameters showed a non-significant decline as BMI increased (Table 2 and Figure 1).

Discussion

It is well established that in patients with T2DM and CKD in all forms and stages, factors such as age, smoking status, obesity, and length of T2DM are strongly associated with the disease's development and risk of complications. How much they impact BMM and raise the chance of problems indirectly is yet unknown. To ascertain the degree of correlation between these demographic features and the values of BMM parameters in this population group, we included a group of patients with T2DM in the early stages of CKD in our study. Several BMM measures serve as biomarkers for the underlying disease's pace of development and early diagnosis of consequences. The topic of whether diabetic factors (DF) influence pathophysiological processes and, consequently, the values of the biomarkers,

is brought up by inconsistent findings and discrepancies in the results obtained in the examined groups, particularly in the early stages of the illness.

Gender and age impact

Research has repeatedly demonstrated a negative correlation between the cumulative incidence of fractures and the severity of renal illness. Fractures are more common in women in all phases of CKD, even those over 65 with comparatively maintained renal function and an eGFR of 45–59 ml/min/1.73m2 as opposed to those with an eGFR of 60 ml/min/1.73m2 or higher. Regardless of the severity of CKD, age and female gender have a significant influence on the disrupted BMM [9]. The results of a different meta-analysis that evaluated the relationship between smoking and the onset of a BMM disease show inconsistent results. In contrast to the general understanding, men smokers regardless of age still seem to have a more noticeable loss of bone strength than female smokers, even if this difference is not statistically significant [10]. The analysis in the prior cohort research did not account for smoking and its effect on BMM.

Our research supported previous findings that blood vitamin D content is inversely correlated with age above 65. This was the only significant correlation found between age and any of the BMM parameters that we chose to examine. A non-significant but still linear negative correlation was found between age and calcium and alkaline phosphatase. Additionally, there was a small but positive linear association found between age, phosphorus, and PTH. With the increasing ages, phosphatemia and PTH rise while levels of vitamin D, calcium, and AP fall. Previous statements are important and it means that patients with T2DM should be screened for vitamin D level particularly in older age. Timely supplementation may prevent further BMM disorders.

In the ratio 1:1.1, there was an equal representation of men and women. There was no other difference between the gender except for a statistical trend that was not statistically significant for men to increase each of these five measures.

The impact of smoking status

There is a conflicting information about how smoking affects BMM parameters. It has been noted that smoking is linked to a decreased bone density and intestinal absorption of calcium and vitamin D in a number of researches with various designs [11,12]. This would suggest that PTH will rise as well, however other research have produced conflicting findings. The fact that PTH levels in smokers are lower than in non-smokers after quitting is marked evidence that smokers have lower serum PTH levels than non-smokers [13].

A recent meta-analysis examined the impact of smoking on vitamin D levels and supplementation. It concluded that smoking was linked to reduced vitamin D levels regardless of age, even in cases when vitamin D supplementation was present [14]. There's no doubt that smoking has an impact on serum phosphorus levels. Smokers with T2DM had blood levels of triglycerides and phosphorus greater than non-smoking T2DM patients, irrespective of the age or gender [15]. The results also imply that smokers could have reduced bone mineralization and/or increased bone resorption, which has been shown to result in higher phosphorus levels [16]. An independent relationship between smoking and serum phosphorus was found in another study that examined CKD-MBD parameters. This relationship persisted even after controlling for the CKD grade factor, although it did not hold true for other CKD-MBD components including FGF23 and Klotho [17]. The results of another study on the effects of smoking on the same parameters are in conflict with these findings [18].

Thirty-five % of the participants in our research were smokers. No parameter showed a significant impact, yet there was a weak but linear negative link between smoking and PTH value and a weak but positive correlation between smoking status and vitamin D, phosphorus, calcium, and AF. Lack of statistical significance may be viewed as result of the number of patients but, one should be quite aware that patients with T2DM and smoking habits may negatively impact MBD and particularly potentiate adynamic bone disease in diabetic patients.

BMI impact

Obesity has a well-established impact on overall health, particularly in individuals with T2DM, where it is thought to play a major role in the development of the illness through the mechanism of insulin resistance. Analysis of adipose tissue's endocrine impacts has been done for a very long time. Studies on its effects on mineral balance, both clinical and experimental, are beginning to corroborate the link between obesity and disturbance of BMM.

New research suggests that adipokines regulate systemic mineral homeostasis via endocrine mechanisms involving leptin, interleukin-6, and now adiponectin. These processes interact with vitamin D and FGF23 to modify the renal regulation of calcium metabolism, as well as phosphate [19]. There is a strong evidence that the overall population, not only those with T2DM and CKD, has a connection between fat and mineral metabolism. Therefore, striving for a balance between obesity and mineral homeostasis is crucial for optimal functioning of both systems [20,21]. There are opposing viewpoints according to which they are adversely associated [22].

On the other hand, the reverse process is also described, i.e. the modulatory effect of the mineral status on the metabolism of adipose tissue, and the new clinical evidence regarding the role of disturbed mineral balance on fat loss in the general population and patients with CKD and T2DM is highlighted [23,24]. By defining these regulatory mechanisms, it is hoped that the complicated imbalance regulating calcium and phosphorus mineral homeostasis and its dysregulation in diseases associated with obesity will be better understood, thereby improving the predictability and prevention of complications in patients with T2DM. The average height of BMI in our study was 28.8±3.6 kg/m2, which puts it in the undernourished category. This study found a non-significant linear negative association between the five BMM parameters and BMI height. Still, we should be aware that BMI control is pivotal for diabetic patients not only from diabetic point of view but also from BMM point of view.

Impact of T2DM duration

While some authors confirmed that the duration of T2DM has no influence on BMM and its parameters, others rejected this thesis and showed that the duration and the therapy modality have an influence on BMM [25,26]. Recent targeted studies have already detected independent predictors of fractures, regardless of the age and disease duration [27].

In our study, the mean duration of T2DM was 12.3±8.4 years, and duration of T2DM was shown to significantly increase the serum phosphorus. Although insignificant, a tendency to decrease serum calcium is also observed with the increase in the duration of T2DM.

According to the information above, this study not only reveals a substantial correlation between DF and bone markers, but it also demonstrates a significant positive link just for the duration of T2DM and serum phosphorus.

Despite the limited size of the analyzed group, the ultimate objective is to observe the effect of DF during the early stages of the disease while the BMM parameter values are still normal or noticeably changed. For this analysis, we used data that are available in almost every clinic for either endocrinology or nephrology. Early screening for BMM parameters is very important and an early detection and intervention may prevent serious complication within the syndrome of BMD.

Conclusion

We may conclude from the reported data that the length of T2DM can raise serum phosphate concentrations and cause alterations in BMM. The length of T2DM was inversely correlated with serum calcium levels, but not significantly. The trend of its decline with the duration of T2DM is apparent, however not statistically significant.

Significantly, vitamin D levels also decline with the age. Nevertheless, we cannot conclude that this is due to T2DM directly, as healthy individuals also exhibit same declines in vitamin D concentration within the aging process.

Considering the size of the studied group, additional research with a larger number of respondents is required in order to be able to draw a final conclusion, even though the remaining studied parameters from BMM did not show a significant difference compared to all the studied demographic characteristics.

Conflict of interest statement. None declared.

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Original article

The Influence of Suboptimal Ultrasound Parameters of Venous Blood Vessel Mapping on the Success of Creating Vascular Accesses for Hemodialysis

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Abstract

Introduction. Vessel mapping in routine preoperative imaging has substantially increased the percentage of successful AVFs according to the Fistula First trend. As part of the mapping procedure, both arteries and veins are examined, and the successful creation of vascular access requires favorable color duplex ultrasound examination (CDS) criteria. The growing number of people suffering from diabetes and hypertension, as well as elderly people, requires finding a solution even in the scenario of unfavorable ultrasound parameters. The aim of our study was to determine the ultrasound parameters of veins during preoperative examination and to analyse the influence of ultrasound parameters of veins on the success in creating vascular access, accordingly.
Methods. In a retrospective, cohort study we included 135 patients treated at the Clinical Department of Nephrology and Metabolic Disorders with Dialysis "Professor Dr. Vasilije Jovanovic", Zvezdara University Medical Center in Belgrade, and provided with vascular access (VA) for HD in the period from January 2016 to December 2018. Preoperative mapping of the blood vessels of the hands was performed according to a standard protocol in accordance with the KDOQI guidelines for vascular access. Blood samples were taken for analysis on the day before surgery. Vascular access maturation was considered successful when the first hemodialysis puncture was performed with an adequate blood flow of ≥200ml/min, without an additional surgical intervention in the postoperative period.
Results. Total of 135 patients with end stage kidney disease (ESKD) were included, 89 males and 46 females. The most common causes of kidney failure were hypertension (48; 35.6%) and diabetes (40; 29.5%). The subjects with favorable CDS characteristics were significantly older. In addition, a significantly higher percentage of patients with favorable CDS characteristics of veins was found in patients over 65 years, as well as in men compared to women. Mature VA at the first puncture was registered in 105(77.8%) patients. Successful maturation was observed more often in men than in women, with larger inner diameter of venous blood vessels and in patients who took antiplatelet therapy in the immediate postoperative period. On the other hand, significantly lower success in the maturation of VA was found in diabetics. Cox's regression analysis found that with the use of antiplatelet therapy, the chances of unsuccessful maturation are increased by 3.6 times, and with an increase in the internal diameter of the vein by 1 mm, the chance of failure in maturation of VP decreases by 43%. Successful creation of VA was observed in 43% of patients with unfavorable at least one CDS parameter as well as in 52% of patients with diabetes, which indicates the delicacy of strict adherence to recommendations.
Conclusion. More effort, skill, and persistence are needed with aim to create successful VA in the growing number of individuals with suboptimal vascular characteristics.

Keywords: Ultrasound preoperative examination, color duplex ultrasound examination, vascular access, arteriovenous fistula, maturation

Introduction

End stage kidney disease (ESKD) is the last, fifth stage of CKD, when estimated glomerular filtration rate (eGFR) is <15ml/min/1.73m2. Timely preparation for the start of any of the renal replacement therapy methods is then indicated [1]. The decision on the modality of treatment with dialysis and transplantation must be individual, with the patient being well informed. Hemodialysis (HD) is the most common modality treatment in Serbia, where in 2016 about 82.7% of patients with ESKD were treated with chronic HD [2]. Worldwide, more than 80% of patients are treated with HD [3]. The exceptions are Mexico, Colombia, Hong

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Kong and New Zealand, where most patients are treated with peritoneal dialysis. In Europe, the percentage of HD patients is similar to ours and in 2016 it was 84% [4]. Vascular access (VA) is a basic prerequisite for successful HD. Therefore, creating and maintaining VA is the most important part of clinical practice when starting dialysis. Native arteriovenous fistula (AVF) created in the forearm according to the Cimino-Brescia, as an anastomosis of the radial artery and cephalic vein, has the longest survival of all vascular accesses and the least complications, so it is the most common and preferred type of VA [5,6]. AVF maturation requires at least 3 weeks until the first puncture, but it is desirable that the maturation process be longer, up to 3 months. According to the annual report from 2016, in Serbia, of the total number of prevalent patients on hemodialysis, 77% had a native AVF, 1.9% had an AV graft, (AVG) 4.4% had a tunneled central venous catheter (CVC), and even 16.7% had a temporary CVC [2].

Due to an increase in the prevalence of patients of advanced age, obese patients, patients with vascular diseases and diabetes, as well as patients with multiple previous VA, the physical examination proved to be insufficient for location of suitable blood vessels for a successful creation and maturation of VA for hemodialysis in up to 25%- 50% of cases [7]. In the diagnostic procedure called 'vessel mapping', color duplex ultrasound examination (CDS) is, along with physical examination, the basic method of preoperative procedure before the creation of VA [8]. Vessel mapping in the routine preoperative imaging has markedly increased the percentage of successfully AVFs according to the Fistula First trend [9]. As part of the mapping procedure, both arteries and veins are examined, and the successful creation of vascular access requires favorable CDS criteria [8,10-12].

Data about preoperative examination were mainly related to defining criteria for the successful creation of VA [13]. The growing number of patients suffering from diabetes and hypertension, as well as elderly people, requires finding a solution even in the scenario of unfavorable ultrasound parameters. However, there is a lack of data how unfavorable or suboptimal ultrasound parameters of veins influence the successful creation of VA. Therefore, the objectives of our study were:

1. To determine the ultrasound parameters of veins during preoperative examination and, based on that finding, to analyse the influence of ultrasound parameters of veins on the success in creating VA
2. To determine the influence of other risk factors on the success in creating VA (age, gender, comorbidities and relevant medication).

Material and methods

Patients

We conducted a retrospective, cohort study that included 135 patients who were treated at the Clinical Department of Nephrology and Metabolic Disorders with Dialysis "Professor Dr. Vasilije Jovanovic", Zvezdara University Medical Center in Belgrade, who underwent VA for HD in the period from January 2016 to December 2018.

Inclusion criteria were: age 18 years, end-stage kidney disease - stage 5D, preoperative mapping of the blood vessels of the arms.

Exclusion criteria were: earlier stages of kidney disease - pre-emptive creation of VA.

Using standard medical documentation, we analyzed regular drug therapy, underlying disease and comorbidities. Among the medications, the use of anti-aggregation drugs, anticoagulants, ACEi and statins were included into analysis. Comorbidities included the presence of diabetes mellitus (DM), arterial hypertension (HTA) and cardiovascular diseases (CVD): previous myocardial infarction (MI), angina pectoris, (AP), cardiomyopathy (CMP), cerebrovascular insult (CVI), myocarditis (MCI) and peripheral arterial disease (PAD).

Patients who had favorable all CDS criteria of venous blood vessels for the creation of VA, were compared with those who had at least one unfavorable CDS criterion.

Blood vessel mapping and analysis: preoperative mapping of the blood vessels of the hands was performed according to a standard protocol in accordance with the KDOQI guidelines for vascular access, using CDS [9]. The examinations were performed by three trained doctors using a linear probe with a frequency of 7.5 MHz, on an ultrasound machine Sonoace brand, Model 8000SE.

Favorable CDS criteria of venous blood vessels for the creation of VP were defined as:

- inner diameter of the vein ≥2mm,
- compressibility with an ultrasound probe,
- the absence of accessory veins with an internal diameter ≥1mm at a distance of <10cm from the anastomosis,
- vein depth less than 6mm,
- regular morphology of the vein.

Blood samples were taken for analysis on the day before surgery, and analyzed by standard laboratory techniques. The following analyses were included: calcium, phosphorus, intact parathyroid hormone (iPTH), glucose, c-reactive protein (CRP), cholesterol, triglycerides and hemoglobin.

VA maturation was considered successful when the first hemodialysis puncture was performed with an adequate blood flow of ≥200ml/min, without any additional surgical interventions required in the postoperative period.

Statistical analysis

Statistical analysis was performed using the Statistical

Package for Social Sciences 22.0 (SPSS Inc., Chicago, Illinois, USA). Data are presented as means ± standard deviation (SD) for continuous variables and percentages for categorical variables. Differences between groups were analyzed using: T-test, Mann Whitney test of independent pairs, Kruskal Wallis and Pearson's $\chi 2$ test. Spearman's correlation test was used to correlate the variables. The Kolmogorov-Smirnov test was used to assess the normality of the variables. All variables found to be statistically significant were entered into a COX regression test to assess the effect on maturation success. A p value of less than 0.05 was considered as statistically significant.

Results

Out of the total of 135 patients with ESKD, 89 patients were male and 46 were female. The most common causes of kidney failure were hypertension (48; 35.6%), diabetes (40; 29.5%), polycystic kidney disease (12; 8.9%), obstructive nephropathy (12; 8.9%), glomerulonephritis (12; 8.9%) and other (12; 8.9%).

In 87 (64.4%) patients favourable CDS characteristics of venous blood vessels were registered, while in others those characteristics were below the values recommended by the guidelines (Table 1).

Table 1. Frequency of individual CDS characteristics of veins in study population

CDS characteristics of veins		No (%)
Vein compressibility	Compressible	133(98.5%)
	Uncompressible	2(1.5%)
Vein depth	<6mm	129(95.6%)
	>6mm	6(4.4%)
Accessory veins	No	116(85.9%)
	Yes	19(14.1%)
Vein morphology	Regular	130(96.3%)
	Pathological	5(3.7%)
Vein inner diameter	≥2mm	115(85.2%)
	<2mm	20(14.8%)
CDS characteristics	Favourable	87(64.4%)
	Unfavourable	48(35.6%)

Data presented as number and percentage

Basic laboratory parameters in patients with favorable and unfavorable CDS characteristics are shown in Table 2.

Table 2. Laboratory parameters of patients with favorable and unfavorable ultrasound characteristics of veins

Parameter	Unfavorable CDS characteristics N=48	Favorable CDS characteristics N=87	P
Age, years	61±12	67±11	0.007
Ca, mmol/L	2.21±0.23	2.14±0.25	0.124
Phosphate, mmol/L	1.57±0.40	1.68±0.59	0.286
iPTH, pg/mL	358±470	281±200	0.251
Hemoglobin, g/dL	9.48±1.60	9.26±1.39	0.407
CRP, mg/L	21.32±29.26	22.80±37.43	0.816
Cholesterol, mmol/L	4.1±1.3	4.2±1.2	0.821
Triglycerides, mmol/L	1.84±1.20	1.814±0.91	0.876
Glucose, mmol/L	7.2±3.9	6.4±2.3	0.135
Time to puncture, days	16±11	19±10	0.084

Data presented as mean ±SD

Patients with favorable CDS characteristics were significantly older than patients from the group with unfavorable CDS characteristics. The other parameters did not show statistical difference.

During the first puncture, successful maturation of the VA was registered in 105 (77.8%) patients, while maturation was not successful in 30 patients (22.2%).

The comparison of basic parameters between these two groups of patients is presented in Table 3.

Table 3. General characteristics of patients with successful and unsuccessful maturation of vascular access for hemodialysis

		Successful VA Br=105	Unsuccessful VA Br=30	p
Age, years		65±12	66±14	0.681
Age limit, years	< 65g.	38(79.2%)	10(20.8%)	0.865
	≥ 65g	67(77.9%)	20(22.1%)	
Gender	Female	31(67.4%)	15(32.6%)	0.037
	Male	74(83.1%)	15(16.9%)	
VA location	Forearm	92(77.3%)	27(22.7%)	0.722
	Cubital	13(81.3%)	3(18.7%)	
Order of creation of VA	First VA	86(79.6%)	22(20.4%)	0.301
	Next VA	19(70.4%)	8(29.6%)	

Data presented as number and percentage

Significantly more successful maturation of VA was found in men compared to women, while there were no differences in the success of maturation according to the age of the patient and between the older and younger patients (cut-off 65 years). Also, neither the location nor the sequence of creation had any influence on the maturation of VA.

A subsequent comparison included ultrasound parameters considered relevant to VA creation and maturation (Table 4).

Table 4. Ultrasound characteristics of veins in patients with successful and unsuccessful maturation of vascular access for hemodialysis

		Successful VA, No=105	Unsuccessful VA, No=30	p
CDS parameters	Favorable	72(82.8%)	15(17.2%)	0.061
	Unfavorable	33(68.8%)	15(31.2%)	
Vein compressibility	Compressible	103(77.4%)	30(22.6%)	0.446
	Uncompressible	2(100%)	0(0%)	
Vein depth	<6mm	101(78.3%)	28(21.7%)	0.503
	≥6mm	4(66.7%)	2(33.3%)	
Accessory veins	Yes	15(78.9%)	4(21.1%)	0.895
	No	90(77.6%)	26(22.4%)	
Vein morphology	Regular	101(77.7%)	29(22.3%)	0.903
	Pathological	4(80%)	1(20%)	
Vein inner diameter	≥2mm	94 (81,7%)	21 (18,3%)	0,008
	<2mm	11 (55,0%)	9 (45,0%)	
Vein inner diameter average, mm		3,0±0,87	2,47±0,71	0,003

Data presented as number and percentage

According to the above data, only the internal diameter of the vein was significant, while the compressibility, depth of the vein, morphology of the vein and the presence of accessory veins were not shown to be significant for the successful maturation of the AVF. It should be noted that successful VA creation was observed in 43% of patients with unfavorable at least one CDS parameter.

Table 5. Applied therapy in patients with successful and unsuccessful maturation of vascular access for hemodialysis

Therapy		Successful VA, No=105	Unsuccessful VA, No=30	p
Antiplatelet therapy	Yes	67(89.3%)	8(10.7%)	<0.001
	No	35(62.5%)	21(37.5%)	
Anticoagulation therapy	Yes	11(91.7%)	1(8.3%)	0.227
	No	91(76.5%)	28(23.5%)	
Statins	Yes	20(80%)	5(20%)	0.775
	No	82(77.4%)	24(22.6%)	
ACE inhibitors	Yes	66(77.6%)	19(22.4%)	0.936
	No	36(78.3%)	10(21.7%)	

Data presented as number and percentage

The analysis shows that the maturation of the VA was significantly more successful in patients who took antiplatelet therapy in the postoperative period. Other types of therapy did not significantly affect the immediate outcome of VA creation.Table 6 shows that the presence of diabetes mellitus is significantly more frequent in patients who had a failure of VA maturation, while the presence of arterial hypertension and other cardiovascular diseases (myocardial infarction, angina pectoris, cardiomyopathy, cerebrovascular insult, myocarditis and peripheral arterial disease) did not influence VA maturation. Nevertheless, the actual creation of VA was observed in 52% of patients with diabetes.

Using COX regression analysis, the significance of antiplatelet therapy, internal vein diameter, diabetes, and gender on the immediate success of vascular access was examined (Table 7).

Table 6. Comparison of patients with successful and unsuccessful maturation of vascular access for hemodialysis according to the comorbidities

Comorbidity		Outcome: Successful No=105	Outcome: Unsuccessful No=30	p
DM	No	69(85.2%)	12(14.8%)	0.011
	Yes	36(66.7%)	18(33.3%)	
HTA	No	1(50%)	1(50%)	0.341
	Yes	104(75.3%)	29(24.7%)	
KV morbiditet	No	66(76.7%)	20(23.3%)	0.702
	Yes	39(79.6%)	10(20.4%)	

Data presented as number and percentage

Table 7. Risk factors for successful creation of vascular access for hemodialysis (COX regression analysis)

	p	OR	CI 95%
Antiplatelet therapy	0.002	3.602	1.585-8.186
Vein inner diameter	0.049	0.575	0.331-0.998
Diabetes mellitus	0.073	0.508	0.242-1.065
Gender	0.205	0.609	0.283-1.312

This analysis showed that, among the previously significant parameters, only the internal diameter of the vein and the use of antiplatelet are significant risk factors for a successfully created VA. Although the number of patients who had successfully created VA more frequently received antiplatelet therapy, Cox regression analysis revealed that antiplatelet therapy increases the chance of unsuccessful maturation of VA by 3.6 times. Also, with an increase in the inner diameter of the vein by 1mm, the chance of maturation failure of the VA decreases by 43%.

Discussion

Presented research established that subjects with favorable CDS characteristics are significantly older. Significantly higher percentage of patients with favorable CDS characteristics of veins was found in patients over 65 years, as well as in men compared to women. Mature VA at the first puncture was registered in 105 (77.8%) patients, while maturation was not successful in 30 patients (22.2%). Successful maturation was observed more often in men than in women, with larger inner diameter of venous blood vessels and in patients who took antiplatelet therapy in the immediate postoperative period. On the other hand, significantly lower success in the maturation of VA was found in diabetics. There was a tendency towards lower success in the maturation of VA in patients with unfavorable CDS characteristics, but without statistical significance (p 0.061). Cox's regression analysis found that with the use of antiplatelet therapy, the chance of unsuccessful maturation increases by 3.6 times, and with an increase in the internal diameter of the vein by 1 mm, the chance of failure in maturation of VP decreases by 43%.

According to the literature data, unsuitable properties of venous blood vessels for the creation of VA for hemodialysis, were confirmed as depth greater than 6 mm, accessory vein with internal diameter greater than 1 mm at a distance of less than 10 cm from the planned anastomosis and visibly altered morphology of the vein, internal vein diameter smaller than 2mm, i.e. below 2.5mm under compression (cuff) for AV fistulas, and >4mm for AV grafts, i.e. inadequate distensibility of the vein [10,14-16]. The results of these studies are consistent with the finding of a significantly larger internal vein diameter in subjects with favorable CDS criteria in the presented study. Patients with chronic kidney disease of older age have been described as a category in which there are a number of problems related to the creation and maintenance of VA for hemodialysis, primarily related to comorbidities (mainly cardiovascular) and altered morphology of blood vessels [17-19]. On the other hand, in the geriatric population, general mortality is high, and the expected life expectancy is short, which is the reason why VA planning in the elderly needs strategies such as an individual plan, placing access in the pre-terminal stage of kidney disease, as well as choosing an approach with the least chance of early complications. This is a strategy to avoid or minimize the use of central venous catheters, which carry the greatest risk of infection, especially in the category of patients with advanced age [20]. The finding that patients with favorable CDS characteristics of veins are significantly older in the present study leads us to the conclusion that this population of dialysis patients should not be excluded from the creation of AVF, as the most adequate VA.

The percentage of successfully matured VA in our study corresponds to the data reported in the literature [21]. Even with adequate internal diameter of the vein, AVF still have a lower chance of successful maturation in women than in men [22]. In a retrospective study by Silpa *et al.*, the relationship between gender and AVF maturation was investigated [23]. Although no signifycant difference was found in the internal diameter of the vein, women had a significantly lower percentage of primary fistula maturation, 25.0% versus 39.6%. Also, women had a significantly higher chance of primary VA failure, as much as 20.0% versus 10.0%. Multivariate analysis found that men have a 2.3 times greater chance of successful AVF maturation. In the HEMO study, female gender was identified as a significant predictor of more frequent use of AVG compared to AVF [24]. The above results are consistent with ours, where we see that there is a gender difference in terms of maturation in favor of males.

The basic preoperatively established criteria for successful maturation of the AVF is the inner diameter of the vein greater than 2mm, i.e. over 2.5mm with an upper arm cuff and >4mm for an AV graft [25, 11]. In the study of Lannery *et al*, the internal diameter of the vein was found as a sole independent predictor of the success of AVF maturation [26]. In the present analysis, we found that the inner diameter of the vein is significantly larger in subjects with successful maturation of the AVF, and this was also confirmed by the Cox regression model, which found that with an increase in the inner diameter of the vein by 1 mm, the chance of maturation failure of the AVF decreases by 43%.

The presented study showed a significantly higher number of successfully matured AVF in patients who postoperatively took antiplatelet therapy, most often Ticlopidine, and less often Acetylsalicylic acid or Clopidogrel. Drug therapy prescribed to reduce early complications and improve the maturation of VA has been

analyzed in other studies, as well. Thus, the study by Dember *et al*., investigated the importance of Clopidogrel therapy during 6 weeks after the creation of AVF. Patients who were taking Clopidogrel had a significantly lower degree of fistula thrombosis compared to placebo in the mentioned period (12.2% vs 19.5%; P = 0.018). However, Clopidogrel did not affect the number of matured AVF for dialysis, so according to this study, routine therapy with this antiplatelet agent did not provide clinical benefit [27].

In a study by Irish *et al*, the use of fish oil as well as aspirin did not reduce the chance of AVF loss within 12 months of creation [28]. In a meta-analysis by Palmer *et al*., the results of 21 studies were examined in which the effects of antiplatelet therapy and placebo or no therapy on the function of VA were compared. Antiplatelet therapy reduced the loss of fistula function by half, but the positive effect on maintaining the function of AV grafts, as well as the influence on fistula or graft maturation for dialysis were not confirmed. The effect of antiplatelet therapy on the frequentcy of serious bleeding during immediate postoperative period was also unclear [29]. In a meta-analysis of three studies with Ticlopidine, with a follow-up of one month after the creation of VA, it was found that Ticlopidine may have a beneficial effect as an adjuvant therapy for maintaining the function of AVF and grafts in this short postoperative period [30]. However, by using Cox regression analysis, the chance of unsuccessful maturation of the vascular access increases 3.6 times with the use of antiplatelet therapy. It is not advised to give antiplatelet therapy in the first week after AV creation, but in some cases, it is continued because of other earlier indications (coronary, cerebrovascular diseases etc.). In a deeper analysis, it was observed that the highest percentage of unsuccessfully created VA was due to the compression of the anastomosis by the hematoma, which can be linked to the antiplatelet therapy [31,32]. Additionally, this seemingly illogical statistical result may be a consequence of a relatively small sample [33].

The incidence of diabetes is constantly increasing, and diabetic nephropathy leads to the development of chronic kidney disease, which over time requires treatment with dialysis [34]. The results of the presented research indicate that diabetics have a significantly lower chance of VA maturation compared to those without diabetes. Although the mechanism is not entirely clear, there are several possible explanations. Diabetes carries a higher risk of platelet aggregation and increases the release of von Willebrand factor, leading to platelet aggregation and endothelial injury [35]. Hyperglycemia and end products of glycosylation affect the reduced elasticity of blood vessel walls and additionally potentiate the flow disorder, platelet aggregation and thrombosis [35]. Also, due to atherosclerosis, which is more often present in diabetics, it is difficult to create VA. Diabetics are more susceptible to the deposition of lipids in the vascular walls near the anastomosis, which leads to an endothelial dysplasia and coagulum development, and due to the hemodynamic effect of the so-called Jet lesions, additional damage to the intima, hyperplasia and consequent stenosis occurs [36]. The Chinese author Yan *et al*., conducted a meta-analysis of 23 papers that addressed the issue of AVF maturation in diabetic patients [37]. This meta-analysis found a significantly higher percentage of AVF maturation failure in diabetic patients compared to non-diabetic patients.

The importance of the inner diameter of the vein and its distensibility for the successful maturation of VA has been confirmed, so in the work of Dageforda *et al*., a multivariate analysis proved that patients with a larger inner venous diameter have a significantly lower risk of unsuccessful AVF maturation, as well as a higher long-term AVF survival [38]. In the study by Kakkos *et al*., it was proven that the internal diameter of the vein with an upper arm cuff, less than 4.3 mm is the only independent predictor of reduced AVF survival [39]. In the study by Misskey *et al.*, it was concluded that the internal diameter of the artery and vein with an upper arm cuff independently predicts the maturation and survival of VA. An internal diameter of vein with cuff <3.4mm was associated with a reduced long-term survival of brachiocephalic AVFs, but not with their maturation [40]. In the work of Hou *et al*., emphasis was placed on the vein distensibility, as the ability of the vein to increase in diameter after placement of an upper arm cuff, especially in cases with previously measured diameter of vein <2.0mm. It was found that the survival of radiocephalic AVF is significantly improved if the distensibility of the vein is >0.52 mm [41].

Conclusion

This work showed that men and older people have more favorable CDS characteristics than women and people younger than 65 years and that CDS parameters were not affected by comorbidities (hypertension, diabetes and cardiovascular diseases). At the same time, patients with successful VA maturation were more frequently male, without diabetes and those taking antiplatelet therapy. A larger internal vein diameter is the only significant predictor of successful VA maturation, but not other CDS characteristics of the vein: compressibility, depth, presence of accessory veins and vein morphology. Successful creation of VA was observed in 43% of patients with unfavorable at least one CDS parameter, as well as in 52% of patients with diabetes, which indicates the delicacy of strict adherence to recommendations. More effort, skill, and persistence are needed with an aim to create a successful VA in the growing number of individuals with suboptimal vascular characteristics.

Conflict of interest statement. None declared.

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Case report

The Nutcracker Syndrome - A Presantation of Two Cases

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Abstract

The nutcracker syndrome (NCS) is a rare vascular anomaly with compression of the left renal vein between the aorta and the superior mesenteric artery and impairment of the venous blood flow. The venous congestion and dilation of the left renal vein may lead to hematuria with or without proteinuria, pelvic congestion, abdominal and pelvic pain in women and varicocele and/or swelling of the left testicle in men. We present two female patients with NCS diagnosed using contrast-enhanced computed tomography for hematuria and abdominal pain since childhood and discuss the diagnostic strategy in such patients.

Keywords: nutcracker syndrome, hematuria, abdominal pain, imaging studies, computed tomography

Introduction

Nutcracker syndrome (NCS) is a rare vascular abnormality with compression of the left renal vein (LRV) between the superior mesenteric artery (SMA) and the aorta due to abnormally sharp aortic-SMA angle (below 35-39°) [1-3]. Venous stasis and distension of the distal part of the vein, congestive erythrocituria and proteinuria and venous thrombosis may develop. Erythrocituria occurs due to the distension and congestion of the periureteral and peripelvical veins with microruptures [1,3,4]. Ureteroscopic investigation may reveal bleeding from the left ureter [1]. Some patients report non-specific abdominal symptoms due to pelvic venous congestion - dull pain in the abdomen, pelvic floor and the left abdominal flank in women and varicocele/edema of the left testicle in men [1-5]. The compression of the LRV between the SMA and the aorta resembles the position of a nut between the two arms of a nutcracker (Figure 1) and this gave the name *nutcracker syndrome*. The term *nutcracker phenomenon* is used in the absence of clinical signs and symptoms and laboratory alterations when this vascular abnormality is an accidental finding during imaging studies for other causes [1,2].

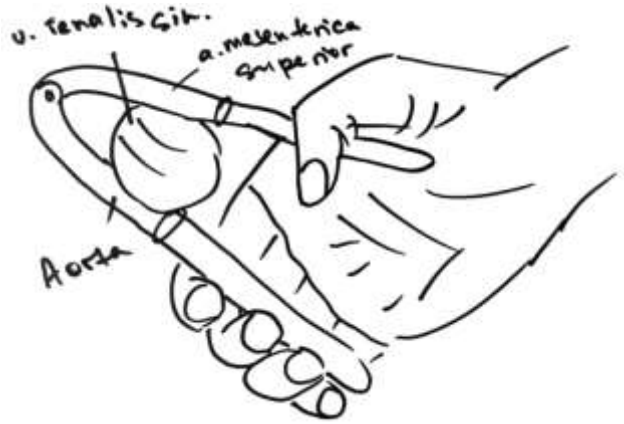

Fig. 1. Nutcracker syndrome. The left renal vein is compressed between the superior mesenteric artery and the aorta, in parallel with a nut compressed between the two arms of a nutcracker. This may happen when an abnormally sharp (below 35-39°) outflow angle the superior mesenteric artery from the aorta.

At the level of the nephron, venous stasis and hypertension may lead to an increased secretion of norepinephrin and angiotensin II in upright position due to the distension of the venous wall and marked orthostatic proteinuria and/or arterial hypertension [1].

NCS was first described in the English literature in 1937 by J.C.B. Grant [6] as abnormally sharp angle between SMA and aorta and entrapment of the LRV between them. Thirteen years later, A.R. El-Sadr and E. Mina [7], described a man with varicocele and compression of the LRV between SMA and the aorta and

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discussed the surgical aspects of the treatment in such cases. In 1969, C. Cope and H. J. Isard [8] named it left renal vein entrapment, and in 1972 A. de Schepper used the term nutcracker syndrome [9].
In 2023, we observed two cases of this rare vascular abnormality. The contrast-enhanced CT was performed as a standard diagnostic procedure in the evaluation of hematuria of unknown origin [4]. Written informed consent was obtained from both patients prior to any diagnostic and therapeutic procedure performed.

Case presentation

Clinical case 1

A 46-years-old female was referred to the Clinic of Nephrology for diagnostic evaluation of recurrent hematuria and proteinuria <0.5 g/24 h since childhood. Past and family history and the physical exam were unremarkable. Clinical-laboratory investigations (hematological, biochemical and coagulation) at the admission were normal, urine investigations revealed mild erythrocituria (14-15 erythrocytes/pf) and proteinuria 0.22 g/l. The abdominal ultrasound presented both kidneys normal in size and vascularization with small calyceal stones (4-5 mm) bilaterally. The urinary bladder was within the normal limits. Due to the presence of erythrocituria with no visible source of bleeding we decided to perform contrast-enhanced computed tomography (CT) of the abdomen and pelvis that also revealed both kidneys normal in size, with small calyceal stones 2.6-3.6 mm, without dilation of renal pelvises and ureters. The aortic-SMA angle was decreased (28.1º), there was marked compression of LRV between SMA and the aorta (Figure 2) and it was diagnosed as *nutcracker syndrome*. After the CT investigation, the patient was referred to vascular surgeon for further treatment.

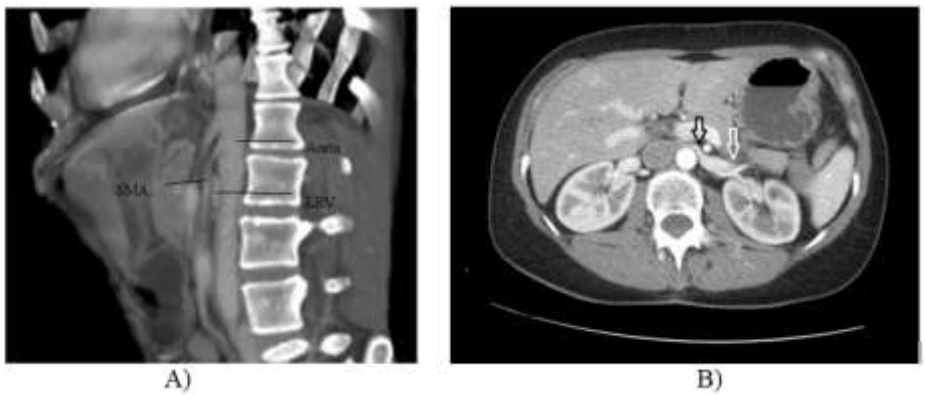

Fig. 2. CT scan of the patient 1. A) Decreased aortic-superior mesenteric artery (SMA) angle with entrapment of the left renal vein (LRV) between the two vessels. B) The left renal vein appears compressed (black arrow) between SMA and the aorta with marked prestenotic dilation of the vein (white arrow).

Clinical case 2

A 42-years-old female was referred to the Clinic of Nephrology for diagnostic evaluation of low abdominal and pelvic pain and hematuria for the past 3 years. The patient had consulted gynecologist, gastroenterologist, and gastrointestinal endoscopies revealed no abnormalities. The general and neurological status were normal. The clinical-laboratory investigations of blood (hemogram, biochemical investigations of serum, coagulation) and urine, and abdominal ultrasound revealed no abnormalities. The contrast-enhanced CT of the abdomen and pelvis revealed the presence of a sharp angle between SMA and the aorta with entrapment of the LRV between the both vessels with marked prestenotic dilation of the LRV (Figure 3) and pelvic venous congestion. After the CT examination the patient was referred to vascular surgeon for further treatment.

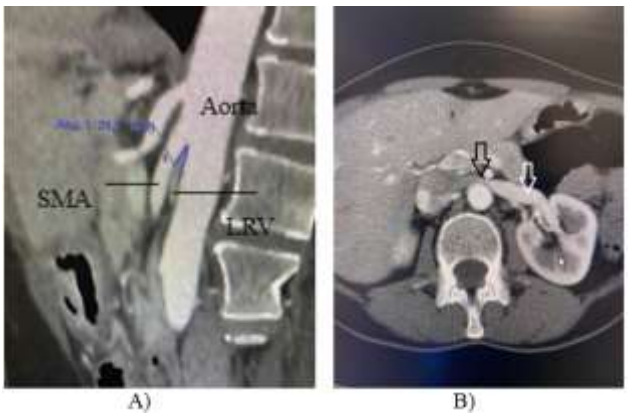

Fig. 3. A) Decreased aortic-SMA angle (21.2º) with entrapment of the LRV between the bot vessels. B) Entrapment of the LRV between the aorta and the SMA (black arrow) and prestenotic dilation of the vein (white arrow).

Discussion

NCS is a rare vascular abnormality in both children and adults, with venous stasis in the left kidney and pelvic area due to compression of the left renal vein between the aorta and SMA [1-4] and subsequent he-

maturia, low-grade proteinuria due to dilation of the peripelvical and periuretral veins [4]. NCS is often associated with varicous veins of the lower legs [10]. NCS is very probable when bleeding from the left ureter is detected, in patients with orthostatic proteinuria, isolated pelvic vein congestion, and in males with isolated left-sided varicocele [4,5,10]. Due to the increased bleeding risk in venous stasis, renal biopsy is not advisable. The golden standard in the diagnosis are the imaging methods-cross-sectional imaging (CT and/or magnetic resonance imaging), Duplex ultrasound and venography [4]. In our patients, the ultrasound examination showed no changes, so they both were referred for contrast-enhanced abdominal CT detecting NSC.

Our patients have anterior type of NSC with classical clinical symptoms. Posterior type of NCS has also been described - compression of the LRV between the aorta and the spine when the vein has abnormal retroaortic position. NCS is rarely observed in the right side - usually during pregnancy when the right renal vein is compressed by the uterus, or in patients with adjacent neoplasms or other aberrant vessels.

In our patients NCS was detected with contrast-enhanced CT years after the first symptoms, and both were referred to vascular surgeon for further evaluation and treatment.

The major steps in the treatment of NCS consist in non-operative and operative techniques [4]. Non-operative approach is indicated in less severe, non-specific clinical symptoms and minimal laboratory abnormalities, and consists in conservative observation and endovascular stenting with subsequent anti-thrombotic prophylaxis (anticoagulant/antiaggregant). The surgical approach consists in transposition of the LRV with or without ligation of the left gonadal vein, if needed [4]. Due to the mild clinical symptoms and laboratory abnormalities, our patients were referred for endovascular treatment with stenting of the LRV.

Conclusion

The presented clinical cases show a rare vascular abnormality - nutcracker syndrome, detected using contrast-enhanced CT of the abdomen and pelvis. Both patients had history of abdominal pain and hematuria and had undergone multiple investigations in the past revealing no cause for their symptoms. These two cases demonstrate the importance of the team work with close cooperation between nephrologist, urologist, vascular surgeon, imaging specialist, gastroenterologist and neurologist in the evaluation of patients with hematuria and abdominal/pelvic pain.

Conflict of interest statement. None declared.

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Case report

Breast Cancer in a Male Kidney Transplant Recipient - A Case Report

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Abstract

Breast cancer is the 5th leading malignant cause of death worldwide. The mechanism behind its development is multifactorial and involves a combination of hormonal, genetic, environmental, and age-related factors affecting DNA damage. Due to their chronic immunosuppressive therapy, kidney transplant recipients are exposed to an increased risk of carcinogenesis. We present an unusual case of an 80-year-old kidney transplant recipient who developed an invasive breast cancer 8 years after cadaveric kidney transplantation. Chronic immunosuppressive therapy at the time of diagnosis included cyclosporine (100mg/day), mycophenolate mofetil (1000mg/day), and prednisolone (5mg/day). A core needle biopsy was performed after the ultrasound examination. The patient was treated with modified radical mastectomy and axillary lymph node resection. The pathohistological report confirmed invasive stage T3N1Mx breast carcinoma with metastatic lymph node involvement. The results of the pathohistological examination demonstrated the presence of breast carcinoma, characterized by positive expression of estrogen receptors (ER) and progesterone receptors (PR), while human epidermal growth factor receptor 2 (HER2) had a negative status. The patient began adjuvant hormone therapy with tamoxifen 10 mg 2x1 per day. He was switched from cyclosporine to an mTOR inhibitor. To date, the patient has been consistently monitored per scheduled follow-up appointments and continues to remain in remission without any further complications. Due to decreased awareness and minimal degree of suspicion, more than 40% of patients with male breast cancer (MBC) first present at the advanced stage, which typically takes the form of a lump or nipple inversion. As a result, MBC patients experience worse prognosis than their female counterparts. This case report demonstrates the importance of thorough and regular clinical examinations of male kidney transplant recipients. Although MBC is rare, the possibility of its existence should be emphasized, especially in a vulnerable population such as kidney transplant recipients.

Keywords: breast cancer, kidney transplant recipient, male patient, immunosuppression, pathohystology

Introduction

Breast cancer is the 5th leading malignant cause of death worldwide [1]. The mechanism behind the development of breast cancer is multifactorial and involves a combination of hormonal, genetic, environmental, and age-related factors affecting DNA damage. Normally, the immune system of a supposedly healthy individual targets cells with abnormal DNA, whereas this protecttive system is absent in immunosuppressed patients [1]. Due to their chronic immunosuppressive therapy, kidney transplant recipients are exposed to an increased risk of carcinogenesis. Neoplasm occurrence is 2-3 times more common among solid organ transplants than in the overall population [2]. Although male breast cancer (MBC) accounts for only 1% of all cases of breast cancer [3] and only 0.2% of all cancer diagnosis in men [4], it is important not to neglect the possibility of MBC, especially in a vulnerable population, such as kidney transplant recipients.

Case presentation

We present an unusual case of an 80-year-old kidney transplant recipient who developed an invasive breast cancer 8 years after deceased donor kidney transplanttation. The patient's medical history included kidney transplantation in 2014, arterial hypertension, atrial fibrillation, mitral and tricuspid valve insufficiency, and hyperparathyroidism. He underwent an electrostimulator implantation in 2015 because of tachycardia-bradycardia syndrome. In 2018, a total hip replacement surgery was performed following a fall and a left hip fracture. During the same year he was hospitalized 3 times due to *Klebsiella pneumoniae* and *Acinetobacter*

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baumannii urinary tract infections, and pneumonia. During the 2nd hospitalization and treatment for pneumonia he developed *Clostridium difficile* colitis. Chronic pharmacologic therapy at the time of diagnosis included: cyclosporine (100mg/day), mycophenolate mofetil (1000mg/day), prednisolone (5mg/day), nebivolol (5mg/day), allopurinol (200mg/day), pantoprazole (20mg/day), urapidil (180mg/day), furosemide (125mg/day), potassium citrate (2.17g/day), warfarin (5mg/day) and cinacalcet (30mg/day). In May 2022, during a regular examination by a transplant nephrologist, the patient reported a history of trauma to the left breast one month prior, resulting in subsequent edema of the affected area. Upon physical examination, no abnormalities were observed, except for the presence of a palpable, firm mass in the left breast, measuring 5x5 cm. The mass was painless on palpation and did not fluctuate. An ultrasound examination revealed a retro-mamillary, irregularly shaped, heterogeneous area of cystic and solid vascular parts measuring 5x5 cm. A modified radical mastectomy and axillary lymph node resection was performed in May 2022. The pathohistological report confirmed invasive stage T3N1Mx breast carcinoma with metastatic lymph node involvement. The results of the pathohistological examination demonstrated the presence of breast carcinoma, characterized by positive expression of estrogen receptors (ER) and progesterone receptors (PR), while human epidermal growth factor receptor 2 (HER2) had a negative status. Among the 11 excised lymph nodes, only the biggest one (size 3 cm) had metastatic lesions that did not penetrate the capsule. After the operation, the patient underwent an MSCT scan which revealed no metastatic lesions in the lungs, liver, or bones. In September 2022, according to the recommendations of an oncologist, the patient began adjuvant hormone therapy with tamoxifen 10 mg 2x1 per day. According to the recommendations of the transplant nephrologist, the patient was advised to switch from cyclosporine to an mTOR inhibitor. The question of adjuvant radiotherapy was discussed at a multidisciplinary tumor board. It was determined that, in the absence of cancer progression, radiotherapy should be avoided due to the patient's comorbidities, personal preferences, and life circumstances. To date, the patient has been consistently monitored per scheduled follow-up appointments and continues to remain in remission without any further complications.

Discussion

The most prevalent type of MBC, accounting for 90% of all cases, is invasive ductal carcinoma [5]. Hormone receptor expression is prevalent in MBC, with approximately 81% of cases expressing PR and 90% expressing ER [5]. Since the majority of MBCs are hormone receptor positive, the mainstay adjuvant hormonal therapy is tamoxifen for a total duration of 5-10 years, according to recent guidelines [3]. Due to decreased awareness and minimal degree of suspicion, more than 40% of patients with MBC first present at the advanced stage, which typically takes the form of a lump or nipple inversion [5]. As a result, MBC patients experience worse prognosis than their female counterparts [3]. There is little data available regarding male breast cancer in general and most diagnostic and therapeutic options are adapted from the management of women's breast cancer [6]. However, it is known that MBC patients are more likely to develop second ipsilateral or contralateral breast malignancy and should be subjected to a regular, long-term examinations [3,7]. MBC typically presents at an advanced age, with the peak age of diagnosis at 71 years [8]. At the time of diagnosis our patient was of an advanced age which, in itself is a risk factor for the development of breast cancer. However, it is important to note that our patient has been on immunosuppressive therapy for 8 years prior to the development of the disease. The process of de novo carcinogenesis in organ transplant recipients are influenced by several interrelated factors. Chronic immunosuppressive therapy is believed to be a major contributing factor to the alteration of the immune response to oncogenic viruses and the dysregulation of immunosurveillance mechanisms of cancer cells [9]. There is an evidence that certain immunosuppressive drugs such as calcineurin inhibitors (cyclosporine and tacrolimus) and azathioprine are directly involved in the development of cancer [10,11]. Nevertheless, the rate of carcinogenesis depends on the type of drug, the degree of immunosuppression and the duration of immunosuppressive therapy [12-14]. According to some studies, it appears that kidney transplant recipients do not have a higher risk of developing breast cancer than the general population [15,16]. However, two cases concerning MBC in kidney transplant recipients have been reported in the literature to date [17,18].

Conclusion

This case demonstrates the importance of thorough and regular clinical examinations of male kidney transplant recipients. Although MBC is rare, the possibility of its existence should be emphasized, especially in a vulnerable population such as kidney transplant recipients.

Conflict of interest statement. None declared.

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Case report

The Importance for Renal Biopsy in Patients with Diabetes Mellitus

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Abstract

The diagnosis of diabetic kidney disease (DKD) is most often made clinically. However, there is a growing awareness of the prevalence of non-diabetic kidney disease (NDKD) with or without concomitant diabetic nephropathy (DN) in patients with diabetes. Proteinuria and renal disfunction is common in patients with diabetes mellitus. In most of the cases diabetic nephropathy is the cause of that disfunction, but some of the cases present other non-diabetic renal disease. Renal biopsy in diabetic patients has presented variety of glomerular changes. Immunosuppressants used for treatment of the glomerular diseases may be associated with complications as well.

Herein, we review the prevalence and presentation of NDKD in diabetic patients, with a focus on glomerular lesions, and discuss the ways in which diabetes can affect the diagnosis and management of these conditions. Diabetic patients with glomerular disease represent a sizable patient population.

We report 2 cases with diabetes mellitus with NDKD confirmed with renal biopsy. The first case was a 58-year-old man, admitted to our Department with a history of diabetes, with actual presence of fatigue, inappetence, edema and proteinuria of 6 g/24 hours. Renal function was diminished, with creatinine values of 226 micromol/l, with eGFR 38 ml/min. Second case was a 53-years -old-man with edema, hypertension, atrial fibriloflater and breathlessness, treated with cardiologic therapy, but with persistent edema. Creatinine value was 126 mcmol/l and proteinuria 4,36 g/l (11,94 g/24hours). In both cases, renal biopsy was performed and the histopatologic analysis showed membranoproliferative glomerulonephritis with simultaneous presence of diabetic glomerulonephritis.

The patients were treated with corticosteroids as pulse therapy, cyclophosphamide, diuretics and after that the clinical signs were stabilized.

The authors suggest that the renal biopsy should be performed in diabetic patients with unusual features, such as proteinuria without other signs of diabetic disorders. Renal histology can be of a fundamental importance to both treatment and prognosis of the disease.

Keywords: diabetes melitus, diabetic kidney disease, non-diabetic kidney disease, renal biopsy, glomerulonephritis

Introduction

Proteinuria and renal disfunction is common in patients with diabetes mellitus. In majority of cases diabetic nephropathy is the cause of such disfunction, but some of the cases present with other than non-diabetic renal disease [1-3].

The diagnosis of diabetic kidney disease (DKD) is most frequently made clinically. However, there is a growing awareness of the prevalence of a non-diabetic kidney disease (NDKD) with or without concomitant diabetic nephropathy (DN) in patients with diabetes. Conversely, a large proportion of NDKD found on kidney biopsies is glomerular diseases. This knowledge draws an attention to the possibility of undiagnosed, potentially reversible lesions in this population. Renal biopsy in diabetic patients has presented with a variety of glomerular changes [4,5]. However, simultaneous presence of non-diabetic renal disease may be seen in up to 28% of patients [6].

Immunosuppresants used for the treatment of glomerular diseases may be associated with complications. Here, we review the prevalence and presentation of NDKD in diabetic patients, with a focus on glomerular lesions, and discuss the ways in which diabetes can affect the diagnosis and management of these conditions.

Reported types of superimposed glomerular diseases include IgA nephropathy, endocapillary proliferative glomerulonephritis minimal change disease, membranoproliferative glomerulonephritis, rapidly progressive glomerulonephritis and cryoglobulinemic glomerulonephritis [7-10]. Diabetic patients with glomerular di-

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sease represent a sizable patient population that, unfortunately, has been excluded from or under-enrolled in many recent clinical trials investigating management of glomerular diseases [11,12].

Case report

Herein, we report on 2 cases with diabetes mellitus with non-diabetic kidney disease confirmed with renal biopsy.

Case 1

A 58-year-old man was admitted to our Department with a history of diabetes, with actual presence of fatigue, inappetence and edema. Proteinuria was 6 g/24 hours. The patient had a 3-years old history of diabetes mellitus, treated with insulin.
Ultrasonography showed normal kidney size, hyperechogenic parenchyma of 19mm, without any obstruction.
Laboratory findings were as follows: hemoglobin 103 g/l, erythrocytes $3,5x10^9$/l, leukocytes $9,6x10^3$/l, CRP 5,2. Serum protein was 63 g/l, albumin 35, globulin 28. Electrolyte status was stable: Na 137, K 5,1, Ca 2,24, phosphate 1,45, lipids were in normal range. Proteinuria was controled several times without any reduction 5,8….7,3….5,4 g/24h. Renal function was diminished with urea 18,8 mmol/l and creatinine levels 226 mcmol/l, and creatinine clearance 38 ml/min. Serum immunogobulins were within the normal range, and cANCA was negative. As the renal function was impaired, we performed a renal biopsy to find the reason for a persistently high levels of proteinuria. The finding presented with glomerulonephritis membranoproliferativa with a high grade of chronic tubulointerstitionephritis. Thirteen out of 17 glomeruli were with glomerular sclerosis with partly mesangial cellularity. The other 4 glomeruli were with a lobular feature and high mesangial cellularity. Two fibrous crescents were also present.
The treatment included corticosteroids as pulse therapy, cyclophosphamide, diuretics and after the stabilization of the clinical signs, the patient continued with a low dose of corticosterod therapy.
Control laboratory findings presented values for creatinine of 244mcmol/l, but the proteinuria was decreased to 0,51g/l and 1,53 g/24 hours. In the next 3 years period of the follow-up, the patient remained as stable chronic kidney disease stage 3, with creatinine of 236 mcmol/l, and proteinuria of 0,34 g/l (0,56 g/24 h).

Case 2

A 53 years -old-man with edema, hypertension, atrial fibriloflater and breathlessness, was treated with cardiologic therapy, but the edema was still present. Although the patient had varices cruris as a thrombophlebitis consequence and edema, the patient was admitted at the Department of nephrology for further examination, with creatinine 126 mcmol/l. The other laboratory finding were: hemoglobin 135 g/l, erythrocytes $4,75x10^9$/l, leukocytes $10,6x10^3$/l, CRP 4,7. Serum protein was 55 g/l, albumin 30, globulin 25. Electrolyte status was stable: Na 139, K 4,2, Ca 2,1, phosphate 1,3, lipids were in normal range with statine therapy. The hypertension was regulated with antihypertensive therapy. Ultrasonographic examination presented enlarged kidneys, with hyperechogenic parenchyma of 20 mm, features for possible diabetic kidney. Proteinuria was 4,36 g/l (11,94 g/24h). Because of the edema persistence with increasing proteinuria, renal biopsy was performed.
The histopathologic findings of the renal biopsy presented 6 glomeruli, 3 of them obsolent and 3 with alterated capillary lumen and thickening of the glomerular membrane with increased cellularity of the mesangial matrix and some of the glomeruli had a lobular feature. Tubules are partly atrophic with interstitial fibrosis and inflamation. The histopatologic analysis showed membranoproliferative glomeurlonephritis with simultaneous presence of diabetic glomerulonephritis.

Discussion

The prevalence of NDKD in patients with diabetes reflects the rising incidence of diabetes in the general population. The most current national prevalence of diabetes in adults is as high as 12.2%. A contributing factor is the obesity, as about 90% of diabetic adults are overweighed or obese. It is well known that CKD and ESKD cause a significant morbidity and mortality in obese and diabetic populations [1,7,13]. The relative risk of ESKD is higher in diabetics, but also the prevalence of NDKD with or without concomitant DN is found in patients with diabetes [2,4].
Membranous nephropathy (MN) is the most common glomerular disease in adults and is always associated with the occurrence of nephrotic syndrome [1,2]. MN is characterized by high levels of proteinuria, edema, hypoalbuminemia, and elevated serum lipids [3]. Also, membranoproliferative glomerulonephritis is reported in patients with diabetes on the renal biopsy. Both studies cited FSGS as the most common pathology in the NDKD-alone group at 22% and 21%, respectively, followed by hypertensive nephrosclerosis, acute tubular necrosis, IgA nephropathy, and MN. Typical renal biopsy of MN involves thickening of glomerular basement membrane (GBM) on the light microscopy. MN is a chronic disease, with spontaneous remissions (occurs in about 30% of patients, usually within the first 2 years after diagnosis in those patients with milder presentations) and frequent relapses. Those who do not undergo remission progress to end-stage renal disease [1,12, 13]. Diabetic kidney disease (DKD) and diabetic nephropathy (DN) are considered major causes of end-stage kidney disease worldwide. To obtain a clear differential diagnosis of either MN or DN has usually become

difficult, and therefore, renal biopsy is regarded as the most effective diagnostic method.

It has been estimated that NDKD causes 40%-60% of ESKD in patients with type 2 diabetes, whereas only 2%-3% of renal disease in type 1 diabetics is of non-diabetic etiology [13,19].

The exclusion of diabetic patients from these trials is predicated on the assumption that the presence of diabetes alters the natural history of primary glomerular diseases. Studies of patients with FSGS, MN, IgA nephropathy, and minimal change disease that characterizes the clinical and histopathologic presentation and long-term outcomes of these diseases, also excludes patients with a history of diabetes at the time of first biopsy [2,14,15].

Furthermore, the presence of DN is associated with a higher rate of progression to ESKD regardless of diagnosis. Our data align with a 2011 retrospective study by Chan KW *et al*., [4] that showed a significantly worse cumulative renal survival in patients with DN alone versus either NDKD alone or with any concomitant disease, suggesting potential prognostic value of biopsy in cases with clinical suspicion for NDKD. The authors noted that nearly half of the patients with NDKD were treated with immunosuppression, mostly prednisolone, with a 67.6% complete or partial remission rate of both proteinuria and renal failure [4]. Although biopsy is not indicated in the diagnosis of DKD, these findings suggest there is a significant proportion of diabetic patients with NDKD for whom biopsy may not only aid in prognosis but also, change disease management.

Although indications for biopsy, such as absence of retinopathy, diabetes duration <5 years, and microhematuria, have been validated in type 1 diabetics, in whom the prevalence of NDKD is only 2%–3%, evidence regarding biopsy criteria for type 2 diabetics is largely retrospective [4,16]. Factors most commonly identified in the literature include younger age, shorter duration of diabetes, higher hemoglobin levels, absence of retinopathy, and sudden onset of proteinuria [2,16,17]. Although none of these factors are diagnostic, presence of one or more can raise suspicion for NDKD and prompt earlier consideration of biopsy in diabetic patients.

Unfortunately, little evidence exists to guide management of primary glomerular diseases in diabetic patients due to under-representation in cohorts (retrospective and prospective) and clinical trials of glomerular disease. Glucocorticoids, the mainstay of primary glomerular disease management, carry a high morbidity risk; many of the toxicities of glucocorticoids, including hyperglycemia and weight gain, are expected to be worse in patients with comorbid diabetes [12,13]. Steroid-sparing immunosuppressive agents, including calcineurin inhibitors and rituximab, have proven to be effective alternatives for treating primary glomerular diseases, and they may be preferred in diabetic patients.

In patients with type 2 diabetes and an atypical presentation of kidney disease, >60% are likely to have NDKD with or without DN [17]. Additionally, diabetic patients with NDKD have greater renal survival compared with those with DN alone. Thus, consideration of kidney biopsy to make a diagnosis and potentially guide treatment for reversible lesions is an important component of evaluating diabetic patients.

There still exists a need to enroll diabetic patients with glomerular diseases in observational studies of glomerular diseases as well as in clinical trials of disease-targeting agents. The exclusion of diabetic patients from these studies under-represents a growing population of patients with higher morbidity and mortality. The nephrologist has to recognized the role of DKD in patients with CKD and ESKD. Now is the time for us to recognize the importance of NDKD, particularly those with glomerular lesions, so that we can slow and postpone the progression of the chronic kidney disease [2,13].

The patients described in this report had diabetes with nephrotic syndrome manifested by edema. Diabetic nephropathy is well recognized complication of diabetes mellitus but in our case the duration of diabetes mellitus was short and the patient did not demonstrate hypertension, or diabetic retinopathy and neuropathy. The incidence of a non-diabetic glomerular disease has varied from 8% to 22% [19].

In reviewing the medical literature, we can find patients with membranoproliferative glomerulonephritis which suggested poor prognosis. Membranoproliferative glomerulonephritis (MPGN) is characterized by a pattern of glomerular injury on the light microscopy, including hypercellularity and thickening of the glomerular basement membrane. The clinical presentation usually consists of mixed nephritic and nephrotic features. Cause is idiopathic or secondary to another disorder. Diagnosis is confirmed by renal biopsy. Treatment is directed at the underlying disorder, when present. For patients with idiopathic disease, treatment may be supportive or include corticosteroids and other immunosuppressive agents.

The prevalence of non-diabetic renal disease in diabetic patients is not known. From a review of the relevant literature, most of which consisted of isolated case reports, a wide spectrum of non-diabetic renal lesions can occur in patients with diabetes. Although proteinuria is frequently the initial urinary abnormality observed in diabetic nephropathy, it can be also a sign of other glomerular abnormality.

Renal biopsy was performed because of the unusual clinical pictures: microscopic hematuria; heavy proteinuria without evidence of diabetic retinopathy; and, in one case, a sudden onset of renal failure. Renal biopsy disclosed pure MGN without glomerulosclerosis. In both cases, clinical history, physical examination and biologic assessment failed to reveal the cause; MGN was thus considered idiopathic. Treatment, in-

cluding glycemic control and angiotensin-II receptor blockers, led to resolution of the proteinuria in one case.

Conclusion

We suggest that renal biopsies should be performed in diabetic patients with a sudden onset of renal failure, proteinuria without retinopathy, or other evidence of microvascular disease.

Conflict of interest statement. None declared.

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Case report

Management of Glomerulopathy Associated with Echinococcosis

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Abstract

A 67-year-old male patient, who had no previous kidney disease and was followed up for a liver hydatid disease for 2 years, was hospitalized because of proteinuria and impaired kidney function tests when he presented with the complaints of a decreased oral intake and deterioration in general condition. Glomerular lesions associated with echinococci have been rarely reported and an established treatment approach is needed.

Keywords: Echinococcus granulosus, hydatid cysts, glomerulopathy associated with echinococcosis

Introduction

Cystic hydatid disease is an endemic parasitosis caused by the larvae of Echinococcus granulosus for which humans are an intermediate host in its life cycle. Lungs and liver are the main sites of involvement. The incidence of hydatid cysts in the kidney is about 1-2% and the disease usually manifests with hematuria and/or flank pain. We think that there is a need for an established treatment approach for echinococcal-related glomerular lesions, which have been reported in limited numbers in the literature.

Case report

A 67-year-old male patient was admitted to hospital with complaints of a decreased oral intake and deterioration in the general condition. The patient had no prior kidney disease and according to his statement he had been followed up for a liver hydatid disease in the last 2 years. At the time of admission his blood pressure was 110/65 mmHg, and his heart rate was 110 beats/min. On the routine physical examination, a mass was discovered on the right upper quadrant of abdomen, and he was dehydrated. He was hospitalized because of proteinuria and his impaired kidney function tests at the admission. The urine output remained normal in the follow-up of the hospitalization. In the computed tomography examination of the patient whose liver functions and serum electrolytes were normal, a 19x15 x11 cm cystic lesion (contours showing lobulation, calcifications on its walls and vesicles in it) was observed in the right lobe of the liver. The indirect hemagglutination (IHA: 1/640) test was positive, and the diagnosis of hydatid cyst was confirmed. Initial laboratory tests of the patient revealed WBC: 20.300, neutrophils 17.8%, CRP: 152, procalcitonin: 0.61. Albendazole treatment was started due to the permanent fever. There was no bacterial growth in the blood and urine samples taken from the patient. Viral serology, TORCH panel, Brucella, Salmonella, Covid-19 tests requested to investigate the etiology of recurrent fever were negative. Bilateral kidney sizes and vascular structures were found to be normal with the renal Doppler ultrasonography. The patient's laboratory tests were as follows: albumin: 2.9 g/dl, globulin: 3.0 g/dl, LDH: 222 g/L, Anti CCP IGG: <0.5 U/ml, erythrocyte sedimentation rate: 82 mm/h, Anti-ds DNA(Elisa): 35.6 IU/ml(0-20), C3:1.05g/L(0.9-1.8g/L), C4:0.15g/L(0.1-0.4g/L). Protein was +2 in the complete urine test and 2.2 grams/day proteinuria was detected. Kidney biopsy was performed because of an acute renal failure of unknown etiology and proteinuria of 2.2 g/day. It was learned that the patient was started on azathioprine and methylprednisolone with a preliminary diagnosis of scleroderma in another center 6 months ago, and the patient left the treatment voluntarily due to the treatment side effects. The kidney biopsy was supportive of crescentic glomerulonephritis due to fibrinoid necrosis and fibrocellular crescent in 2 glomeruli (Figures 1, 2 and 3). In addition, it was recommended to be evaluated clinically in terms of membranous nephritis by the pathologist due to the association of IgG and C3 accumulation, although it is a weak one. The patient was treated with prednol at 1 mg/kg and azathioprine 75 mg after 3 days of pulse steroid with a preliminary diagnosis of vasculitis. There was no significant decrease in creatinine values in the follow-up. He was consulted with surgery in order to be evaluated in terms of

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operation related to hydatid cyst disease. Before heaptectomy, azathioprine was discontinued and prednol dose was reduced. Right hepatectomy (segments 5-6-7) was performed. Drug levels were re-arranged after the first week postoperatively.

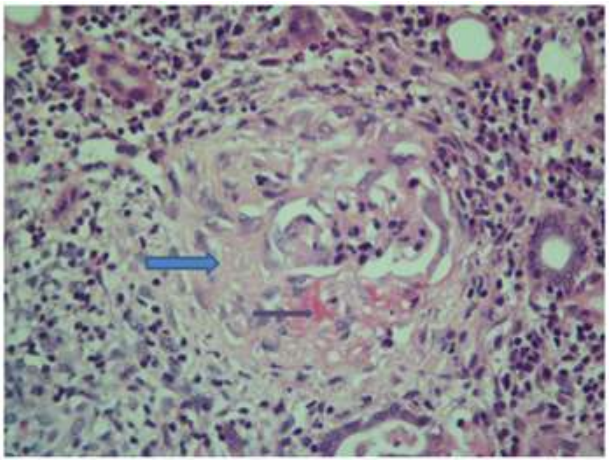

Fig. 1. Blue arrow: Fibrocellular crescent area, Red arrow: Fibrinoid necrosis

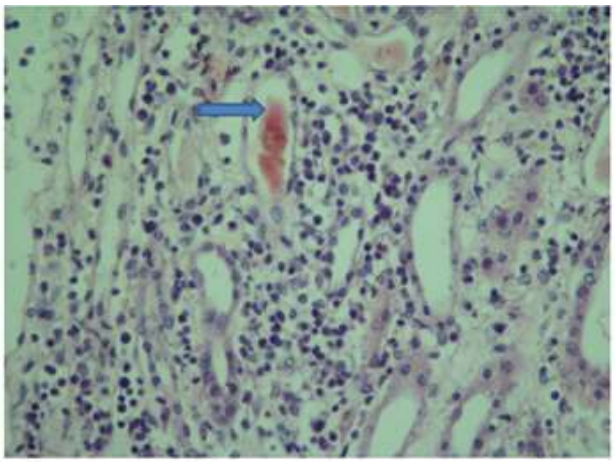

Fig. 2. Intense mixed inflammation in the tubulointerstitial area, arrow, erythrocyte cast in tubule

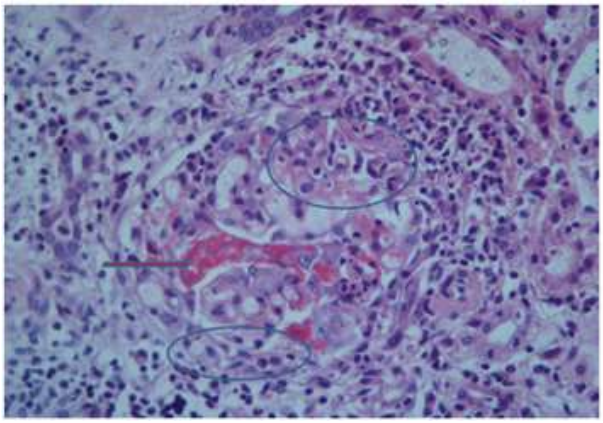

Fig. 3. Cellular crescent areas, arrow: Fibrinoid necrosis

After the third post-op day, the creatinine values started to decrease, and the creatinine level was 1.6 mg/dl after the post-op 7th day and similar levels were observed in the follow up visits after its discharge. The patient's proteinuria at 1-month later was below 1 g/dl and creatinine level was 1.33 mg/dl.

Discussion

The clinical presentation of Echinococcosis usually depends on the size and location of the cysts. In general, hydatid cysts show a slow growth in parenchymal tissues in humans and when they reach a certain size, they show signs of compression on adjacent tissues. Although rare, glomerular lesions such as Ig A nephropathy (IgAN), membranous nephropathy (MN), membranoproliferative glomerulonephritis (MPGN) in the form of secondary glomerular involvement can be seen in response to the presence of Echinococcus in other parts of the body [1].

In the present case, the prior serum creatinine values of the patient were around 2.4 mg/dl at the highest, and the kidney size and kidney echogenicity were normal in the performed imagings. Therefore, an acute nephropathy was thought for the patient, but without any obvious reasons. Consequently, after the surgical removal of his hydatid cyst, the glomerulopathy was resolved.

Covic *et al.*, reported a case of nephrotic syndrome due to mesangiocapillary glomerulonephritis with a hepatic hydatid disease whom her proteinuria was abolished by surgical removal, but later returned with a relapse of hydatid disease. An ultimately definitive treatment for hydatid disease has abolished all proteinuria and restored the patient to sustained good health [1]. Their case demonstrates a relapsing-remitting course of hydatid-associated mesangiocapillary glomerulonephritis according to the presence or absence of hepatic echinococcal cysts, and positive serum ELISA [1].

There are few reports of hydatid-associated glomerular lesions in the literature, and most do not have a clear relationship with clinical disease activity [1]. Secondary glomerular involvement due to Echinococcus is seldomly reported such as IgA nephropathy [2], membranous glomerulopathy [3,4], mesangiocapillary glomerulonephritis [1,5], postinfectious glomerulonephritis [6]. Membranous glomerulonephritis and echinococcal disease have been reported in an adult with early membranous glomerulonephritis after kidney biopsy at liver cyst resection [3]. Immunoperoxidase studies have demonstrated the echinococcal antigen and corresponding antibody in the glomeruli [1,4]. Ibarrola *et al.*, showed that the immunofluorescence study with anti-hydatid serum gave positive results for a finely granular pattern in the glomerular basement membrane, when renal tissue from the patient had been used as a substrate [4]. The authors suggested the positivity of indirect immunofluorescence studies using the antihy-

datid serum and renal tissue from the patient, is valid evidence for the presence of hydatid antigen in the glomerular basement membrane [4]. In addition, their case report documents the role of hydatid antigen in the pathogenesis of glomerulonephritis in human for the first time [4]. The possibility of an incidental association of the parasitosis with glomerulonephritis is due to trapping of the antigen in the glomerulus [4]. The authors stated that they observed the resolution of the nephrotic syndrome and the underlying glomerulonephritis once the antigen source has been eliminated [1,4]. Rincon *et al*., described the clinical association of MCGN with hydatid disease in a 70-year-old gentleman having nephrotic syndrome, hypertension, and renal failure treated with antiplatelet drugs without much benefit and progressed to an end-stage renal failure, requiring hemodialysis within a year [5].

In another case, des Grottes *et al*., reported a 71-year-old female patient presenting with repeated bronchopneumonia and terminal renal failure related to immunological glomerulonephritis. In this case, autopsy also revealed mediastinal and pulmonary hydatid cysts [2]. Edelweiss reported a patient with hydatid cyst with a normal renal function and no significant proteinuria. In the performed kidney biopsy during the surgery, the immunofluorescence and immunoperoxidase studies revealed glomerular deposits of hydatid antigen and corresponding antibody. In conclusion, a case of grade I immune complex type membranous glomerulonephritis without obvious clinical manifestations associated with liver hydatid disease has been reported [3].

Öner *et al*., reported a 15-year-old boy with clinical and laboratory findings of acute post-streptococcal glomerulonephritis (APSGN) associated with a cystic mass of 78x55x52 mm in size in the lower pole of the right kidney. The patient, whose hemagglutination test was positive for Echinococcus, was treated with percutaneous drainage and oral mebendazole [6)]. At the end of 22 months, cyst sizes reduced and hemagglutination test was negative without recurrence.

Uslu *et al*., reported a case with loss of renal function and non-nephrotic level proteinuria, and diagnosed as hepatic hydatid cyst, with mesangioproliferative glomerulonephritis [7]. They could not achieve remission with albendazole, but improvement in renal function and regression in proteinuria were observed following the surgical resection [7]. The authors claimed that nephropathies secondary to parasite invasion include acute kidney injury due to the physical invasion of the parasite and the systemic effects of infection, and glomerulonephritis results from immune interaction [7,8]. The authors suggest that although the pathogenesis of glomerular dysfunction resulting from hydatid cyst has not yet been fully elucidated, the most widely accepted view is an immune complex-related mechanism [3,7]. The antibody associated with the echinococcal antigen was demonstrated in the glomeruli by immunoperoxidase studies [3,4,7,9].

A few studies have reported that glomerular pathology caused by hydatid cyst disease is improved or reversible with treatment of infection such as cyst resection [10,11] or albendazole therapy [12]. Altay *et al*., investigated the frequency and characteristics of renal involvement in 80 patients with hydatid disease [8]. In their study, hematuria was found in 11 patients (13.75%) and proteinuria was found in nine patients (11.25%). Percutaneous kidney biopsy was performed in nine patients. They detected 4 immunoglobulin A nephritis (together with tubulointerstitial nephritis in one patient), one membranoproliferative glomerulonephritis, one immunoglobulin M nephritis together with mesangiocapillary glomerulonephritis, one membranous glomerulonephritis, one amyloidosis and one tubulointerstitial nephritis [8]. Glomerular involvement due to the immune complex deposition in echinococcosis has also been described in sheep as glomerular cellular proliferation, capillary wall thickening and hematic cylinders [8,13]. In addition, mesangial and subendothelial granular echinococcosis were detected in all of them by immunofluorescence staining [13]. Although hydatid cyst associated with glomerulopathies usually shows an acute, self-limiting course or resolves after the treatment of hydatid disease, very few chronic and progressive glomerulonephritis have been reported in the literature [2,5,13]. In this context, Altay *et al*., reported that progressive glomerulonephritis and chronic kidney disease developed in two cases (IgAN and vasculitis in one case and amyloidosis in the other case) in their series [8]. Other sporadic cases of the association of echinococcosis with amyloidosis have been reported in the literature [8,10,14-17]. As detailed above, echinococcal antigen and corresponding antibodies in the glomeruli were demonstrated by immunoperoxidase studies [3,10,13]. It has been reported that continual antigen presentation may cause long-lasting antibody response that causes antigen-antibody deposition in the glomerular basement membrane. Considering hydatid disease affecting the kidney is not very rare when sporadic cases reported in the literature are compiled, urinalysis and, if necessary, kidney biopsy in appropriate clinical conditions are recommended in patients with suspected liver hydatid disease [8]. In addition, it is recommended that surgical treatment of hydatid cyst disease should be considered in appropriate clinical conditions in patients who are thought to have glomerular involvement secondary to hydatid cyst disease [7].

Conclusion

We described a case of crescentic glomerulonephritis associated with hydatid disease. In our case, with fibrinoid necrosis and fibrocellular crescent, the surgical removal of hydatid cyst after the immunosuppressive

therapy reduced proteinuria and regressed serum creatinine levels. We believe that it would be appropriate to include hydatid cyst serological tests in the proteinuria and nephrotic syndrome work up in patients with a history and clinical picture compatible with hydatid cyst disease especially in regions where hydatid cysts are endemic.

Conflict of interest statement. None declared.

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