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Review

A Disturbed Phosphate Metabolism in Chronic Kidney Disease Progression and after Kidney Transplantation - What should the Clinicians be Aware of?

Merita Rroji[1], Andreja Figurek[2] and Goce Spasovski[3]

[1]Department of Nephrology, University Hospital Center" Mother Tereza" Tirana, Albania, [2]Institute of Anatomy, University of Zurich, Zurich, Switzerland, [3]University Department of Nephrology, Medical Faculty, University of Skopje, Skopje, N. Macedonia

Abstract

Phosphate (P) is an essential element of life and both reduced and elevated serum P levels will reduce lifespan. Kidneys are very important to maintain phosphorus homeostasis. There is sustained evidence showing that high serum phosphate concentration is associated with cardiovascular disease, all-cause and cardiovascular mortality in chronic kidney disease (CKD), basically by vascular aging and altered mineral metabolism both before and after initiation of renal replacement therapy. On the other hand, the hyperphosphatemia was reported to have a detrimental impact on kidney function *per se*. It might have an independent pathogenic role in the onset and progression of CKD and might even attenuate the renoprotective effect of ACE inhibitors in proteinuric CKD patients. The phosphorus reduction has been shown as strongest determinant of improvement in proteinuria. There is some evidence that both post-transplant hypo-and hyperphosphatemia could be the reason of adverse outcome in transplanted patients, but there are still not enough data on disturbed phosphate metabolism that could bring the guidelines that are needed. Enhancing the knowledge on P balance and toxicity is challenging to improve the care of renal patients, to protect the graft and enable longer kidney and patient's survival. A healthy phosphate balanced diet may be important for a healthy life and longevity.

Keywords: phosphate, fibroblast growth factor 23, chronic kidney disease, proteinuria, kidney transplantation

Introduction

Phosphate (P) is an essential element of life. This is a nutrient required for critical biological reactions that maintain the normal homoeostatic control of the cell, an important component of different cellular structures, so both reduced and elevated serum P levels will reduce lifespan [1]. Regulation of the P pool in humans depends on the homeostatic balance between nutritional intake, intestinal absorption, and kidney excretion. Besides, the distribution of P concentrations is highly heterogeneous between body compartments and strictly regulated by hormonal assets [2]. Kidneys are very important to maintain phosphorus homeostasis where tubular handling is a leading regulator of P equilibrium. In normal subjects, about 75 and 10% of the filtered P is reabsorbed in the proximal convoluted tubule, and in the distal convoluted tubule, respectively and around 15% of the filtered P being excreted in the urine. Furthermore, there is an intestinal-glomerular feedback and the 24 h urinary P excretion (UPE) is considered equivalent to daily intestinal absorption (not intake) of P in normal subjects at a steady state [3,4].

Phosphate balance in CKD patients

In chronic kidney disease (CKD) stages 2 and 3 patients, serum phosphate is not usually increased, due to increased fractional excretion of phosphate. The further reduction of GFR, requires compensatory reduction of tubular resorption of P (TRP), which was mediated by fibroblast growth factor 23 (FGF23) [2] which directly enhances tubular excretion of phosphorus and reduce 1-25 hydroxylase activity which in turn reduces calcitriol production and intestinal phosphorus absorption. The determinants of the FGF-23 increase in CKD are still unclear and may involve a low availability of its cofactor Klotho, expressed in the same tubular sites where FGF-23 acts [1,3,5]. Due to the important role of the kidneys in maintaining phosphorus homeostasis, hyperphosphatemia is a common manifestation of advanced CKD. There is sustained evidence showing high serum phosphate concentration is associated with cardiovascular disease, all-cause and cardiovascular mortality in CKD basically by vascular aging and altered mineral metabolism both before and after initiation of renal replacement therapy [6-8]. The maintenance of phospho-

Correspondence to: Merita Rroji, Department of Nephrology, University Hospital Center, "Mother Tereza", Tirana, Albania; Phone: 00355692314086; E-mail: meritarroji@yahoo.com

rus concentrations within an optimum range is considered as a standard of care in this patient population [9].

Hyperphosphatemia and progression of kidney dysfunction

Hyperphosphatemia *per se* was reported to have a detrimental impact on kidney function. Older experimental studies in rats have shown high dietary phosphorus can initiate and/or worsen the progression of kidney dysfunction [10], whereas dietary phosphate restriction reverses and/or restricts the dysfunction [11]. Podocyte injury due to overexpression of pituitary-specific positive transcription factor 1 (Pit-1) was one of the proposed mechanisms which go apart of the effect of phosphorus on inducing calcifications [12]. It is also possible that the deleterious effect of acute phosphorus loading on systemic endothelial function might also extend to the glomerular endothelium. There are few clinical studies about the potential effect of serum phosphorus concentrations on the rate of progression of CKD. Shwarz *et al.* showed that higher serum phosphorus was associated with a significantly higher risk for progression of CKD, even after adjustment for multiple potential confounders supporting earlier studies that showed a beneficial effect of dietary phosphorus restriction on the progression of CKD in experimental animals [13]. The relative risk of developing ESRD was demonstrated to be much higher in CKD patients with serum phosphorus concentrations >1.29 mmol/l in NHANES participants free of CKD [14]. Bellasi *et al.* utilizing the "Prevenzione Insufficienza Renale Progressiva" (PIRP) database, underlined the strong association between serum phosphorus and a rapid decline in the kidney function and mortality [1,15]. Besides, they reported that the relative contribution of hyperphosphatemia in predicting progression to ESRD or death might differ between women, persons with diabetes, older patients, and those in more advanced stages of CKD.
In a post hoc analysis of the Ramipril Efficacy in Nephropathy (REIN) Study, Zoccali *et al.* [16] found that just 1-mg/dl [0.3226 mmol/l] increase in serum phosphate level was associated with an 85% excess risk for progression to ESRD [16]. Their data suggested that phosphate is an independent risk factor for the progression of kidney disease among patients with proteinuric CKD, and high levels of phosphate might even attenuate the renoprotective effect of ACE inhibitors. In addition, it was shown that the strongest determinant of improvement in proteinuria was the phosphorus reduction (either in urine or serum) and not the protein intake per se [17]. One explanation might be the stimulation of the FGF receptor which may activate the RAS. In this way it might facilitate the onset and progression of kidney damage in subjects at risk and, at the same time, might overcome the effects of RAS inhibitors on angiotensin II activity and production, in particular at the tissue level. On the other hand, nitric oxide (NO) production due to direct or indirect effect (through FGF23) of phosphate might also limit or prevent the effects of ACE inhibitor therapy mediated by NO activation. In addition, the coreceptor of FGF23, Klotho, a well recognized renoprotective factor which reduces angiotensin-II-induced renal damage, is downregulated at an early stage in CKD patients. Klotho -/- mice suffer premature aging, and this phenotype may be corrected with a low phosphate diet raising the hypothesis that lower phosphate in CKD patients with reduced Klotho may mitigate the progression of kidney damage and ameliorate the response to ACE inhibition [16-20].
A meta-analysis and systematic review of 12 cohort studies with 25,546 patients published from 1950 to 2014 reported that every 1-mg/dL [0.3226mmol/l] increase in serum phosphorus level was associated independently with increased risk of kidney failure (hazard ratio, 1.36; 95% CI, 1.20-1.55) and mortality (hazard ratio, 1.20; 95% CI, 1.05-1.37) suggesting that large-scale randomized controlled trials should target disordered phosphorus homeostasis in CKD [21].
Dietary phosphate overload never causes persistent hyperphosphatemia unless renal function is critically impaired, because the kidney as it was mentioned above, excretes excess phosphate into the urine (more precisely in the tubular luminal fluid), and maintains phosphate balance [1-3]. Two possible ways exist to increase the amount of phosphate excreted in the urine. One way is due to an increase in the total volume of urine by increasing GFR (hyperfiltration) and the second might be due to increasing the fractional excretion of phosphate (FEP). Being not always able to increase GFR, patients with CKD mostly can increase the FEP per nephron to maintain phosphate balance [22]. This is mediated mainly by increased level FGF23 in early stages but also by PTH later on [20].
In experimental rat studies it was shown that raised phosphate intake and/or decreased nephron number increases the rate of phosphate excretion per nephron, potentially causing calciprotein particle formation in the tubular lumen, epithelial tubular injury, and fibrosis [20,22-25]. In heminephrectomized rats, a high phosphate diet increased phosphaturia resulting in kidney tubular damage associated with inflammation, oxidative stress, and low klotho expression [26]. High FGF23 was found to be associated with a significantly higher risk of end-stage renal disease in patients with relatively preserved kidney function [22] and C-terminal and intact FGF23 was shown that independently predict the progression of CKD after adjustment for age, gender, GFR, proteinuria, and serum levels of calcium, phosphate, and parathyroid hormone [27]. Based on these data a new paradigm for phosphate restriction was proposed suggesting that phosphate binders be used in patients with CKD and an abnormally high FGF 23 level, regardless of serum phosphate level to prevent preventing

histological kidney damage and progression of CKD [20,28]. Decision to treat patients should be based on a risk-benefit assessment taking account harmful side effects of available compounds [28]. Given the potential toxicity of excess phosphate, the general population may also be viewed as a target for phosphate management. Enhancing the knowledge on P balance and toxicity is challenging to improve the care of general population and renal patients [1-3,25]. A healthy phosphate balanced diet may be important for a healthy life and longevity.

Disturbed phosphate metabolism post-transplantation

Phosphate metabolism is changed even after kidney transplantation and could be manifested either as hypophosphatemia (even severe one with phosphate level <0.5 mmol/l) or as hyperphosphatemia (levels >1.5 mmol/l) [29].

In the first three months after kidney transplantation usually hyperphosphaturia and consequent hypophosphatemia occur as the results of high FGF-23 and PTH levels, ischaemia-reperfusion injury, immunosuppressive drugs and metabolic acidosis [29]. Studies have shown that glucocorticoids induce decrease expression of sodium-phosphate (NaPi) cotransporters in the apical membrane of tubular epithelial cells as well as the intestinal NaPi activity, resulting in decreased phosphate absorption in the intestine and reabsorption in the proximal tubule [30-32]. Experimental studies in rats have shown that cyclosporin inhibits NaPi activity [33]. In the similar manner, the usage of calcineurin inhibitors and mTOR (mammalian target of rapamycin) inhibitors is linked to a post-transplant hypophosphatemia [34,35].

In later months after transplantation, hyperphosphatemia can occur as the result of impaired graft function with secondary or tertiary hyperparathyroidism [29].

It is important to know that higher serum phosphate level, calcium-phosphate product and FGF-23 levels after kidney transplantation present a higher risk of graft failure [36-38]. Analysis of the FAVORIT (Folic Acid for Vascular Outcome Reduction in Transplantation) Trial Cohort study indicated that elevation for every 1 mg/dl [0.32 mmol/l] in serum phosphate level increases transplant failure, when adjusted for confounding factors (treatment allocation, traditional cardiovascular disease risk factors, kidney measures, type of kidney transplant, transplant vintage, use of calcineurin inhibitors, steroids and lipid-lowering drugs) [39].

At both 6 and 12 months post-transplant serum phosphate is found to be the independent predictor of graft lost, even when adjusted for age, sex, blood pressure, proteinuria, donor type and HLA mismatches and phosphate level greater than 1.2 mmol/l resulted in worse graft survival [40].

Hyphophosphatemia can be maintained even later after transplantation. Low serum phosphate 1 year post-transplantation is induced by both PTH and FGF23, but with greater impact of persistent hyperparathyroidism [41].

Acute phosphate nephropathy is also described as an entity that could cause graft failure due to phosphate overload and calcium phosphate deposition (with or without hypercalcemia) [42,43]. On the other hand, there are studies that reported no association between serum phosphate, serum calcium and calcium phosphate products with poor patient and graft outcomes during the first year after kidney transplantation [44].

There is some evidence that both post-transplant hypo- and hyperphosphatemia could be the reason of adverse outcome. In the large study on 2786 kidney transplant recipients 1 year after transplantation (with the follow-up of 78.5 months) both, higher (≥5 mg/dl i.e. 1.6 mmol/l) and lower serum phosphate (<2.5 md/dl i.e. 0.8 mmol/l) were associated with death-censored graft failure, but also with patient mortality (exhibiting U-shape association) [45].

Furthermore, it is suggested that higher serum phosphate is a predictor of all-cause mortality in patients with kidney transplant after adjustment for traditional cardiovascular risk factors, estimated glomerular filtration rate, high sensitivity C reactive protein and renal graft failure [46]. Serum phosphate is negatively associated with graft function in CKD stage 4-5 in 990 patients with median 72 months post-transplantation [47].

Treatment of hypo- and hyperphosphatemia after kidney transplantation is very challenging. Phosphate supplementation early post-transplant is recommended when serum phosphate is between 0.5 and 1.0 mmol/l with the presence of muscle weakness [29]. However, it is reported that phosphate supplementation may play a role in the calcification of native or transplanted kidney [48,49]. On the other hand, usage of phosphate binders in patients with transplanted kidney should also be very careful as they can reduce the concentration of mycophenolate mofetil [50].

Compared to the data known for CKD population, in kidney transplant recipients there are still not enough data on disturbed phosphate metabolism that could bring the guidelines that are needed. Therefore, more attention should be paid on the discovering the "nature" of phosphate metabolism in a long run after kidney transplantation and the best approach for treating the potential disturbance in order to protect the graft and enable longer kidney and patient survival.

Conclusion

Exploring the association between serum phosphate levels and renal outcomes based on the available data indicate that phosphate might have an independent pathogenic role in the onset and progression of CKD and might even attenuate the renoprotective effect of ACE inhibitors in proteinuric CKD patients. The phosphorus reduction was shown to be the strongest deter-

minant of improvement in proteinuria. Compared to the data known for CKD population, in kidney transplant recipients there are still not enough data on disturbed phosphate metabolism that could bring the guidelines that are needed. Enhancing the knowledge on P balance and toxicity is challenging to improve the care of renal patients. Finally, a healthy phosphate balanced diet may be important for a healthy life and longevity.

Conflict of interest statement. None declared.

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Original article

Appearance of Primary and Secondary Glomerulonephritis: Single Centre Experience

Hasan H. Yeter[1], Elif Gecegelen[2], Vural Bastug[2], Berfu Korucu[1], Farabi C. Fettahoglu[2], and Ulver Derici[3]

[1]Nephrology fellowship, Department of Nephrology, Gazi University, [2]Research assistant, Internal medicine, Gazi University, [3]Department of Nephrology, Gazi University, Ankara, Turkey

Abstract

Introduction. Glomerular disease could lead to chronic kidney disease. The aim of this study is to determine the frequency of kidney biopsy proven glomerulonephritis (GN) to create better database in Turkey.
Methods. We evaluated 1273 native kidney biopsies from 2008 to 2017. Renal disease were divided into five major categories: primary GN, secondary GN, tubulointerstitial nephropathies (TIN), vascular nephropathies (VN) and acute tubular necrosis (ATN).
Results. 756(59.4%) patients had primary GN, 353 (27.7%) patients had secondary GN, 101(7.9%) patients had TIN, 32(2.5%) patients had VN and 31(2.4%) patients had ATN. While, the most frequent pathological diagnosis was focal segmental glomerulosclerosis (FSGS) (31.35%), followed by membranous GN (27.65%) in primary glomerulonephritis, amyloidosis (27.2%) was the most frequent pathological diagnosis followed by systemic lupus erythematosus (SLE) (20.68%) in secondary glomerulonephritis. FSGS is the most common kidney pathology in younger than 50 years old (18-30 years old, 22% and 31-50 years old, 20%). Membranous glomerulonephritis (MGN) is the most common kidney pathology after the ages 50 years old (50-64 years old, 20% and >65 years old, 17%).
Conclusion. FSGS is the most common primary GN in Turkish population and the frequency is gradually increasing.

Keywords: adult, glomerulonephritis, kidney biopsy, registry

Introduction

Chronic kidney disease (CKD) is increasingly prevalent worldwide with high morbidity and mortality [1]. Early diagnosis of CKD, determination and treatment of possible risk factors for progression to end stage kidney disease have prime importance [1,2]. Glomerular diseases can result from many inherited or acquired disorders and could lead to CKD. Glomerular disease frequency varies between countries. National databases such as European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) in Europe, United State Renal Data System (USRDS) and Italian Registry of Renal Biopsies (IRRB) provide comparison of frequency of glomerulonephritis (GN) in these countries. The main causes of CKD in the USA are diabetic nephropathy (46.7%), hypertensive nephropathy (23.8%) and GN (9.4%). On the other hand, the most common cause of CKD in China is GN and the frequency varies between 50% and 68.6% [3,4]. The most common cause of GN vary from country to country in epidemiological studies. While the most common cause of nephropathies is IgA nephropathy (IgAN) in Italy (43.5%) [5], focal segmental glomerulosclerosis (FSGS) in USA [6], IgAN in Japan (%50) [7] and FSGS in Brazil (29.7%) [8].
One of the basic condition to conduct good clinical epidemiological studies is the ability to create databases for specific diseases. National epidemiological studies provide opportunity for determination frequency of specific disease and develop strategies, as well as making it possible to compare with other nations. The aim of this study is to determine the frequency of kidney biopsy proven GN to create better database in Turkey and compare with the data of other countries.

Material and methods

This descriptive study was conducted in Gazi University Nephrology and Pathology departments. We evaluated data relating to 2028 kidney biopsies collected from 2008 to 2017. Patients, who were older than 18 years of age with abnormal urine findings and/or decreased renal function of unknown etiology were evaluated. Data of 114 renal biopsies could not be classified due to inadequate sampling of materials. We analyzed age, gender and biopsy proven diagnosis.
Nephrotic range proteinuria (>3.5 g/d proteinuria), asymptomatic urinary abnormalities (persistent low grade proteinuria with or without microscopic hematu-

Correspondence to: Hasan Haci Yeter, Nephrology department, Gazi University, Ankara, Turkey,
Orchid: 0000-0002-5787-1048; Phone: 905542397449; E-mail: hasanyeter@hotmail.com

ria), nephritic syndrome (hematuria, arterial hypertension and reduced kidney function), acute kidney failure (sudden increase of serum creatinine and/or reduced urine output) and chronic kidney disease with unexplained reason were kidney biopsy indications. Renal biopsy was not performed if kidney length was below 9 cm. Renal biopsy cores were obtained with ultrasonography accompanied standard procedure by the nephrologist. Fresh biopsy cores were fixed in formaline and evaluated under light microscopy. Paraffin sections were prepared and stained with hematoxylin eosin, Kongo red and Jones silver methenamine stains. Small renal cortical tissue was separated for immunofluorescence study. Immunofluorescence studies on cryostat sections using polyclonal antisera against IgG, IgM, IgA, C3, C1q, C4d, kappa and lambda light chains have been used. Electron microscopy was not used for all biopsy specimens so that reason minimal change disease (MCD) and alport 's syndrome could not be included.

Renal disease were divided into five major categories: (1) primary GN; (2) secondary GN; (3) tubulointerstitial nephropathies (TIN); (4) vascular nephropathies (VN); and (5) acute tubular necrosis (ATN). Primary GN were classified as IgA nephropathy, FSGS, membranous GN (MGN), MCD, membranoproliferative GN (MPGN), diffuse proliferative GN (DPGN), mesangioproliferative GN (MePGN) and acute poststreptococcal GN (APSGN). Systemic lupus erythematosus, Hench-Schönlein purpura, amyloidosis, Goodpasture's syndrome, vasculitis, diabetes mellitus, Alport's syndrome and dysgammaglobulinemia associated GN (Waldenstrom's macroglobulinemia, monoclonal gammopathy and light chain disease) were considered as secondary GN. Acute and chronic TIN evaluated in TIN. While thrombotic microangiopathy, malignant nephrosclerosis, cortical necrosis and preeclampsia were considered among VN, acute tubular necrosis considered among ATN.

Categorical variables are expressed as percentage. Data distribution was determined by using Kolmogorov-Smirnov test. Homogenity of variables were determined by using one way anova homogenity of variance test. Chi-square test was used to compare categorical variables. The Kruskal-Wallis one way analysis of variance was used if more than two groups were being compared. Analyses were performed with Statistical Package for the Social Science (SPSS version 20.0.0, IBM) software for Windows.

Results

We examined 2028 kidney biopsies which were performed between 2008 and 2017 in Gazi University, department of Nephrology. Biopsies, which were non-diagnostic (n=114), non-specific (n=128) and transplant kidney (n=126), were excluded from the study. Also, biopsies reported as CKD and patients who were younger than 18 years old were excluded (Figure 1). Finally, 1273 native kidney biopsies were examined. Among all adults, 756(59.4%) patients had primary GN, 353 (27.7%) patients had secondary GN, 101(7.9%) patients had tubulointerstitial nephropathies, 32(2.5%) patients had vascular nephropathies and 31 patients (2.4%) had ATN.

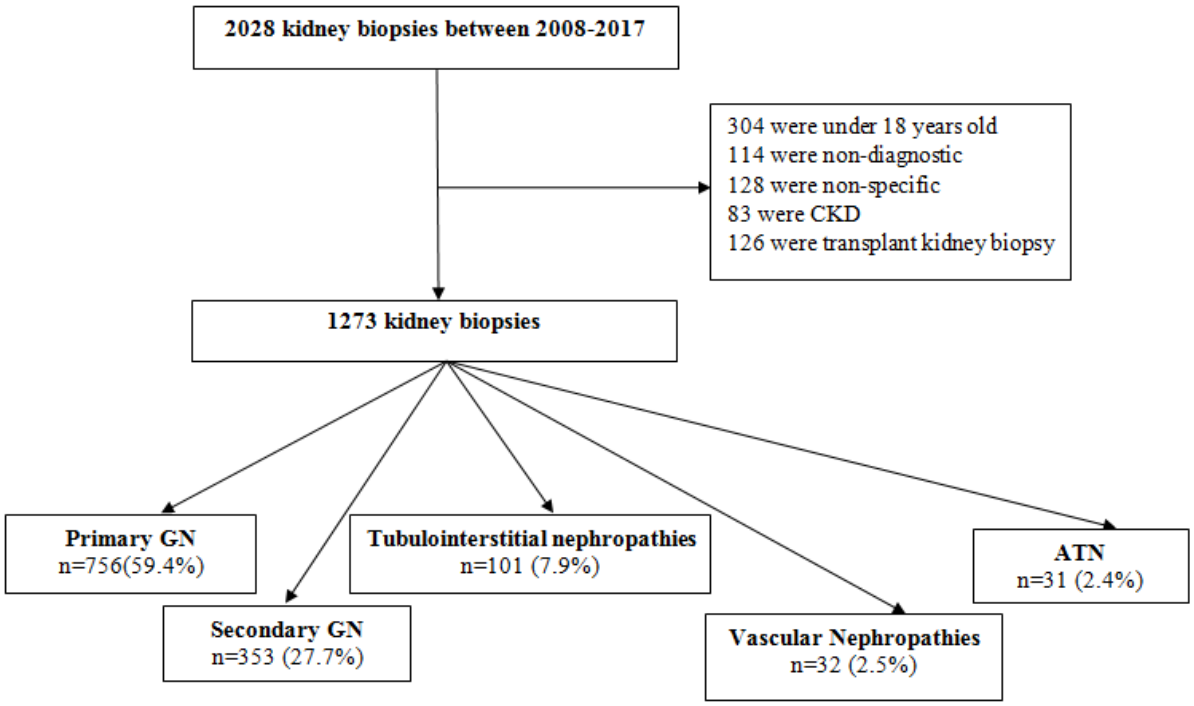

Fig. 1. Distribution of kidney biopsies between 2008 and 2017

While, the most frequent pathological diagnosis was FSGS (31.35%), followed by membranous GN (27.65%) and IgAN (22.09%) in primary glomerulonephritis, amyloidosis (27.2%) was the most frequent pathological diagnosis followed by SLE (20.68%) and diabetic nephropathy (15.01%) in secondary glomerulonephritis (Figure 2). About 53% (n=679) of total patients who had done kidney biopsy were male. Primary GN were more frequent in males (54%), when compared to females (46%); similarly, secondary GN (males 55% and females 45%) and ATN (males 61% and females 39%) were more frequent in males. On the contrary, TIN (females 54% and males 46%) and VN (females 69% and males 31%) were more frequent in females. Glomerular diseases, whose frequency was significantly higher in males, were IgAN (p<0.001), amyloidosis (p=0.02) and diabetic nephropathy (p=0.03).

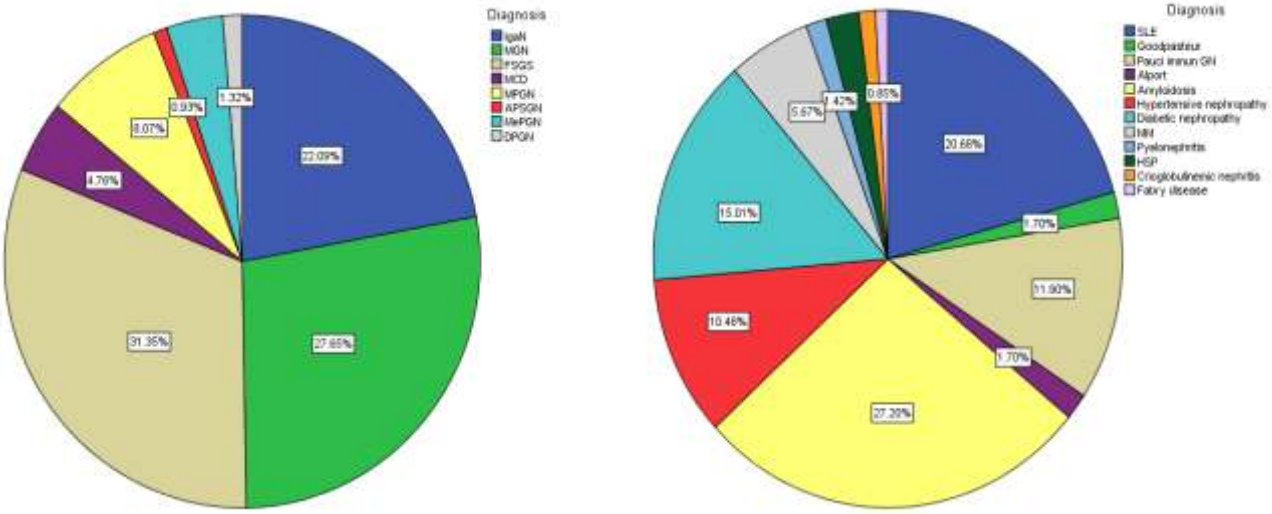

Fig. 2. Frequency of histological diagnosis of primary and secondary nephropathies: Abbreviations are: IgAN, IgA nephropathy; MGN, membranous glomerulonephritis; FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MPGN, mesengioproliferative glomerulonephritis; APSGN, acute poststreptococcal glomerulonephritis; MePGN, mesengioproliferative glomerulonephritis; DPGN, diffuse proliferative glomerulonephritis; HSP, Hench-Schönlein purpura; SLE, systemic lupus erythematosus; MM, multiple myelom

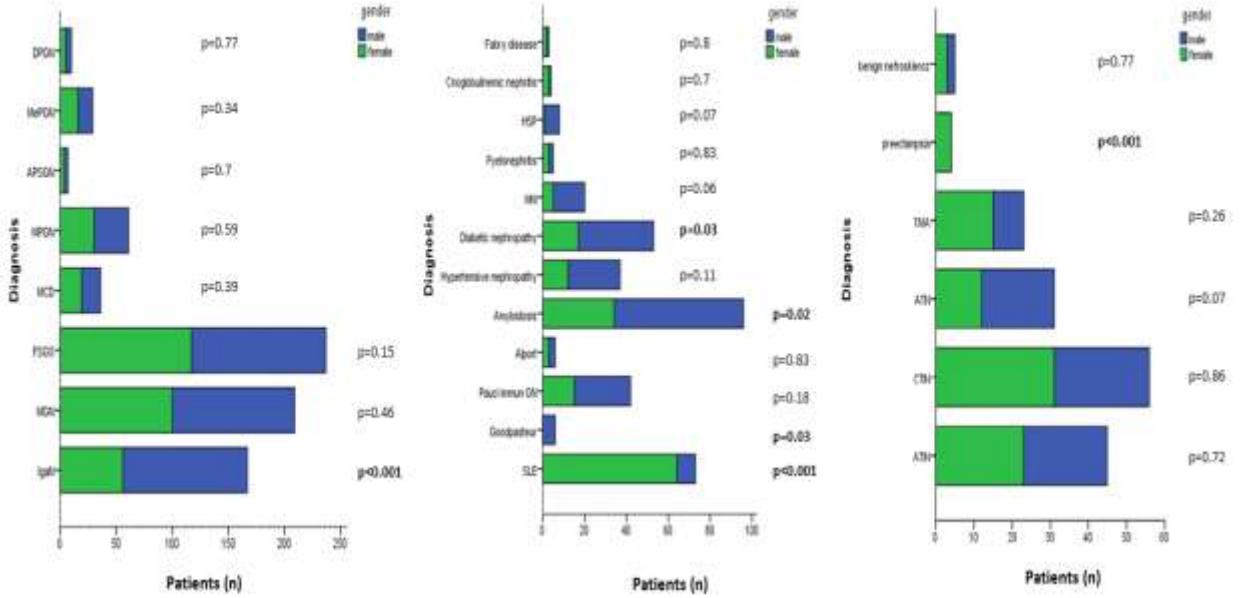

Fig. 3. Gender distribution of patients in kidney diseases: Abbreviations are: IgAN, IgA nephropathy; MGN, membranous glomerulonephritis; FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MPGN, mesengioproliferative glomerulonephritis; APSGN, acute poststreptococcal glomerulonephritis; MePGN, mesengioproliferative glomerulonephritis; DPGN, diffuse proliferative glomerulonephritis; HSP, Hench-Schönlein purpura; SLE, systemic lupus erythematosus; MM, multiple myelom; ATIN, acute tubuinterstitial nephritis; CTIN, chronic tubulointerstitial nephritis; ATN, acute tubular necrosis; TMA, thrombotic microangiopahty

Besides, SLE (p<0.001) was significantly higher in females (Figure 3). When the age distribution of primary glomerulonephritis was examined, it was observed that frequency of MGN increased significantly with age and frequency of IgAN decreased after the age of 50 (p<0.001 and p=0.01, respectively). While, the frequency of SLE and Goodpasture Sy, decreased significantly with the age (p<0.001 and p=0.009), frequency of amyloidosis, hypertensive nephropathy, diabetic nephropathy and MM increased significantly with the age (p=0.01, p=0.002, p=0.007 and p=0.02, respectively) (Figure 4). When the age distribution of primary and secondary GN is examined within themselves; FSGS is the most common primary GN between the ages of 18-30 and 31-50 [35% (n=78) and 30% (n=109) respectively]. On the contrary, MGN is the most common primary GN in patients who are 50 years and older [37% (n=41) and 42% (n=25]. And also frequency of IgAN decreases with the age. SLE is the most common secondary GN in the age of 30 [42% (n=37)], whereas amyloidosis is the most common secondary GN after the age of 30 [31-50 years old, 28% (n=36), 50-64 years old, 33% (n=22) and >65 years old, 36% (n=25)]. When the frequency of kidney pathology is examined in all biopsy population; we showed that FSGS is the most common kidney pathology until 50 years old [18-30 years old, 22% (n=78) and 31-50 years old, 20% (n=109)]. While MGN is the most common kidney pathology between the ages 50-64 years old [20% (n=41)], MGN and amyloidosis are the most common glomerular pathology after the age of 65 [17% (n=25) and 17% (n=25) respectively] (Table 1). When the prevalence of diabetic nephropathy is examined among the secondary GN, the prevalence gradually increases until the age 65, but it decreases slightly after this age. When we analyzed the frequency of primary GN according to years, we showed that MGN is the most common primary GN until the end of 2013, whereas FSGS became the most common primary GN with a marked increase in the frequency after 2013 (Figure 5).

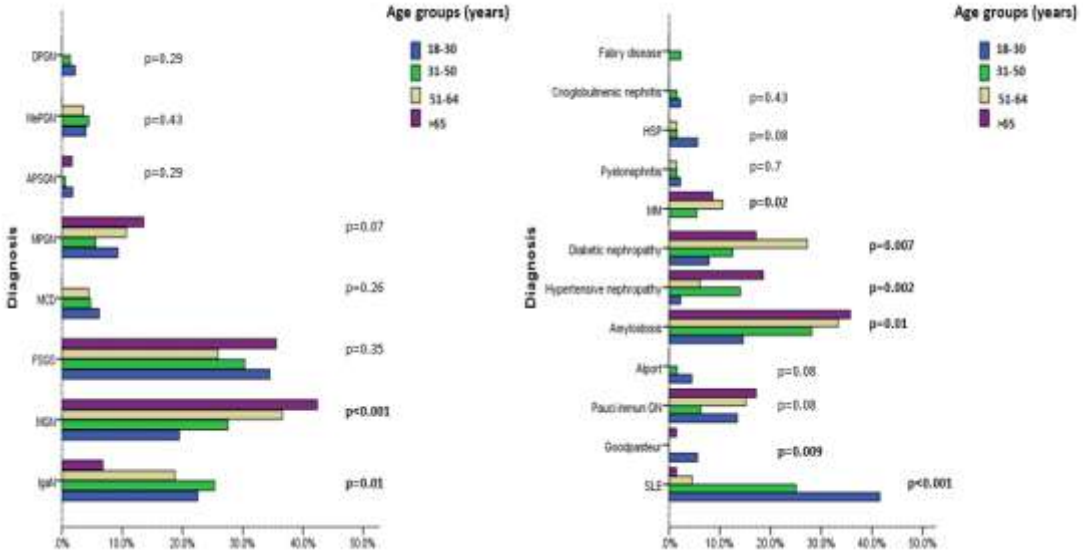

Fig. 4. Age distribution of primary and secondary glomerulonephritis

Table 1: The frequency of glomerulonephritis among age groups

	Primary Glomerulonephritis			Secondary Glomerulonephritis		
	IgAN	**MGN**	**FSGS**	**Amyloidosis**	**SLE**	**DN**
Age (years)	37(18-75)	44(18-84)	38(18-84)	50(21-81)	30(18-48)	53(22-79)
Age groups(years)						
18-30	23%(51)	20%(44)	35%(78)	15%(13)	42%(37)	8%(7)
31-50	25%(91)	28%(99)	30%(109)	28%(36)	25%(32)	13%(16)
50-64	19%(21)	37%(41)	26%(29)	33%(22)	5%(3)	27%(18)
>65	7%(4)	42%(25)	36%(21)	36%(25)	1%(1)	17%(12)
Total	22.1%(167)	27.6%(209)	31.3%(237)	27.2%(96)	20.7%(73)	15%(53)
	General Biopsy Population					
	IgAN	**MGN**	**FSGS**	**Amyloidosis**	**SLE**	**DN**
Age groups(years)						
18-30	14%(51)	12%(44)	22%(78)	4%(13)	10%(37)	2%(7)
31-50	16%(91)	18%(99)	20%(109)	6%(36)	5%(32)	3%(16)
50-64	10%(21)	20%(41)	14%(29)	11%(22)	2%(3)	9%(18)
>65	3%(4)	17%(25)	15%(21)	17%(25)	1%(1)	8%(12)
Total	13%(167)	16%(209)	19%(237)	8%(96)	6%(73)	4%(53)

IgAN: IgA nephropathy; MGN: membranous glomerulonephritis; FSGS: focal segmental glomerulosclerosis; DPGN: diffuse proliferative glomerulonephritis; SLE: systemic lupus erythematosus; DN: diabetic nephropathy

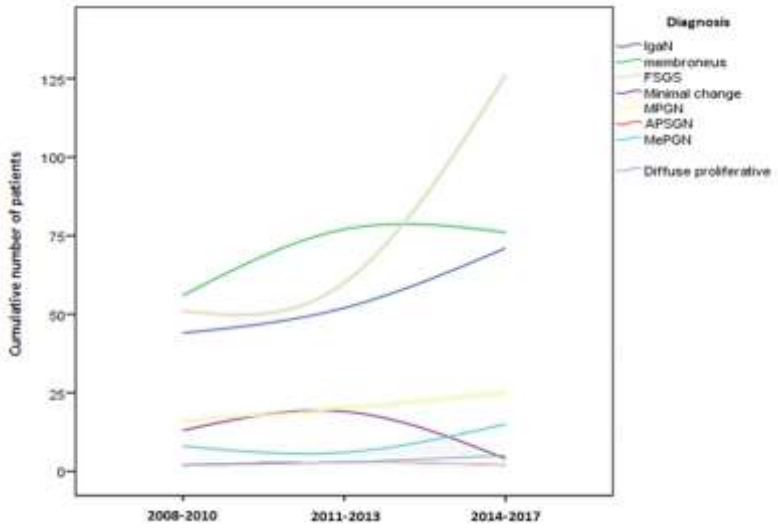

Fig. 5. Changes in the frequency of primary glomerulonephritis over the years

Discussion

Renal biopsy registries can provide very important information about epidemiology of renal diseases. Our observational study showed that FSGS is the most common GN in Turkey and it is followed by MGN and IgAN. The frequency of FSGS has increased during the last 5 years period. It has also been demonstrated in our study that FSGS is a type of GN that can be seen similar in both genders and every age groups.

Ozturk *et al*. demonstrated that MGN is the most frequent primary GN in Turkey in 2014 [2]. In subgroup analysis of age they showed that FSGS is the most common primary GN in patients who are younger than 40 years old. Similar to their study, we showed that MGN is the most leading cause of primary GN until 2014. But, we also demonstrated that the frequency of FSGS has increased steadily and became the most leading cause of primary GN after 2014. Many factors may have contributed to increase in FSGS frequency. Development in biopsy techniques and increased number of glomeruli colected by biopsy may have facilitated the diagnosis. On the other hand, Borges FF *et al*. showed that the frequency of FSGS is increasingly high and the obesity may be the cause of this rise [9]. We can also hypothesize that epidemic of obesity can facilitate the disease occurrence. Secondary forms of FSGS are potentially preventable, through promoting healthy lifestyle choices and reducing obesity [10]. The latest observational study, which is conducted with 264 patients, in Turkish population was published by Ayar Y *et al*. in 2016 to our knowledge [11]. They showed that MGN is the most common cause of primary GN in Turkey. Although the study was published in 2016, they have reviewed the medical records of their Clinics until 2014. On the other hand, Hur *et al*. reported that FSGS is the most common primary GN in Turkey and followed by MGN and IgAN [12]. Similar to our study, they showed that amyloidosis is the most common secondary GN followed by SLE. The frequency of GN varies regional. In a review, which was published in 2017, they showed that the most common GN was IgAN in Asia and Europe; FSGS in USA and Latin America [10]. Braden GL *et al*. showed that there was a significant increase in the percentage of Afircan-american, Hispanics and Caucasions with FSGS [13]. Similar to our result, Sim JJ *et al*. demonstrated that FSGS frequency has increased steadily between 2000 and 2011 and it is the most common primary GN in USA [14].

It is known that, diabetic nephropathy is the most common cause of CKD, but it is usually found low in kidney biopsy specimens. In our series, diabetic nephropathy prevalence was found to be low. The most important reason is that clinical diagnosis is still important in diabetic nephropathy and renal biopsy is usually not performed unless there is evidence of the presence of accompanying primary GN in these cases.

In conclusion, FSGS is the most common primary GN in Turkish population and the frequency is gradually increasing. Among the secondary GN, SLE in early ages and amyloidosis in middle and older ages are more frequent. The reason for the less frequent observation of diabetic nephropathy in secondary GN is the small number of biopsy cases.

Conflict of interest statement. None declared.
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Original article

Organ Donation in India: The Organ transplant coordinator's Perspective

Vaishaly Bharambe[1], Vatsalaswamy Puranam[2], Vasanti Arole[3] and Manvikar Purushottam Rao[4]

[1]Department of Anatomy, Dr D Y Patil Medical College, Hospital and Research Centre, Pune, India;[2]Academic Director, Dr D Y Patil Medical College, Hospital and Research Centre, Pune, India; [3]Dept of Anatomy, College of Medical Sciences, Bharatpur, Nepal; [4]Head of the Departmen of Anatomy, Dr D Y Patil Medical College, Hospital and Research Centre, Pune, India

Abstract

Introduction. The Transplant of Human Organ Act has made appointment of a transplant coordinator as mandatory for every transplant hospital for coordinating all matters related to removal or transplantation of human organs or tissues or both. The present study was undertaken to investigate the challenges of organ donation from the perspective of organ transplant coordinators.
Methods. The study consisted of detailed discussion with organ transplant coordinators regarding their experiences related to problems in organ donation activity in Pune. The discussions were analyzed for insights into the organ donation.
Results. 14 OTCs participated in the study. The analysis of the discussion revealed that challenges for organ donation in Pune region revolved around themes such as poor awareness about details of organ donor categories and organs each could donate, non declaration of brain death, financial status of the potential recipient, willingness or not to counsel the donor family for organ donation, myths and misconceptions related to organ donation and fear of organ trade, among others.
Conclusion. The people of this region needed to be educated about the details of categories of organ donors and the organs each category may donate. The OTCs must be trained in counselling for organ donation. The doctors of the region need to be encouraged to diagnose brain death. There was need to counsel people to help overcome the myths. The police or lawyers who were trained in document verification could be involved in document scrutinisation process to combat problem of organ trade.

Keywords: organ donation, organ transplant coordinator, organ trade, transplant of human organs act

Introduction

The Transplant of Human Organ or Tissue or both Act (2011) of India (THO Act 2011) states that no hospital shall be registered under the Act unless the authorities were satisfied that the hospital had appointed a transplant coordinator with requisite qualifications. An organ transplant coordinator (OTC) has been defined by the THO Act as a person appointed by a hospital for coordinating all matters related to removal or transplantation of human organs or tissues or both and for assisting the authorities for removal of human organs in accordance with the related sections of the Law [1].
Organ shortage for purpose of organ transplant surgeries is a universal problem worldwide [2]. However, Asia lags behind the rest of the world in efforts to solve this problem. Authors believe that the way to raise the organ donation rates in Asia, especially in India is to raise ground level awareness, dispel misinformation in the minds of people and encourage people to register for organ donation [3]. An OTC is a person who does all of this besides carrying out grief counselling for the family of the patient and broaching the subject of organ donation with the family of the brain dead patient.
The present study was undertaken to investigate the challenges to organ donation and transplant activity in Pune district of Maharashtra, a state in western Maharashtra, from the perspective of OTCs.

Aims

The present study was aimed at studying the challenges that impact Organ Donation in India and to find solutions to combat them from point of view of OTCs. After reviewing the findings the study attempted to suggest solutions to improve the rate of organ donation.

Material and methods

Pune division is one of the 6 divisions of Maharashtra state of India. The present study was carried out in select cities of this Pune division of Maharashtra. All those Cities in the Pune division of Maharashtra which

Correspondence to: Vaishaly Bharambe, D 9 State Bank Nagar. Panchvati, Pashan Road, Pune 411008, India;
Phone: +982 2 91 08 45; 02 02 58 99 168; Fax: 02 02 78 05 100;
E-mail: vaishalybharambe@yahoo.co.in

had registered organ transplant centres or had a non transplant organ retrieval centre (NTORC) were selected in this study. Thus the cities selected were Pune, Karad, Sangli-Miraj, Kolhapur and Solapur cities of Pune district.
Institutes Ethical committee clearance was obtained before starting of the study.
This study consisted of detailed discussion with organ transplant coordinators (OTCs) to bring out individual experiences related to organ donation and problems encountered by them as they dealt with recipients and possible donors and their families.
The inclusion criterion for this study population was that all participants had to be OTCs residing in Pune division of Maharashtra. Only those consenting to participate were involved in the study. The exclusion criterion was those who refused to consent to participate in the study. The sampling method used here was convenience sampling.
The OTCs residing and working in each of the cities in Pune division of Maharashtra were requested for time to discuss challenges involved in process of organ donation in Pune division of Maharashtra and they were requested to share their experiences in the field of organ donation. The respondents were assured that confidentiality of identity would be maintained and ethical principles would be followed. The interviews were noted down during the discussion and the suggestions of the OTCs were noted. The discussions were immediately analyzed thereafter, for the themes generated during the discussions and for insights into the organ donation activity. This qualitative research was done till the point of saturation.

Results

A total of 24 OTCs were approached for participation in the study. 18 agreed to participate in the study. However 4 refused to sign the consent and the discussion with them has not been included in this study. Thus the number of OTCs participating in present study was 14. The themes generated during the discussion are seen in Figure 1.

Discussion

An OTC is a person appointed by the hospital for coordinating all matters related to removal or transplantation of human organs or tissues or both as per the THO act, 2011 [4]. The OTC may also coordinate between the brain dead patient's relatives and the ZTCC which is a non government organization that maintains the waiting list of registered patients awaiting an organ for transplant in Maharashtra [5]. The OTC must assist the authority in every way to facilitate the organ donation [2].
The OTCs can be doctors, nurses, allied health science graduates or social workers [4]. The success of organ donation and transplant program depends upon effective coordination by trained OTCs. The THO Act- 2011 has made nomination of a transplant coordinator mandatory for any hospital which wishes to be a transplant centre. [1] The OTC needs to coordinate between the related medical, paramedical and non medical personnel to bring about effective organ retrieval and transplant. They are also accorded to the job of raising awareness levels among people of that region regarding organ donation. They are the ones often conducting awareness camps in the locality [2].
The present study reports that while 24 OTCs were approached for participation in the study, 18 agreed to participate in the study out of which only 14 actually signed the consent form for participation in the study. With greatest respect for every participant and non-participant in the present study the researchers would like to state that this non participation shows the fear and curiosity that lurks in the minds of some OTCs regarding organ donation. While there is documentation of fear about organ donation in the minds of people, there is not much literature about fear that medical personnel feel when dealing with the topic of organ donation [6].
The present study finally carried out discussions with 14 OTCs in Pune division of Maharashtra. These were analysed and the discussion is being presented under the themes that emerged from the analysis. At places the discussion on some themes was interrelated and is therefore presented together.
The themes generated during discussion are seen in Figure 1.

Awareness about organ donation, Awareness among people about brain death

The OTCs felt that people of this region though aware of organ donation, lacked understanding of details of the concept of organ donation. They did not know about the different categories of donors and the different organs that could be donated by each category of donor. Some OTCs reported that often elderly persons approached them with doubts regarding organ and body donation process.
Balajee *et al.* in a study conducted in Puducherry found that 88% of the participants were aware of organ donation [7]. However, Wig *et al*. studied the awareness of brain death among people in Delhi and found that awareness regarding this definition of death was very poor. Very few respondents in his study were aware of importance of the state of brain death in the organ donation programme [8].

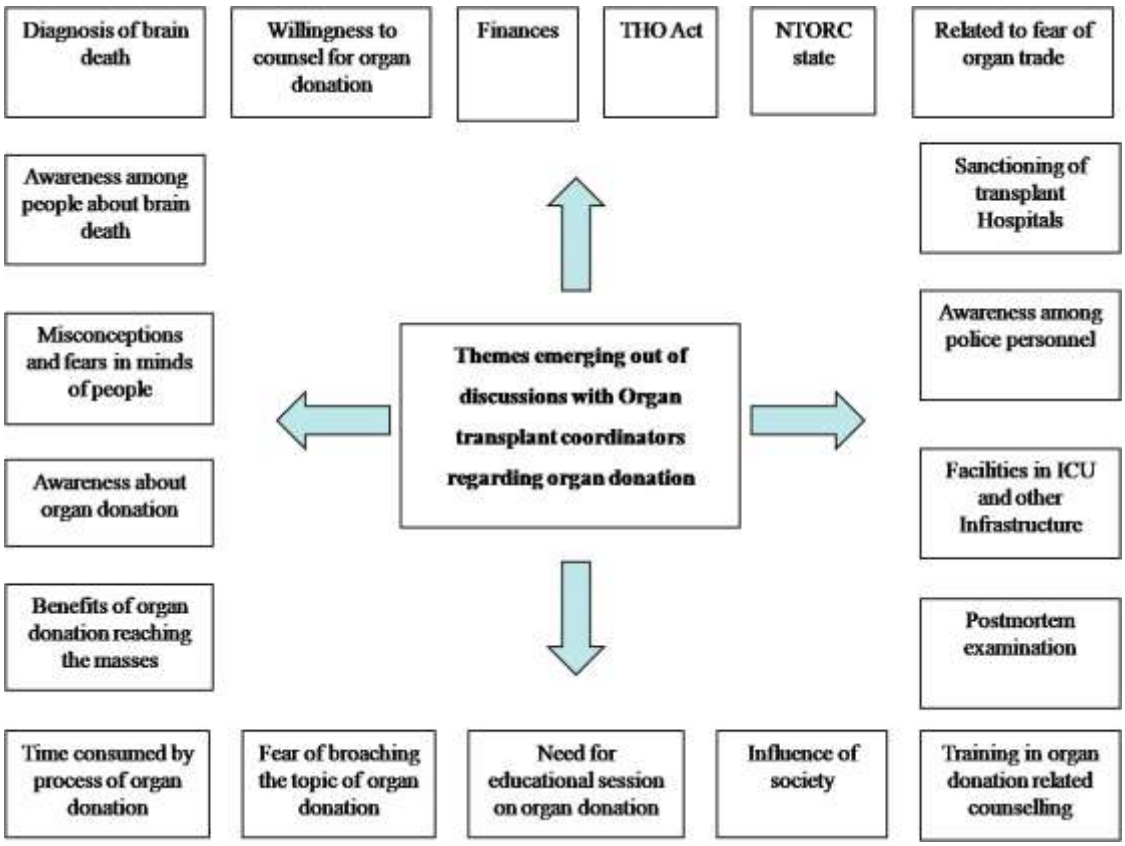

Fig 1. Themes generated during discussion with the organ transplant coordinators

Thus though people may be aware of idea of donating organs, they need to be educated about the details of categories of organ donors and the organs each category may donate.

The OTCs suggested that it was necessary to raise awareness levels about both organ donation and brain death among the people. For this purpose many suggested interesting strategies such as displaying of brochures/posters at key places in the hospital, making pamphlets easily available so that people could read and ask doubts related to organ donation. Some suggested that interesting videos could be played on the television screens of hospitals describing the process of organ donation in the form of small skits, interviews etc. Many OTCs suggested that lectures could be taken in housing societies near their respective hospitals on eye, kidney, liver donation etc, and posters could be displayed in societies for further percolation of knowledge. Some OTCs suggested putting up stalls in fairs held during Ganapati (religious) festivals to promote organ donation and handing out of organ donation related information pamphlets and forms.

Training in Organ donation related counselling, Necessity for educational session on organ donation

The OTCs felt it was important to build a rapport with the relatives of patients in ICU before declaration of brain death. Once the relatives began to trust the OTC, the relatives of the brain dead patient would feel confident to discuss their doubts with the OTC. It was important that during the trust building process, the OTC never uttered a word about organ donation. This rule should be strictly followed. Enough time should be given to relatives to accept the calamity and discuss among themselves. Also relatives should not be judged for their reactions related to the patient's health condition. Wojda *et al*. in their review of clinical practices in relation to organ donation at a high-performing healthcare organization, state that it is necessary to support the family of the potential donor in the very difficult time when their relative is critically ill in the ICU. There is a set protocol when the coordinator from the organ procurement organization, approaches the family with the possibility of organ donation [9]. The Ramchandra protocol designed at Sri Ramchandra Hospital, Chennai states that it is essential when approaching a grieving family for organs to first make a request for eye donation. If the family agrees to eye donation, then only should a request be made for organs to be donated. If eye donation is refused, efforts for organ donation should be abandoned [10].

One OTC who participated in the present study belonged to rural part of Pune division. The individual was a medical social worker, who was acting as the OTC at the hospital in the rural region of Pune division. He

explained that uneducated people of that region believe that donating any organ means being born without that organ in the next life. The OTC felt that if his own family member was in such a situation (brain dead), he would not be able to give consent for organ donation. He frankly expressed his opinion that "one should not expect from others what one will not be able to do himself/herself". Here the OTC himself was not convinced about cadaver organ donation. There was a dire need to educate the OTC himself regarding organ donation followed by training in organ donation related counselling.

Diagnosis of brain death

The OTCs stated that one of the biggest challenges in organ donation was the diagnosis of brain death. In Pune division of Maharashtra, doctors were reluctant to diagnose brain death. The OTCs stated that only relatives of a certified brain dead patient could be counselled for organ donation in deceased donor programme.
The THO Act of India describes brain stem death as the stage at which all functions of the brain stem have permanently and irreversibly ceased and is so certified. [1] In India as per the THO Act, the brain death is declared by the brain death committee which involves team of four doctors recognized by the Indian Government and who are not part of the transplant [1].
Sheerani *et al*. conducted a study so analyze the knowledge of health professionals in Sindh, Pakistan regarding concept of brain death. The study reported that 54% of the participating health care professionals did not have a clear idea of brain death, 47% would not turn off the ventilator even in a brain dead patient, 26% doctors considered it as euthanasia. The study highlighted the confusion among health care professionals regarding brain death and stressed on including these aspects in medical curricula [11].
In a ruling in 2012 the Public Health Department of Government of Maharashtra observed that Brain-Death was not being declared promptly. Patients who were brain dead were being kept on life support needlessly, delaying organ transplant to needy patients. Following this observation, orders were issued making it mandatory to declare brain-death and certify it, and the certification of the same be conveyed to the ZTCC for distribution of the organs [12].

Misconceptions and fears in the mind of people

The OTCs observed that there were some misconceptions because of which patients or their relatives refused to give consent for organ donation such as a misbelief that with parts of the body transplanted into different individuals, the soul may not get "mukti". The word "Mukti" means freedom, indicating in this case that with the organs transplanted into various individuals, the soul would remain tethered onto the earth and may not get release or freedom as is believed happens following any death. There is also a misbelief that the person may be born without those organs in their next birth (related to concept of rebirth in Hinduism).
This situation and related myths was faced by a family in India. After consultation with related priests and pundits in India, they were explained that only the body was considered mortal and not the soul which therefore could not be fractured into bits by the organ donation process and also that it was the soul that moved on into next life and not the body. Therefore any organs donated or otherwise would not affect the next life of that individual (donor) [13].

Time consumed by process of organ donation and Postmortem examination

The OTCs reported that the relatives of the brain dead organ donor felt that since they had consented to organ donation, the process of organ donation from that patient should be fast and be given preference over other activities of the hospital. They in fact were often very upset with the time consumed during the process of organ retrieval till the final handing over of the body to the family. There is a need to speed up the process of organ donation to bring about early handing over of the body to the relatives to carry out the last rites.
The relatives also often complained that not only did the process of organ donation involve cutting of body (for organ retrieval in the operation theatre) but in a medicolegal case it was followed by tearing and mutilation of the body (their perception) again during post-mortem examination. The OTCs stated that often the relatives would agree to organ donation only if post-mortem examination could be waived off.
Murty in an article on guidelines for post-mortem work in India, stated that medicolegal post-mortem work is done on request of Police/Magistrate or Court. It is not in the power of the Medical officer to waive off post-mortem examination. His role may be that of an advisor at the best, but still the final decision of waiving off the post-mortem examination lies with the Investigating agency [14].

Fear of broaching topic of organ donation, Unwillingness to counsel for organ donation

The OTCs stated that they often had to face violent reactions from the distraught relatives on broaching the subject of organ donation. The relatives believe that since the patient had a heart beat and the body felt warm, the patient was still alive and feel that the OTCs are requesting for organ donation from a living patient. The OTCs described incidences where police had to intervene to prevent casualty. Hence many OTCs found themselves reluctant to raise the topic of organ donation.

The OTCs suggested that efforts need to be taken to raise the awareness levels among the people to prevent such incidences which lead to unwillingness to counsel for organ donation. Organ donations can only rise if no opportunity is lost to request for organ donation [15].

Awareness levels among the police personnel

For the process of organ donation to go through, there is need for a no objection certificate (NOC) from the local police. They sometimes refuse this because patient's heart is beating, so he/ she is not dead according to them. OTCs stated that convincing the police personnel takes up precious time and sometimes the opportunity for organ donation is lost.
Recently there were reports that the Maharashtra state organ transplant cell was considering a proposal for a separate organ transplant module for the police during their training sessions, to sensitize them [16].

Related to organ trade/related fears, THO Act [1]

Some of the OTCs observed that in case of a live organ donation, they (the OTCs) had to be very alert. They observed that many patients would claim that a person was their near relative and would bring necessary documents proving the relationship. The OTCs stated that they found it very difficult to check the authenticity of the documents. The OTCs suggested that a lawyer and representative of police should be involved in verification of documents before the recipient and donor appeared before the approval committee.
People also often came trying to misuse the "friend clause" in the THO act. Shroff analysed the legal aspects of organ donation and transplantation, and stated that to a large extent there has been a flawed interpretation of the law by the authorization committee and the registered medical practitioners [17]. The Law states that if any donor authorizes the removal of any of his human organs before his death under subsection [1] of section 3 for transplantation into the body of such recipient, not being near relative as is specified by the donor, by reason of affection or attachment towards the recipient or for any other special reason, such human organ shall not be removed without prior permission of the authorization committee [1]. Shroff states that this clause has been misused or misinterpreted by many over the years since the act was passed. He emphasizes that it is very difficult to judge the affection being claimed. Therefore, every friend, using the deep affection clause in organ donation activity, needs to be considered carefully to avoid legal sale of human organs.
The dilemma of the OTCs was that by doubting the sanctity of the proclaimed relations or friendship, one may be preventing someone from getting a chance at life. At the same time, accepting of an erroneous document could make the OTC party to organ trade.

All OTCs felt that it was crucial that allocation of organs in deceased donor programme be a fair and transparent process and as per the rules set down by the THO act [1].

Finances

Financial status of recipient affects the outcome of the transplant surgery: The OTCs stated that in case of a poor patient on the waiting list, it is important to inform the recipient about the necessity to take immunosuppressant medicines after the transplant surgery. If the recipient is unable to afford these medicines and doesn't take them, the body will soon reject the transplanted organ. The patient will be back on the waiting list, or worse, may die. Meanwhile the next patient on the waiting list who could have afforded the medicines continues to wait for an organ to become available. The OTCs have to discuss these practical aspects also with the patients and their relatives. Gordon *et al*. have stated that adherence to immunosuppressant therapy is crucial for graft survival in transplant recipients [18].

Influence of society and interpersonal relations

Many OTCs observed that in case of live donors, majority of the recipients were males and majority of the donors were females. The OTCs stated that they and the examining doctors too, tried to ask the female donors if they were being forced into organ donation. However mostly they were so well rehearsed for these questions that they point blank refused any such suggestion. Doctors often even assured them that if they are being coerced into organ donation, they could be declared unfit preventing the organ donation at the same time helping them to maintain their family relations with the recipient. The OTCs felt that this was a reflection of our social system.
Some gender specific observations were made by Pouti *et al*. in relation to live organ donation. They observed that women seem to have more self-sacrifice and sense of responsibility compared to men and were more predisposed to donate their organs. The gender disparity in living donor transplantation was because of higher proportions of wife to husband donations. This was also observed because of disproportionate female to male donations among biological relatives and also unrelated pairs. A study suggested that men and women donated roughly by the same rate when asked to become organ donors, but women were asked more often, hence the higher incidence of female donors. Today the number of female transplant recipients continues to decline. However, in renal transplantation, female donor kidneys had a worse 5 year survival [19].

Conclusion

The present study was undertaken to investigate the

challenges for organ donation and transplant activity in Pune district of Maharashtra, a state in western Maharashtra, from the perspective of OTCs and to find solutions to combat these challenges. The study revealed several challenges and solutions, many of which were also suggested by the OTCs participating in the study. The OTCs stated that though people were aware of idea of donating organs, they needed to be educated about the details of categories of organ donors and the organs each category may donate. For this purpose many suggested interesting strategies. The OTCs must be trained in counselling for organ donation. There was a dire need to educate the OTCs themselves regarding organ donation and related counselling.

The OTCs stated that one of the biggest challenges in organ donation was the diagnosis of brain death. People had misconceptions and fears in their minds associated with organ donation. This needed discussions and counselling of the people by respective religious leaders. The process of organ retrieval and final handing over of the body of the brain dead donor was very time consuming. The distraught relatives waiting to receive the body had advised that the process needed to be speeded up and that no post-mortem examination should be conducted of the brain dead donor following organ retrieval (in case of medicolegal cases) as it meant further cutting of the body.

Many times relatives had been known to get violent on broaching the subject of donation of organs. Fearing these reactions, many OTCs were reluctant to broach the topic of organ donation. At times, the police personnel were unaware of concept of brain death and therefore refused to accept the patient as dead, as the heart continued to beat. This lead to delay in the organ donation process or at times the braindead patient's heart stopped beating leading to loss of opportunity for organ donation.

The OTCs reported that they had to be alert to people attempting through falsified documents to pass another person as their relative or as a friend to bring about organ donation. They felt themselves inadequate to detect falsified documents and suggested that police or lawyers who were trained in document verification be involved in document scrutinisation process.

The PTCs observed that more females were donors as compared to males and suggested that it was time for society to change and more number of males should come forward to be organ donors.

Finally the OTCs stated that the financial status of the recipient is an important matter to be considered while planning transplant surgery as the recipient must be able to afford the immunosuppression medicine without which the body would anyway reject the transplanted organ.

Conflict of interest statement. None declared.

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Original Article

Determining the Risk Factors for Acute Rejection and Glomerular Filtration Rate after Kidney Transplantation

Payam Amini[1], Abbas Moghimbeigi[2], Farid Zayeri[3], Hossein Mahjub[4], Hojjat Sayyadi[5], Mohsen Mohammadrahimi[6] and Saman Maroufizadeh[7]

[1]Department of Biostatistics & Epidemiology, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran; [2]Modeling of noncomunicable Disease Research Center, Department of Biostatistics, Faculty of public health, Hamadan University of Medical Sciences, Hamadan, Iran; [3]Department of Biostatistics, Member of Proteomics Research Center, School of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran;. [4]Research Center for Health Sciences, Department of Biostatistics, Faculty of public health, Hamadan University of Medical Sciences, Hamadan, Iran; [5]Department of Biostatistics and Epidemiology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran; [6]Department of Kidney Transplantation, University of Medical Sciences, Tabriz, Iran; [7]Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Abstract

Introduction. Several factors cause low estimated glomerular filtration rate (eGFR) as well as acute rejection after kidney transplant. This study aims to determine risk factors affecting low eGFR as well as the frequency of acute rejections during one year after kidney transplantation recruiting a longitudinal joint modeling approach.

Methods. Using the information from 129 kidney transplant patients, the eGFR and the frequency of acute rejections were recorded for three time points of 4, 8 and 12 months after kidney transplant. Using a longitudinal joint model, the adjusted effects of predictors were assessed on both the eGFR and the frequency of acute rejections, jointly.

Results. The results demonstrated that being one year younger reduces the risk of higher stages of eGFR (OR=1.053, p<0.001). Males were more prone to experience lower stages of the eGFR (OR=3.571, p<0.001). Patients with chronic allograft necrosis were at risk of higher stages of the eGFR (OR=3.048, p=0.001). The frequency of acute kidney transplant rejections for a patient without anti-thymocyte globulin is 6.398 times higher than with anti-thymocyte globulin (p=0.048). The absence of urinary tract infection was the only factor leading to zero rejections. Acute rejection was more potential for a BIL-D liver dysfunction patient with a factor of 2.487 in comparison to BIL-T.

Conclusions. Our study revealed that the absence of urinary tract infection strongly results in zero rejections. Factors such as chronic allograft necrosis cause lower eGFR score. The frequency of rejections is affected by anti-thymocyte globulin, BIL-T liver dysfunction.

Keywords: kidney transplantation, transplant rejections, glomerular filtration rate, longitudinal study

Introduction

Chronic Kidney Disease (CKD) is a global public health problem [1]. The increasing prevalence and incidence of CKD may result in complications in kidney function and cardiovascular disease [1]. It is well known that CKD can be a very important candidate for development of cardiovascular disease and end-stage renal disease [2,3]. Chronic renal failure is defined as less than 60 ml/min estimated glomerular filtration rate (eGFR) for more than three months [4]. The prevalence of CKD varies across different regions such as 10.8% in China in 2012, 11.2% in Australia in 2006 and 10 to 15% in united states of America in 2009 [5-7]. Moreover, chronic kidney dysfunction and recurrence of glomerulonephritis can be the main reasons for long-term graft loss [8]. Many factors such as leukopenia and human leukocyte antigen (HLA) type, type of donor (e.g., living or cadaver) and donor's age and race, recipient's serum creatinine level, sex, age and health status may cause a chronic loss of graft. Reaching end-stage chronic kidney disease is the main reason for kidney transplant in which the eGFR is less than 15 ml/min [4]. It is argued that the prevalence of end-stage renal disease in Iran is high [9]. According to the reports more than 2000 kid-

Correspondence to: Abbas Moghimbeigi, Associate Professor, Modelling of noncomunicable Disease Research Center, Department of Biostatistics, faculty of public health, Hamadan University of Medical Sciences, Hamadan, Iran. ORCID: 0000-0002-3803-3663, P.O. Box: 6517838736; Phone: +98-9125683772, Email: moghimb@gmail.com

ney transplants were performed in 2012 in Iran [10]. Kidney transplant has been introduced as one of the most efficient treatments for patients who suffer from CKD in the end-stage. However, not all of the transplants are successful. Depending on the immunosuppressive strategies, 10-15% of patients in the first year experience an acute rejection [11]. In spite of the morbidity of patients, immunosuppressive therapy has been advanced and has resulted in decrease of acute rejection [12]. During the first 10 years, an approximation of 40% of renal allografts fail. Moreover, the number of transplants decrease annually [13]. Assessing and observing the changes in eGFR is necessary for the care of renal transplant recipients. The progression of kidney disease can be predicted by eGFR. In the early stages after transplantation, several important clinical factors may influence kidney graft function such as blood residues, rejection episodes, and acute immunosuppressive drug toxicity. Hence, after transplantation, the number of acute kidney transplant rejections may be associated to the eGFR [14].

Count and ordinal data are recorded in a multitude of settings. Using the GLMMs, variety of models can be performed based on the distribution of the response variable. For a count data, a Poisson distribution with a log-linear link function is commonly assumed, while the mean and variance of count response are equal. In the case of inequality due to the extra heterogeneity, overdispersion would be considered in the modeling process. Negative binomial is a common choice to address this issue. In the case of inflated zeros in the data, zero-inflated models are performed [15]. Ordinal data is a special form of categorical data while the order of the response categories is of importance. Analyzing methods for binary data has been extended to nominal and ordinal categorical outcomes [16]. Longitudinal joint models have been applied extensively in medical area. Some studies have used longitudinal joint modeling to model serial echocardiographic measurements of aortic gradient, aortic regurgitation and measurements of the occurrence of death jointly [17], to investigate the joint evolution of longitudinal pulse and respiratory rate of congestive heart failure patients [18], to assess the hemodynamic effect on diastolic blood pressure, systolic blood pressure and heart rate over time [19], to evaluate the development of longitudinal occurrence and prevalence of antimicrobial resistant zoonotic agents [20], and to investigate the children body weight and number of days of diarrhoeal illness recorded at 7 time-points of follow-up [21].

The main objective of this study is to evaluate the joint evolution of acute kidney transplant rejection and eGFR of patients after one year of kidney transplantation. Besides, identifying the potential risk factors for the two longitudinal outcomes is carried out through the joint model.

Materials and methods

Participants and study design

A total of 129 patients records were checked and used in this historical cohort study. The patients referred to the kidney transplant center of the Imam Khomeini Hospital of Urmia University of Medical Sciences from 2003 to 2014. More details about the inclusion and exclusion criteria, sample size, the study protocol and ethical considerations are well explained by Sayyadi *et al*. [22].

In the current study, two main longitudinal response variables, acute kidney transplant rejection as the count variable and estimated glomerular filtration rate as an ordinal variable in 5 states were assessed. These variables were recorded every 4 month after the transplantation for one year. Glomerular filtration rate was estimated from abbreviated prediction equation provided by the Modification of Diet in Renal Disease study (MDRD). The estimated glomerular filtration rate among an Iranian population is calculated as follows:

The stages were determined using the National Kidney Foundation (NKF) criteria as stage 1 with normal or high eGFR (eGFR > 90 mL/min), stage 2 Mild chronic kidney disease (eGFR=60-89 mL/min), stage 3 Moderate chronic kidney disease (eGFR=30-59 mL/min), stage 4 Severe chronic kidney disease (eGFR=15-29 mL/min) and stage 5 end stage chronic kidney disease (eGFR <15 mL/min) [1].

The independent and predictor variables were the type of kidney donor (relative/ non-relative), recipient's age and sex, anemia (yes/no), type of medication (Azathioprine/ Cellcept/both/none), diabetes (yes/no), and anti-thymocyte globulin (yes/no), as well as complications after transplantation, such as proteinuria (yes/no), hyperkalemia (yes/no), hyperuricemia (yes/no), leukopenia (yes/no), myocardial infarction(yes/no), delayed graft function (yes/no), acute tubular necrosis (yes/no), urinary tract infection (yes/no), chronic allograft necrosis (yes/no), dyslipidemia (TG/ CHOL), liver dysfunction (BIL-T/ BIL-D), and hypercalcemia (yes/no).

Statistical analysis

The descriptive statistics of the patients are shown as frequency (percentage). Repeated measures over time and across several cases causes a special kind of variance which must be analyzed through longitudinal analysis methods. Among different kinds of models, Generalized Linear Mixed effects Models (GLMMs) deal with the intra-class correlation caused by longitudinal repeated measures using random effects in the model. These approaches provide subject specific interpretation as well as population average [23]. In the present study, random intercepts in the models were used to consider the correlation between repeated measures for the same patient.

Separate modeling of associated response variables provide less accurate estimations. Joint modeling approaches

increase the accuracy of estimations by reducing their standard errors [15].

Let y_{1ij} and y_{2ij} represent the frequency of acute rejections and eGFR respectively, for subject *i* at the occasion j. In the following models, x_{ij} and z_{ij} are the covariates, β and α are the coefficients, θ_c is the estimated threshold of the c^{th} category of eGFR, w_i and b_i are the random effects. The random intercepts are correlated through the correlation parameter ρ and hence the association between eGFR and frequency of acute rejections are considered. The two associated response variables follow the general form as follows:

$$E(y_{1ij}) = e^{x_{ij}\beta + w_i}$$

$$pr(Y_{2ij} \leq c) = \frac{e^{b_i + \theta_c - z_{ij}\alpha}}{1 + e^{b_i + \theta_c - z_{ij}\alpha}}$$

In the case of inflated zeros in the frequency of acute rejections, zero-inflation models are used such as zero-inflated Poisson (ZIP). To check the zero inflation, Vuong test is used.

Results

A total number of 129 patients were followed for one year after kidney transplantation; their rejection status as well as eGFR were recorded on months 4, 8 and 12. The hospital records from September 2003 to December 2014 were checked. About 63% of patients were male (79 patients), 72% with hypertension, 10.9% relative donors, 3.1% with delayed graft function (DGF), 5.4% with acute tubular necrosis (ATN), 2.4% with myocardial infarction, 54.8% with urinary tract infection (UTI), 68.2% with chronic allograft necrosis (CAN), 61.9% with Hyperuricemia, 19.4% with anti-thymocyte globulin (ATG), 65.1% with Proteinuria, 6.2% with Hyperkalemia, 39.5% with liver dysfunction, 82.2% with dyslipidemia, 17.1% with Hypercalcemia, 48.8% with anemia and 21.75 with diabetes. The distribution of two response variables, eGFR and number of acute rejection of kidney transplantation during months 4, 8 and 12 is illustrated in Table 1. No significant difference was detected longitudinally.

Table 1. The frequency (percentage) of eGFR stages and acute rejections at months 4, 8 and 12 among 129 patients

Response variables	Stage	time			p-value
		4	8	12	
Estimated glomerular filtration rate	1	25(19.4)	37(28.7)	26(20.2)	0.697
	2	55(42.6)	50(38.8)	55(42.6)	
	3	35(27.1)	32(24.8)	36(27.9)	
	4	6(4.7)	4(3.1)	4(3.1)	
	5	8(6.2)	6(4.7)	8(6.2)	
acute kidney transplant rejection	0	112(86.8)	111(86)	115(89.1)	0.966
	1	10 (7.8)	10(7.8)	8(6.2)	
	2	3(2.3)	4(3.1)	2(1.6)	
	3	1(0.8)	3(2.3)	2(1.6)	
	4	1(0.8)	0(0)	1(0.8)	
	5	2(1.6)	1(0.8)	1(0.8)	

The results from longitudinal joint model of eGFR and acute rejection of kidney transplantation are shown in Table 2. The simple GLMM was separately used to find the potential factors for the joint model. To do so, variables with p-value <0.15 were included in the joint model. Moreover, the Vuong test was applied (z-value =3.35, p-value=0.0004) and the application of ZIP model for the eGFR was confirmed. According to the outputs, the odds ratio of being in a lower stage of eGFR for one year older patient is 0.949. It means that being one year younger increases the odds of being in a lower stage of eGFR with the odds ratio of 1.053 (p<0.001). Females were less prone to experience a lower stage of eGFR compared to males. Being in lower stage of eGFR for females was 0.280 times more than men. In other words, men were 3.571 times more likely to experience lower stages of eGFR (p<0.001). Patients without chronic allograft necrosis experienced lower stages of eGFR with the odds ratio of 0.328. That is so those with chronic allograft necrosis were 3.048 times more likely to be in the lower stages of eGFR (p=0.001). The results exposes that time, DGF, ATN and being diabetic did not affect eGFR. The acute rejection of kidney transplantation was assessed by ATG, DGF, UTI, CAN, liver dysfunction and time. The expected number of rejections for a patient without ATG is 6.398 times the expected number of rejections for a patient with ATG (p=0.048). In patients without UTI, the expected number of rejections would decrease by a factor of 0.443. Moreover, rejection was more potential for a BIL-D liver dysfunction patient with a factor of 2.487 in comparison to BIL-T. UTI was the only factor caused zero rejections significantly. The odds of zero rejections for patients without UTI was 0.358 times than those with UTI. In other words, patients with UTI are luckier to experience rejections with the odds ratio of 2.793 (p=0.049). Although the correlation between the random intercepts was not significant (correlation=0.252, p=0.671), a positive correlation between eGFR stages and the frequency of acute kidney transplant rejection was observed.

Table 2. The results of longitudinal joint modeling of eGFR and acute kidney transplant rejection

Response	Parameter	Estimate	SE*	p-value	95% CI†		OR‡
Estimated glomerular filtration rate	Age	0.052	0.011	<0.001	0.028	0.075	1.053
	Sex (female)	1.272	0.338	<0.001	0.603	1.941	3.568
	Diabetes (No)	0.238	0.362	0.511	-0.955	0.478	1.269
	ATN§ (No)	-0.372	0.631	0.555	-0.874	1.618	0.689
	DGF‖ (No)	-2.162	0.157	0.063	-0.127	4.452	0.115
	CAN¶ (No)	1.114	0.341	0.001	-1.791	-0.438	3.047
	Time (month)	-0.005	0.030	0.851	-0.066	0.054	0.995
	Standard deviation of the random intercept	0.451	0.071	<0.001	0.309	0.593	-
Acute kidney transplant rejection	ATG# (NO)	1.856	0.931	0.048	0.012	3.699	6.398
	DGF (NO)	-1.290	1.684	0.445	-4.623	2.043	0.275
	UTI** (NO)	-0.815	0.390	0.038	-1.587	-0.043	0.443
	CAN (NO)	0.385	0.357	0.283	-0.322	1.093	1.470
	Liver Dysfunction	0.911	0.404	0.026	0.111	1.712	2.487
	Time (month)	-0.054	0.051	0.291	-0.156	0.047	0.947
	Standard deviation of the random intercept	0.440	0.275	0.112	-0.104	0.985	-
Zero Inflation	ATG (NO)	1.184	1.576	0.453	-1.934	4.303	3.267
	DGF (NO)	-1.341	1.552	0.389	-4.413	1.730	0.262
	UTI (NO)	-1.026	0.518	0.049	-2.051	-0.001	0.358
	CAN (NO)	0.579	0.463	0.199	-0.319	1.514	1.784
	Liver Dysfunction	0.645	0.556	0.248	-0.455	1.746	1.906
	Time (month)	-0.021	0.070	0.761	-0.161	0.118	0.979
Correlation between the random intercepts		0.252	0.593	0.671	-0.921	1.425	-

* Standard Error; † 95% Confidence Interval; ‡ Odds Ratio; § acute tubular necrosis; ‖ delayed graft function; ¶ chronic allograft necrosis; # anti-thymocyte globulin, ** urinary tract infection

Discussion

In the current study, longitudinal data of patients with kidney transplants were utilized to find significant factors of eGFR and the frequency of acute kidney transplant rejection by recruiting longitudinal joint modeling approach. The distribution of patients across five stages of eGFR did not differ during three time points (one year) as well as the frequency of acute kidney transplant rejection. At the end of the first, second, third and four months the majority of patients were observed in stage two of eGFR. However, a slight difference was shown between month eight in comparison to months four and 12. In other words, except for the stage four, the distribution of eGFR is almost the same for months four and 12. The frequency of acute kidney transplant rejection was the same longitudinally during one year and the majority of patients had no rejections.

The results from longitudinal joint model showed that older patients and females are more likely to experience higher stages of eGFR. O'Hare *et al.* assessed the influence of age among chronic kidney patients using a large number of cases followed for more than three years. They showed that age strongly affects patients with eGFR more than 60 ml/min per 1.73 m^2 resulting in a higher rate of death among older patients [24]. Stevens *et al*. evaluated the performance of the CKD epidemiology collaboration in renal disease study equations to estimate GFR levels above 60 ml/min/1.73 m^2 and revealed that participants with higher eGFR were younger [25]. In another study, Hallan *et al.* exposed the association of kidney measures with mortality and end-stage renal disease regarding age. Considering age as a factor, they aimed to investigate the association of eGFR and albuminuria with clinical risks. They showed that mortality risk for reduced eGFR decreased with increasing age [26]. Our study assessed the adjusted effects of age and sex beside to several factors in a joint model with multiple effects. Although the main determining factor of eGFR is creatinine which remains constant daily, it is a function of creatinine, sex and age. Therefore, a reverse association between eGFR and female gender and older age is expected [27,28].

The results showed that DGF, ATN and being diabetic did not affect eGFR. Hollmen *et al*. evaluated whether donor neutrophil gelatinase-associated lipocalin could be a predictor of DGF after transplantation. Applying a multivariate analysis, they introduced eGFR as a risk factor for prolonged DGF [29]. Esson and Schrier discussed the diagnosis and treatment of acute tubular necrosis which is associated with decrease in glomerular filtration rate [30].

It is well known that ATG prevents acute rejection in organ transplantation [27,28]. It is argued that, patient

without ATG experienced more rejections. In a study by Brennan *et al.* ATG was compared with Basiliximab in rabbits with renal transplantation and they demonstrated that the ATG group had lower incidences of acute rejection [31].

The frequency of rejections was more potential for a BIL-D liver dysfunction patient. Those cases without an experience of UTI had less number of rejections and using the zero-inflated model, UTI was the only factor resulting in zero rejections. Parikh et al. conducted a retrospective review to evaluate the urinary tract infections after renal transplantation. They concluded that UTI can increase mortality risk in renal transplant recipients. The authors showed that although chronic immunosuppressive medications are necessary for patients after kidney transplantation, this may increase infections rates such as UTI [32]. Kidney transplantation is followed by some adverse outcomes such as bleeding, acute rejections and infections. Taking immunosuppressant drugs increases the risk of infections after transplantation and this might lead to rejections. Hence, there is a negative association between infections and acute rejection [27,28].

Longitudinally recording of the data benefits the researchers to investigate the development of response variables over the time and provides a significant more amount of information about medical and clinical problems in contrast to cross sectional type of studies [15, 23]. Generalized linear mixed-effects models are frequently used to analyze longitudinal data. Joint modeling approaches take the natural associations among response variables into account which is ignored by univariate approaches. This procedure causes a smaller standard errors for estimated coefficients resulting in a true significance of effects [15,33]. Evaluating several response variables, Fieus *et al*. applied a joint random effects model approach to assess the development of responses jointly, over the time [34]. Analyzing Lin *et al*. used GLMMs to model clustered continuous and binary response variables jointly to analyze the development of ethylene glycol in mice [35]. Azen and Budescu suggested utilizing joint approaches in order to consider the association of response variables [36]. Badiru compared computational survey of the various univariate and multivariate learning curve models showed that the bivariate model provides a slightly better fit than the univariate model. Moreover, the bivariate model provided more detailed information about the data [37]. Thorp used longitudinal joint and univariate mixed-effects models for metabolic syndrome data in which multiple outcome variables were assessed using several predictors. He found that the multivariate model is able to deal with the same questions addressed as the univariate model. Joint models answer additional important questions about the association in the evolutions of the response variables as well as the evolution of the associations. He showed that taking the association between the responses reduces the standard errors in estimations and leads to more reliable results [38].

The limitations of our study were the inaccessibility to the cases to reach a relative large sample size and also to follow the cases for over one year after transplantation. Moreover, the side effects of the drugs have not been assessed. However, the data was collected in one of the most referral centers and a powerful statistical approach was used to analyze the data.

The present study showed that after adjusting the association between acute kidney transplant rejection and eGFR, females, older patients and those with chronic allograft necrosis were more likely to experience lower eGFR score. Patient with ATG, BIL-T liver dysfunction and without UTI experienced less number of rejections. UTI was determined as the only significant factors causing zero acute rejections.

Conflict of interest statement. None declared.

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Case report

Why Could there be a Significant Difference between the Results of Spot Urine Albuminuria and 24-Hour Urine Quantitative Proteinuria?

Banu Yilmaz[1], Yalkin Dalda[2], Cengiz Ceylan[3], Emre Coluoglu[2] and Harun Akar[2]

[1]University of Health Sciences, Department of Nephrology, Antalya Training and Research Hospital, Antalya, Turkey, [2]Departments of Nephrology, [3]Department of Hematology, Izmir, Turkey

Abstract

Patient's urine analysis revealed trace amounts of proteinuria and 17.22 g/day proteinuria in 24-hour urine. Given the dramatic difference between these two tests (spot urine albuminuria and 24-hour urine quantitative proteinuria), an investigation of plasma cell dyscrasia was planned. Patients who do not have albuminuria but have significant proteinuria on 24-hour urine analysis should be examined for plasma cell dyscrasia and bone marrow examination should be performed.

Key words: Spot urine albuminuria, 24-hour urine quantitative proteinuria, multiple myeloma

Introduction

Multiple myeloma (MM) is a disease characterized by plasma cell neoplasia, which accounts for 1% of all cancers and 10% of all hematological malignancies, respectively. At the time of diagnosis, the median age is 66 years, and abnormal laboratory values may not always be observed, even if it is accompanied by anemia, impairment of renal function, hypercalcemia, hypoalbuminemia and hypergammaglobulinemia. While investigating the etiology of renal failure, we wanted to present a case of myeloma, even though it did not show the characteristic laboratory values mentioned above.

Case

A 69-year-old male patient was referred to the outpatient-clinic for nephrological evaluation after completing pneumonia treatment. During the follow-up after pneumonia in Chest Disease Hospital, high serum creatinine level was noted. The patient was admitted to the Nephrology Clinic to investigate renal insufficiency etiology. Creatinine was found to be 6.2 mg/dl, urea was 97 mg/dl, calcium was 9.3, albumin was 3.9 g/dl, globulin was 2.5 g/dl and hemoglobin was 8.5 g/dl. Renal ultrasonography showed normal kidney size and parenchymal thickness. As first impression, the clinical presentation of the patient was thought to be due to acute renal failure secondary to pneumonia and hypoxia. During the follow-up period, the patient did not require hemodialysis and regression was observed in renal function tests with intravenous hydration. In the patient's spot urine and 24-hour urine analyzes, trace amounts and 17,22 g/day proteinuria were detected. It was planned to investigate in terms of plasma cell dyscrasia from the inconsistency between these two tests, albuminuria and quantitative protein measurement methods in spot urine and 24-hour urine tests, respectively. Bone marrow aspiration and biopsy were performed and evaluated by a hematology specialist. When bone marrow aspiration was assessed, approximately 60% of plasma cells were reported to be detected. Histopathological evaluation of bone marrow biopsy indicated that 80% of atypical plasma cell infiltrates were distributed in the interstitial space. A diagnosis of multiple myeloma was made and the patient was scheduled to undergo chemotherapy in the hematology clinic.

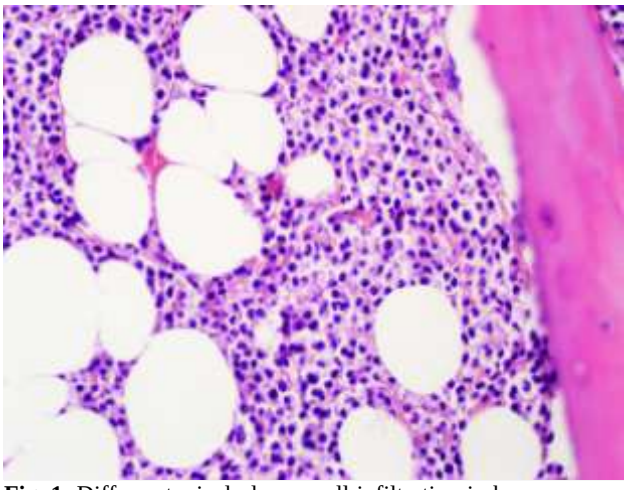

Fig. 1. Diffuse atypical plasma cell infiltration in bone marrow biopsy, H & E, x100

Correspondence to: Yalkin Dalda, Saglık Bilimleri Universitesi, Izmir Tepecik SUAM, Ic Hastaliklari Klinigi, Izmir; E-mail address: ylkndld@hotmail.com

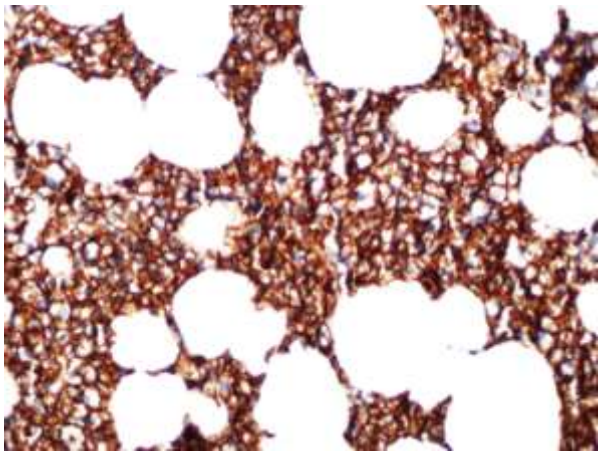

Fig. 2. Immunohistochemical staining: CD138 positive, DAB, x200

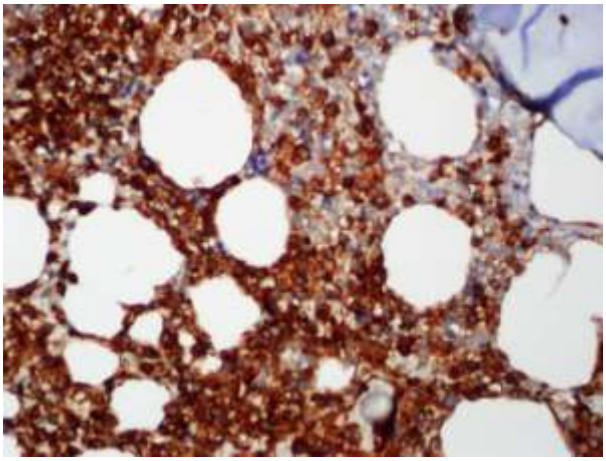

Fig. 3. Immunohistochemical staining: Kappa positive, DAB, x200

Discussion

The most appropriate method for measuring urine protein is uncertain and there are inconsistencies in the guidelines for recommending total urinary protein excretion or only urinary albumin excretion for risk assessment and therapeutic decisions. The urine essentially contains 2 groups of proteins; plasma proteins that cross the filtration barrier, and non-plasma proteins that originate from the renal tubules or urinary tract [1]. The major ones are albumin and Tamm-Horsfall proteins, respectively. In addition to albumin and Tamm-Horsfall proteins, immunoglobulins, low molecular weight proteins and light chains are also present in variable proportions. Dipstick method is a proteinuria assay using a pH sensitive dye impregnated strip that changes color in the presence of negatively charged urinary proteins. Although the use of dipsticks is very practical and easy, they are not very sensitive for proteinuria. On average, dipsticks have low sensitivity and variable specificity for detection of total protein levels and that positively charged proteins such as immunoglobulin light chains may not be detected even when the concentrations of these proteins are high [1]. In general, to detect albumin, a negatively charged protein, their sensitivity remains low. The standard urine dipstick test is not sensitive enough to detect non-albumin proteins, only albumin can be detected [2]. On the other hand, the detection of proteinuria by precipitation is performed by adding acid based on the measurement of turbidity. Among the most commonly used acids, sulfosalicylic acid and trichloroacetic acid are more sensitive for albumin, light chains and globulin, respectively. Precipitation methods have low sensitivity and precision for immunoglobulins. Electrophoresis is the preferred method when focusing on the detection of immunoglobulin and immunoglobulin light chains in the urine. Urine electrophoresis and immunoelectrophoresis, respectively show monoclonal peak and define the specific protein with very high sensitivity [1,3].

Plasma cell dyscrasias are a consequence of the clonal expansion of the neoplastic plasma cell and can be detected by the presence of one of the following: monoclonal light chain in the serum by serum immunofixation electrophoresis, monoclonal light chain in the urine by urine immunofixation electrophoresis or monoclonal plasma cells in the bone marrow by immunohistochemistry [4]. Each abnormally expanded malignant plasma clonal cells produce an excess of intact immunoglobulin or a single type of free light chains. These free light chains of a single type, called monoclonal protein (M-protein) or paraprotein [5]. Practically, plasma cell dyscrasias can be diagnosed by bone marrow aspiration, biopsy and clinical laboratory tests [4,6]. However, plasma cell dyscrasias including multiple myeloma (MM) are diseases with many faces. Renal functions are often impaired in plasma cell dyscrasias [7]. Acute renal failure due to multiple myeloma may rarely be a chatacteristic of the disease [8]. Hutchison and colleagues have suggested that measuring the concentrations of serum monoclonal free light chains (FLCs) and calculating the serum kappa/lambda ratio is a convenient, method for determining monoclonal FLC production in patients with multiple myeloma and acute renal failure [9]. Since multiple myeloma may be a disease that can be diagnosed without characteristic physical examination and laboratory findings, patients should be evaluated for multiple myeloma, while the underlying cause of renal failure is investigated. Patients who do not have albuminuria but have significant proteinuria on 24-hour urine analysis should be examined for plasma cell dyscrasia and bone marrow examination should be performed. Diagnostic evaluation of MM includes extensive history and physical examination, laboratory tests such as proteinuria (in spot and 24 hour urine specimens), peripheral blood smear, erythrocyte sedimentation rate, plasma albumin, globulin, calcium, urea, creatinine levels, serum immunoglobulin levels, serum and urine immunofixation electrophoresis, skeletal radiography and bone marrow aspiration and biopsy. Renal failure is one of the most common complications of MM and occurs in about 20% of patients and in 40-

50% of patients throughout the course of the disease. Multiple myeloma, a plasma dyscrasia, can be diagnosed in the process of investigation of unexplained renal disease that can not be underestimated. Plasma cell dyscrasia is becoming increasingly recognized as a cause of kidney damage. In order to prevent misdiagnosis, adults with unexplained. Acute kidney injury or proteinuria should also be tested for plasma dyscrasia in addition to vasculitis and autoimmune disease serologies. In a patient with renal failure whose etiology is unknown, immunofixation electrophoresis should also be included among the tests to be performed. As with this case, the possibility of underlying multiple myeloma should be considered in the case of acute renal failure and anemia of unknown etiology.

Conflict of interest statement. None declared.

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Case report

Per Magna Cystic Renal Cell Carcinoma

Dimitar Trajkovski[1], Saso Dohcev[1], Sotir Stavridis[1], O Stankov[1], Aleksandar Trifunovski[1], Skender Saidi[1], Jovan Janculev[1] and Liljana Spasovska[2]

[1]University Clinic of Urology, [2]Institute of Patology, Medical Faculty, Skopje, N. Macedonia

Abstract

Renal cell carcinoma (RCC) rarely presents with a cystic appearance. Its cystic form accounts for 3-14% of all RCC. We present a rare case of an enormous 30 cm in diameter cystic renal cell carcinoma (cRCC) that was successfully removed with a nephron-sparing surgery in our institution. The pathology report revealed a 1330 g cyst, size 22x15x9 cm containing two solid tumor formations 4x3 cm and 3x2 cm located on its inner wall and diagnosed as Clear Cell Papillary Renal Cell Carcinoma (CCP-RCC) a subtype of RCC recently established by WHO in 2016. It was our goal in this case report to emphasize that occasionally RCC could present atypically by displaying unusual imaging and clinical appearances even to bizarrely large size and weight.

Keywords: per magna cystic renal tumors, RCC, clear cell papillary renal cell carcinoma, nephron-sparing surgery

Introduction

Renal cell carcinoma (RCC) represents 3-5% of all cancers being the 6th most frequently diagnosed cancer in men and the 10th in women [1].

RCC rarely presents with cystic appearance and this form accounts for only 3-14% of all RCC [2-4]. Cystic RCC commonly present at a lower stage and grade, with almost no loco regional and distant metastases at the time of presentation (localised RCC pT1-pT2, N0/x, M0/x, Fuhrman nuclear grade I-II) with a favorable prognosis after operative treatment [3]. Most patients are asymptotic and are detected incidentally. It was our aim to present an extremely rare case of a bizarrely 30 cm in diameter big and 14 L heavy cystic renal cell carcinoma and emphasize the wide range of possibilities it can present in the human body.

Material and methods

We report a case of a 58 year old male with a per magna cystic tumor of the right kidney. He presented mostly asymptomatic, with only a largely distended abdomen and a mild abdominal discomfort gradually developing in the past year. The patient had no history of any associated pain, hematuria, loss of appetite or weakness. On palpation, a non-tender tensed mass was felt on the entire anterior and lateral abdominal wall. He was aware of having a cyst in his right kidney for a minimum of 5 years incidentally discovered on a routine US examination. Due to his negligence over the time the cyst grew to bizarre size. US and CT scan revealed a massive unilocular cystic lesion in size 30x29x28 cm with extensive dislocation of the surrounding organs and no signs of loco regional lymph node or contact visceral infiltration nor distant metastases. Contrast phase CT confirmed compressed but not obstructed right kidney and ureter with a free contrast passage. A nodule enhancement within the inner cystic wall was reported by the radiologist placing it in a category IV of the Bosniak classification of renal cystic masses with a clear malignant prediction. Preoperative serum creatinine and GFR level were within the normal range. A chest X-ray was performed and revealed no abnormalities. Urinalysis was also normal.

Due to the large proportions of the cyst and the inability to access laparoscopically an open nephron sparing surgery (NSS) was performed through a right subcostal incision, seen on Figure 1. The cyst was punctured and 14 L of chocolate fluid was carefully aspirated. It was then by sharp and blunt dissection removed in-toto and the kidney residue was properly preserved. No signs of visceral metastases were found intraoperatively. Operative material (cyst and aspiration fluid) was sent to pathology for histopathology and cytology examination. The patient had a good postoperative recovery and was discharged on the fifth postoperative day.

Results

The pathology report of the specimen revealed a 1330 g cyst, size 22x15x9 cm containing two solid tumor formations 4x3cm and 3x2 cm located on the inner cyst wall. It was diagnosed as Clear Cell Papillary Renal Cell Carcinoma (CCP-RCC) a subtype of RCC recently established by WHO in 2016. In the report no malignant infiltration outside the cyst was confirmed.

Correspondence to: Dimitar Trajkovski, University Department of Urology, Skopje, R.N. Macedonia;
Phone: +389 78 70 70 70; E-mail: diki987@yahoo.com

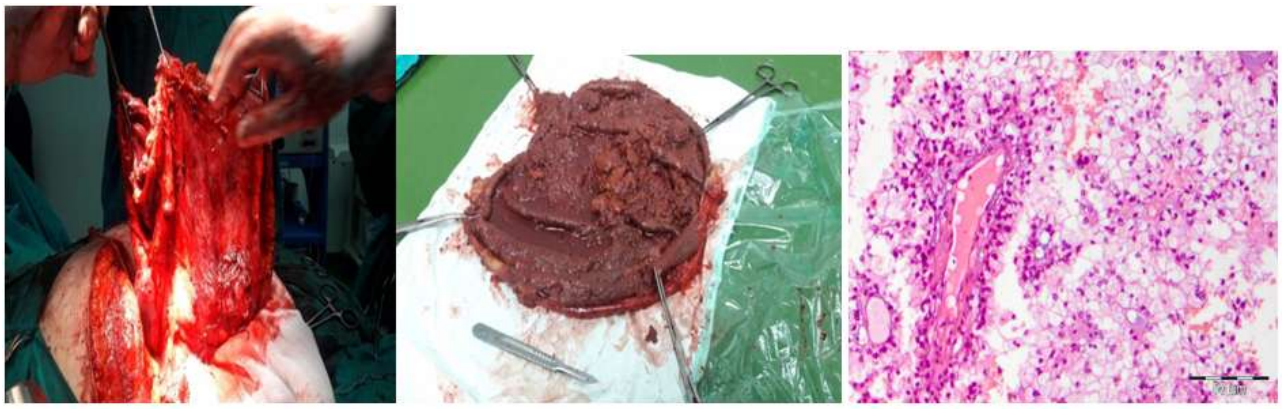

Fig. 1. Nephron sparing surgery of cystic tumor of the right kidney (H.Ex100 Papilla and clear cells)

Malignant cells were not present in micro lymphovascular area. (pTNM=pT2b pNx pMx Fuhrman II). Cytopathology report proved no cell presence in the cyst aspirate (Classification group I).

Discussion

About 50% of individuals over 50 years have cystic renal disease. However, CRCC is relatively rare [5]**.** Accurate diagnosis and treatment are sometimes difficult because clinical manifestations and imaging characteristics of CRCC can be similar to those of benign renal cystic disease. The Bosniak system of cyst classification is a widely followed system for imaging classification of renal cystic masses. It classifies renal cysts into five categories based on CT imaging appearance to predict malignant risk and advocates treatment for each category. Category I and II are treated as benign ad don't warrant treatment or follow up. Category IIF cyst are rarely malignant and request follow up up to 5 years. Category III cysts are over 50% malignant and request surgery or active surveillance. Bosniak category IV are considered clearly malignant with surgery as method of treatment. Alternatively, contrast enhanced US and MRI are additional method used in diagnosing cystic RCC [2,6].

Cystic RCC comprises a wide category of renal cancers, including multilocular cystic RCCs, unilocular cystic RCCs, RCCs with extensive cystic necrosis, and unilocular cysts with mural tumor nodules [3,4]. Cystic RCCs represent a mix of various histological subtypes similar to the solid RCC, most commonly clear cell RCC, papillary RCC and chromophobe RCC and other less common subtypes. They all commonly present at a lower stage and grade, with almost no loco regional and distant metastases at the time of presentation (localised RCC pT1-pT2, N0/x, M0/x, Fuhrman nuclear grade I-II) and come with a favorable prognosis after operative treatment. [7].

In our case a pathology report confirmed a cystic type of Clear Cell Papillary Renal Cell Carcinoma (CCP-RCC). It is a recently recognized histological subtype of RCC and encompasses only 1-5% of all RCC. That makes it the fourth most common histological subtype of RCC after clear cell RCC (ccRCC), papillary RCC (pRCCr) and chromophobe RCC (chRCC). [8,9]. Microscopicaly it is characterized by clear cell cytology in papillary architecture with a low-grade nuclei (Fuhrman grade 1 or 2). Macroscopicaly it presents mainly as encapsulated by variably thick fibrous capsule limited to the renal parenchyma (low stage localized predominantly T1). The low stage and low grade parameters of CCP-RCC and the cystic RCCs in general, provide an excellent prognosis of these tumors following surgical treatment [10,11].

Based on current available oncological and quality of life outcomes, localised RCC stage T1is best managed by partial nephrectomy (PN) (nephron-sparing surgery-NSS) rather than radical nephrectomy (RN). Partial nephrectomy can be performed, either with an open, laparoscopic or robot-assisted approach, based on surgeon's expertise and skills. Patients with localized T2 tumors not treatable by partial nephrectomy should be offered radical nephrectomy performed laparoscopically. [2,12].

Koga *et al*. [13] reported an excellent 5-year survival rate of 80-100% for cystic RCC vs 55% for conventional solid RCCs as well as Onishi *et al*. [14] with an overall 5-year survival rate of 88,6% vs 50-60% for conventional RCCs. No evidence of recurrent or metastatic disease was reported in these groups.

Conclusion

It was our goal to emphasize that occasionally RCC could present atypically by displaying unusual imaging and clinical appearances even to bizarrely large size and weight. In this particular case we encountered and removed a cystic renal tumor grown to enormous proportions of 30cm in diameter containing 14L of fluid. Due to the short postoperative terms, no imaging follow up was conducted at the moment of presenting this report.

Conflict of interest statement. None declared.

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Case report

Renal Transplantation in Patients with Rare Diseases

Tea Vukic, Petar Kes and Nikolina Basic-Jukic

School of medicine, University of Zagreb and Clinical hospital centre Zagreb, Zagreb, Croatia

Abstract

Kidney transplantation is the best treatment option for patients with end-stage renal disease, owing to its effect on quality of life and survival. In order to have a successful transplantation it is necessary to set a proper diagnosis. Leading cause of end-stage renal disease in developed countries are diabetes and nephroangiosclerosis. Considering that more than one third of patients with end-stage renal disease do not have a diagnosis of primary kidney disease, we encounter the issue of transplanting these patients without knowing the etiology of patient's condition, which in the end may lead to graft failure. Misdiagnosed patients may have rare diseases for which tests are not routinely available. Very often we fail to recognize the disease due to the lack of awareness even though there are signs and symptoms that point to the possibility to a certain rare disease. Some of the rare diseases mentioned in this case series report are Fabry disease, primary hyperoxaluria type 1, atypical hemolytic uremic syndrome, tuberous sclerosis and syndromes such as Denys-Drash syndrome, Prune-Belly syndrome, Turner syndrome and Branchio-oto-renal syndrome.
Rare diseases are frequently chronic and many start in childhood. Minimizing diagnostic delays by improving genetic testing and disease recognition will prolong both the patient's life and the life of a graft. Raising awareness of rare diseases will aid early and accurate diagnosis.

Keywords: renal transplantation, rare diseases, Fabry disease, Denys-Drash syndrome, Turner syndrome, primary hyperoxaluria

Introduction

Renal transplantation is the best method of renal replacement therapy. Many patient factors influence graft successfulness. Some of these are age, gender, diabetes, cardiovascular status, smoking status and HLA matching, but one of the most important factors is primary disease itself [1,2]. When primary disease is unknown, it imposes great risk for recurrence of disease in transplanted kidney and graft failure [1], while appropriate precaution measures may be omitted. Recognition of a rare disease is of upmost importance due to its influence on the prognosis, course of treatment and successfulness of transplantation.
The most common diseases in developed world that lead to end-stage renal disease are diabetes, nephroangiosclerosis and glomerulonephritis without biopsy [3]. More than one third of people with end-stage renal disease do not have a proper diagnosis or the exact diagnosis is unclear. Additionally, rare diseases may be hidden in diagnoses of diabetes, nephroangiosclerosis and glomerulonephritis without biopsy, considering that one sign or symptom may be shared between two completely different diagnoses. Diagnosing a rare disease affects choice of treatment and immunosuppression as well as the recurrence of a disease after transplantation. Some rare diseases require multisystem approach and other may be treated with specific therapy that stops further deterioration of kidney function. Because some of these diseases are genetic, it is even more important to recognize the patient in order to perform screening on the other members of the family.

Case-series

As an example of importance of accurate and timely diagnosis, we present a 28 year old patient treated at Clinical Hospital Centre Zagreb where he had kidney transplantation. He received renal allograft from a deceased donor after eighteen years on hemodialysis. Primary kidney disease was unknown. Allograft function was immediate with basiliximab induction and cyclosporine, mycophenolate mofetil and steroids used for maintenance immunosuppression. Twenty days after the transplantation he developed severe proteinuria (51 g/day). Biopsy revealed collapsing form of focal segmental glomerulosclerosis (Figure 1).
Disease was resistant to plasma exchanges and it was necessary to perform graftectomy considering the extent of proteinuria.
One of the most familiar rare diseases is Fabry disease that is caused by alpha galactosidase gene mutation [4]. This mutation causes diminished or complete absence of effect of alpha galactosidase A enzyme that degrades globotriaosylceramides. Consequently, there is accumu-

Correspondence to: Nikolina Basic-Jukic, Department of nephrology, arterial hypertension, dialysis and transplantation, University hospital centre Zagreb, Kispaticeva 12. Zagreb, Croatia; E-mail: nina_basic@net.hr

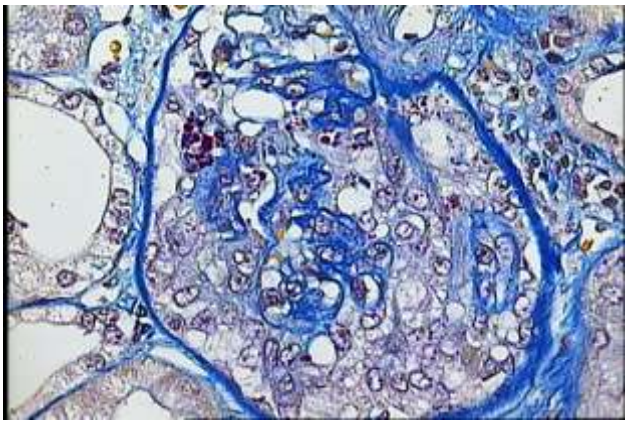

Fig. 1. Collapsing focal segmental glomerulosclerosis in a renal transplant recipient

lation of fatty acids that already in childhood cause symptoms of this disease that appears as pain in their arms and legs. Hence, the children often visit psychiatrist. On the skin of these patients we frequently observe angiokeratomas [5]. Proteinuria is one of the early signs of this disease [6]. Fabry patients can present as neurological patients with transitory ischemic attack or stroke. They suffer early from cardiac diseases [5,6]. Treatment of end-stage renal disease is performed by dialysis or kidney transplantation. In Fabry patients, we have to emphasize the importance of early enzymatic replacement therapy: human alpha galactosidase A [7]. This enzyme is administered intravenously every two weeks, but nowadays there are new peroral medications that will facilitate administration of therapy. A male patient born in 1962 was diagnosed with slowly progressive glomerulonephritis when he was 12. In 2006 diagnosis of Fabry disease was established and since then this patient has been on enzyme replacement therapy. From 2006 to 2010 he was on a regular haemodialysis due to end-stage renal disease. In 2010 he received a renal allograft from a deceased donor. Posttransplant course has been uneventful.

Primary hyperoxaluria type 1 is caused by mutation in alanine-glyoxilate and serin-pyruvate aminotransferase gene. This causes disruption in pathways that convert glyoxilate to glycine and consequently, a product called oxalate accumulates in liver and kidney [8]. Characteristic clinical appearance is a patient with frequent kidney stones [9]. This disease, if not discovered on time, has devastating consequences on kidney graft in post-transplantation period. Conservative treatment is recommended until end-stage renal disease has been reached, when dialysis and preparations for transplantation must be included [10]. Surgery can involve simultaneously liver and kidney transplantation or sequentially liver, then kidney. It is possible to carry out kidney transplantation only, if the liver is not severely damaged. Even though most of procedures of this kind were without success, there are some known existing surgeries with positive outcome. It is also possible to perform liver transplantation only, where kidney damage is not yet present [11]. One of our patients, a female born in 1959, was suffering from multiple urinary and gallbladder stones since 2006. Following cholecystectomy in 2008, she developed acute renal failure and end-stage renal disease of unknown origin. After five years of hemodialysis, she had kidney transplantation. Only seven days after the transplantation the patient stopped urinating and developed acute rejection. By renal biopsy oxalate cristals were found in a renal allograft, but also in bone biopsy specimens (Figure 2).

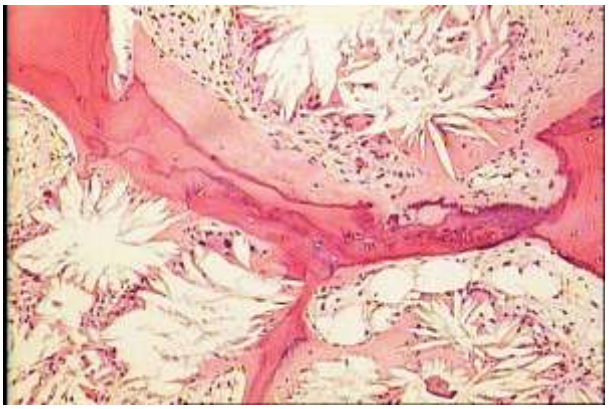

Fig. 2. Bone biopsy revealing oxalate crystals in a patient with primary hyperoxaluria type I

Another patient, a female born in 1958, was healthy until 2009, when suddenly first symptoms appeared along with end-stage renal disease. Etiology of ESRD was unknown. This patient is on hemodialysis since then. Skin biopsy revealed oxalate crystals. A sample was sent abroad for genetic analysis which proved primary hyperoxaluria type I. Good example may be given by comparing these two patients, both female patients, of same age and both suffering from primary hyperoxaluria type 1. The only difference being that one patient was diagnosed prior to transplantation and the other was not. As we could conclude, the consequences of undiagnosed disease in first patient had huge impact on transplanted kidney and overall outcome. Enormous difficulty lies in fault diagnostic tests, lack in screening program and undeveloped genetic tests.

Atypical hemolytic uremic syndrome is a disease primarily caused by genetic mutation in complement system, yet an environment has a huge impact and may provoke this disease [12,13]. Even though this disease present suddenly, it is not an acute disease, but rather chronic multisystem disease. Overly activation of complement system leads to endothelial damage and abnormal clotting. Most patients present with hemolytic anemia, thrombocytopenia and kidney failure [14,15]. Treatment and prevention of atypical hemolytic uremic syndrome after the transplantation is done by plasmapheresis and eclizumab-human monoclonal antibody [16,17]. We must keep in mind that transplantation, together with immunosuppression therapy, activates complement system.

A female patient born in 1967 was admitted to Immunology ward in 2010 where kidney biopsy was done and chronic thrombotic microangiopathy was diagnosed. CKD grade 3 was established at that time and she was treated with plasmapheresis and corticosteroids. Hemodialysis treatment started in 2012 and two years later a diagnosis of aHUS was confirmed by genetic testing. She was treated with eculizumab and successfully transplanted in 2016. Expensiveness of the medication such as eculizumab makes treatment difficult and inconvenient considering that all patients should receive existing treatment. She has already been presented in details [18]. One additional female patient is currently on our waiting list for kidney transplantation with established diagnosis of aHUS.

Tuberous sclerosis is a disease caused by mutations in genes called tuberous sclerosis gene 1 and 2. In 80% of cases there is de novo mutation [19]. It causes benign tumor growth in many organs. Kidney is affected by development of angiomyolipomas and cysts [20]. The bleeding from angiomyolipoma presents a difficulty. This bleeding may require nephrectomy despite the fact that the rest of the kidney may be healthy. In the end, it requires renal replacement therapy. Here, we have to take into account that transplantation could lead to transformation of benign tumors into malignant. mTOR inhibitors play a huge role in cell growth. Tuberous sclerosis does not possess inhibition of cell cycle and cell growth that is why mTOR inhibitors are used in treatment of tuberous sclerosis. These inhibitors treat etiology of tuberous sclerosis [21]. A female patient born in 1982 was diagnosed with TS in childhood following multiple epileptic attacks. Primary disease and arterial hypertension lead to decrease in renal function. Due to the bleeding from angiomyolipoma in the right kidney it was necessary to perform right nephrectomy. This was followed by emergency left nephrectomy because of renal bleeding and development of retroperitoneal hematoma. After few years on dialysis, this patient was successfully transplanted in 2016. Another patient at our Center, also a female, born in 1995 was diagnosed with tuberous sclerosis when she was 2. At the same time polycystic kidneys were noted as well. Due to the decrease in renal function the patient was transplanted in 2016 without precedent dialysis. Transplantation was successful.

Along all diseases mentioned here, there are many syndromes that could lead to end-stage renal disease. These patients are diagnosed in early age. One of these syndromes is Turner syndrome that has a characteristic phenotype due to monosomy X [22]. Turner patients often present with structural kidney malformations such as horseshoe kidney [23]. Clinical Hospital Centre Zagreb holds the only known case of kidney transplantation in a patient with Turner syndrome. Then there is Dandy Walker syndrome, which causes urogenital malformations [24]. These patients have mental retardation; therefore require someone to take care for them in order to have a successful transplantation. At Clinical Hospital Centre there is one patient diagnosed with Denys-Drash syndrome. These patients may have congenital nephropathy, Wilms tumor and gonadal dysgenesis [25]. Two patients are diagnosed with Prune-Belly syndrome that present with abdominal wall defects, criptoorchidism and urinary tract defects-some of which are megaureter, hydroureter, hydronephrosis, vesicoureteral reflux or megacystitis [26]. Branchio-oto-renal syndrome is autosomal dominant disease that appears as hearing impairment together with clefts, fistulas or cysts in the lip or palate region. It affects kidney development and causes malformations such as hypoplasia or dysplasia of the kidney, or kidney cysts [27]. Three patients with this syndrome are transplanted in our Centre.

Conclusion

It is very important to be aware that rare diseases exist and that patients with rare diseases are around us. We always have to consider the possibility of diagnosing a rare disease due to its effect on the outcome. Available treatment exists for many rare diseases. In order to recognize these patients, we need screening program for rare diseases that would simplify diagnosis process. Screening for rare diseases in pre-transplantation period would improve overall outcome of renal transplantations in these patients. It is very likely that screening of transplanted population would show many different genetic mutations that result in deterioration of graft function. An individual approach to each patient is mandatory due to different factors influencing the successfulness of transplantation.

Conflict of interest statement. None declared.

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Case report

Long-Term Hemodialysis Survivor: 33 Years of Maintenance Hemodialysis in a Diabetic Female with a Non-Cuffed Catheter for the Last 18 Years

Bojan Medved, Milena Andonova, Marija Malgaj, Rafael Ponikvar and Jadranka Buturovic Ponikvar

Department of Nephrology, University Medical Center Ljubljana, Ljubljana, Slovenia, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Abstract

Introduction. Arteriovenous fistula (AVF) is considered the "gold standard" for dialysis access. In cases of frequent fistula thrombosis or when the possibility for the creation of a new native AVF or arteriovenous graft (AVG) is exhausted, one or two "temporary", precurved, non-tunnelled, non-cuffed, single-lumen jugular dialysis catheters can serve as long-term vascular access for selected patients, with a complication rate comparable to that of "permanent" tunnelled catheters. Double-needle hemodialysis can also be performed with this type of "temporary" catheter (for blood take) and peripheral vein (for blood return).

Case report. In this article we present the case of a 63-year-old patient with diabetes mellitus type 1 and a 33-year history of end-stage renal disease and hemodialysis treatment with different vascular accesses. During her lifetime on hemodialysis, she had two native AVFs on the right arm, one Gore-tex AVG on the left arm, three Gore-tex AVGs on the right arm, one Gore-tex AVG on the right thigh, and a tunnelled Ash-split catheter inserted into the right jugular vein that later adhereed to the right atrium wall. Because of exhausted options for the creation of a new native AVF or AVG, she has been on double-needle hemodialysis with single, precurved, non-tunnelled, single-lumen jugular catheters and the peripheral vein for the last 18 years. During this time, she had one case of bacteriemia and one case of Staphylococcus aureus sepsis and, for the second time, adherence of the catheter tip to the right atrium wall (the first being with Ash Split catheter). Our Dialysis Center's experience, including the case we are presenting, suggests that "temporary", precurved, jugular hemodialysis catheters locked with 30% trisodium citrate and the routine use of antibiotic ointment (gramicidin, bacitracin and polymyxin B mixture) at the exit site can be an efficient long-term access for hemodialysis in patients not eligible for AVF or AVG creation.

Conclusion. The patient described is living evidence that long-term survival on hemodialysis is also possible with the described 'temporary' catheter and peripheral veins as vascular access.

Key words: arteriovenous fistula, arteriovenous graft, hemodialysis, hemodialysis catheters, non-tunnelled catheters, tunnelled catheters

Introduction

A well-functioning vascular access is a mainstay for an efficient extracorporeal procedure and long- lasting hemodialysis therapy. Native arteriovenous fistula (AVF) is considered the "gold standard" as a dialysis access. There are three main types of hemodialysis access approaches: AVF, arteriovenous graft (AVG), and central venous catheter (CVC). AVF remains the first choice and the best approach towards the longevity of patients. AVF has the lowest morbidity and mortality, and its use is therefore strongly recommended in the guidelines of different countries. However, when the options for the creation of AVF or AVG are exhausted, a CVC can be used. Among hemodialysis catheters, double-lumen, tunnelled, cuffed catheters are predominantly used worldwide as permanent vascular access because of the lower incidence of exit site infections and bacteriemias compared to non-tunnelled "temporary" catheters.

Much effort has been devoted in past years to improving function and reducing infectious complications when using "temporary" dialysis catheters. At our Center, we have excellent experience with the use of one or two precurved, non-tunnelled, single-lumen jugular catheters that can serve as long-term vascular access. Double-needle hemodialysis can also be performed with this type of jugular catheter (for blood take) and peripheral vein (for blood return). These "temporary" dialysis catheters with trisodium citrate locking may function as an

Correspondence to: Bojan Medved, Department of Nephrology, University Medical Centre Ljubljana, Zaloska cesta 7, 1000 Ljubljana Slovenia; Phone: + 386 1 522 3328; Fax: + 386 1 522 2292; E-mail: drbmedved@gmail.com, bojan.medved@kclj.si

important long-term vascular access for hemodialysis in selected patients. The removal or exchange of such catheters is much easier to perform in comparison with tunnelled catheters.

Case report

A sixty three-year-old female patient was diagnosed with diabetes mellitus type 1 at the age of twelve. She was very tired, had often urinated, and has been inadvertently losing body weight. Since then, she has been continuously treated with insulin. 12 years after the onset of diabetes, she was diagnosed with diabetic retinopathy and, 28 years later, besides being poor sighted in the right eye, she became blind in the left eye after vitrectomy. She also underwent cataract surgery on the right eye. In October 1985, at the age of 30, she was put on chronic hemodialysis due to end-stage renal disease (ESRD). She had arterial hypertension for many years, ischemic heart disease with 4 myocardial infarctions, several coronary percutaneous coronary interventions, generalized atherosclerosis, hyperlipidemia, paroxysmal atrial fibrillation, parathyroidectomy due to secondary hyperparathyroidism, carpal tunnel surgery on both hands, peripheral occlusive artery disease, and several percutaneous transluminal angioplasty procedures on the arteries of the lower limbs. She had no amputations. Despite all the comorbidities, the patient was able to take care of herself and did not depend on foreign aid.

The first vascular access in the patient was a single-lumen femoral catheter. Two months after she started hemodialysis treatment, a native radiocephalic AVF was created on the right forearm, and thrombosed the next day. After five days, a new anastomosis was created proximally, 10 cm below the right elbow. The fistula was used for a few months. She was then on two non-tunnelled, single-lumen jugular catheters for 5 years. The catheters were routinely exchanged over the guidewire every 1.5-2 years, usually due to mechanical damage. Afterwards, we attempted to create a native AVF on the left upper arm, but because of vein fibrosis the operation failed. A few days later, a Gore-tex AVG was successfully created on the right upper arm, but unfortunately thrombosed after a few months. Later in the same year, a Gore-tex AVG was created on the left upper arm and was successfully used for 3 years. Soon afterwards, 2 pseudoaneurysms developed and the AVG thrombosed. Thrombectomy was unsuccessful. Nine years after starting hemodialysis, a Gore-tex AVG was created on the right thigh, but unfortunately also thrombosed after one month. The next year, a Gore-tex AVG was created on the right upper arm, which also thrombosed soon after surgery. Over the next 5 years, hemodialysis was performed using one precurved, non-tunnelled, single-lumen jugular catheter and the peripheral veins of the legs (for blood return). After 14 years on hemodialysis, a tunnelled Ash-split catheter was inserted into the right jugular vein. Soon afterwards, the arterial line of the catheter thrombosed and the venous line began to be used as an "artery", thus enabling the blood to return to the peripheral vein. Sixteen months after insertion of the tunnelled catheter, an attempt was made to exchange the catheter over the guidewire. The exchange was unsuccessful, as the catheter tip adhered to the right atrium wall. The catheter was removed conservatively by cardiovascular surgeons. After 21 years of hemodialysis, another attempt was made to create an AVF with the end of the right ulnar vein and the side of the ulnar artery, where a Gore-tex jump graft helped to prolong the too short ulnar vein. The surgery was unsuccessful.

Due to exhausted vasculature preventing the creation of an AVF or AVG, double-needle hemodialysis was performed with a single, precurved, non-tunnelled, single-lumen jugular catheter inserted into the right or left jugular vein as the "artery", and the peripheral veins as the "vein" for the last 18 years. The patient has been dialysed in this way 4 times a week for 3 hours. Nine years after using this vascular access, bacteremia occurred with an infection of the thrombus in the right atrium at the tip of the dialysis catheter. The thrombus was reabsorbed with appropriate antibiotic and anticoagulant therapy. Four years ago, during an attempt to exchange the catheter by guidewire, adherence of the catheter tip to the right atrium wall was detected for the second time. After perfusing the catheter with alteplase, it was successfully removed. Due to an ultrasound-visible fibrin formation in the right atrium, she received a therapeutic dose of low-molecular weight heparin for several weeks. After removal of the catheter, a dialysis catheter was inserted into the left jugular vein. Three years ago, the patient developed catheter-related Staphylococcus aureus sepsis. Since then, the jugular catheter has been regularly exchanged by guidewire every 6 months in order to avoid adherence.

During the entire 18-year period, the jugular catheters were locked with trisodium citrate solution-4% from 2001 to 2005, 30% from 2005 to the present day. Antibiotic ointment was used at the exit site-mupirocin from 2000 to 2016, mixture of gramicidin, bacitracin and polymyxin B from 2016 to the present day.

The patien thad never decided to have a transplant. She is also the mother of a 38 year-old daughter.

The patient has signed a Slovenian translation of a BMJ Consent Form concerning all personal and medical information used in this publication.

Discussion

Our case report demonstrates that it was possible to use a precurved, non-tunnelled, single-lumen jugular catheter (Figure 1), with trisodium citrate as locking solution and antibiotic ointment at the exit site, as long-term vascular access, providing double-needle

hemodialysis and using the peripheral vein (Figure 2) for blood return, for 18 years.

Although the best vascular access for chronic hemodialysis is AVF, the incidence and prevalence of hemodialysis catheters in ESRD patients is increasing [1]. Catheters are needed because of the long maturation of AVF, or when AVF or AVG cannot be created due to exhaustion of vasculature [2]. Some patients develop a rapidly progressive kidney disease, which precludes timely planning for arteriovenous access surgery. For these reasons, 68% of patients in Europe initiate dialysis using a central venous catheter as vascular access

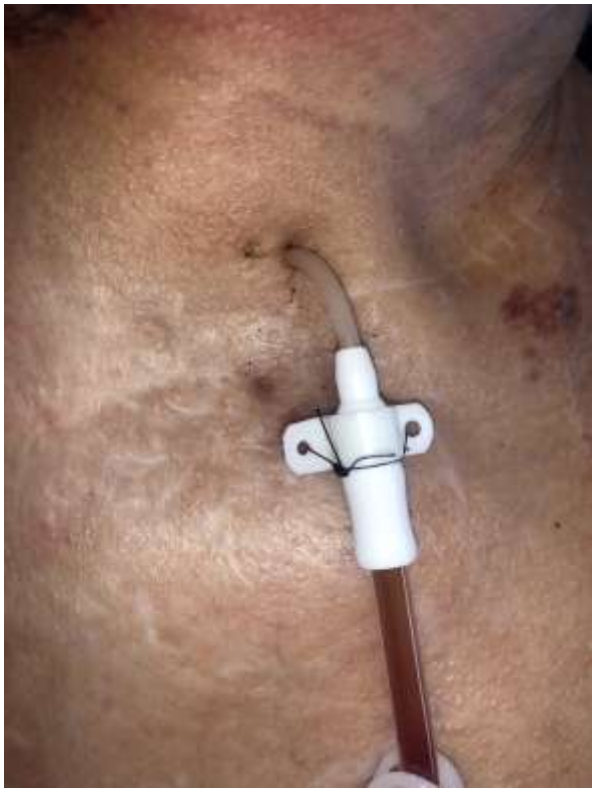

Fig. 1. Patient's exit site of the precurved, non-tunnelled, single-lumen jugular catheter

[3]. Tunnelled, cuffed, double-lumen, central venous catheters are recommended among catheters for chronic hemodialysis [4].

Furthermore, when a catheter is to serve as permanent hemodialysis vascular access, a tunnelled catheter is recommended by various guidelines [5,6], which also strongly discourage the use of temporary catheters, mainly due to higher infection rates compared to tunnelled ones [7]. Despite the guidelines and recomendations, our Center's experience with tunnelled, double-lumen catheters has taught us that dialysis patients and personnel are often confronted with the malfunction of at least one (arterial) or both catheter lumens in tunnelled, double-lumen catheters. The use of the "temporary", non-tunnelled, single-lumen jugular catheter was advised to last for less than 1 week, and femoral catheters for less than 5 days [6]. However, clinical practice at our Center and elsewhere has shown that "temporary" precurved catheters can be used for a much longer period of time, even several months or years, with a complication rate comparable to that of "permanent" tunnelled catheters.

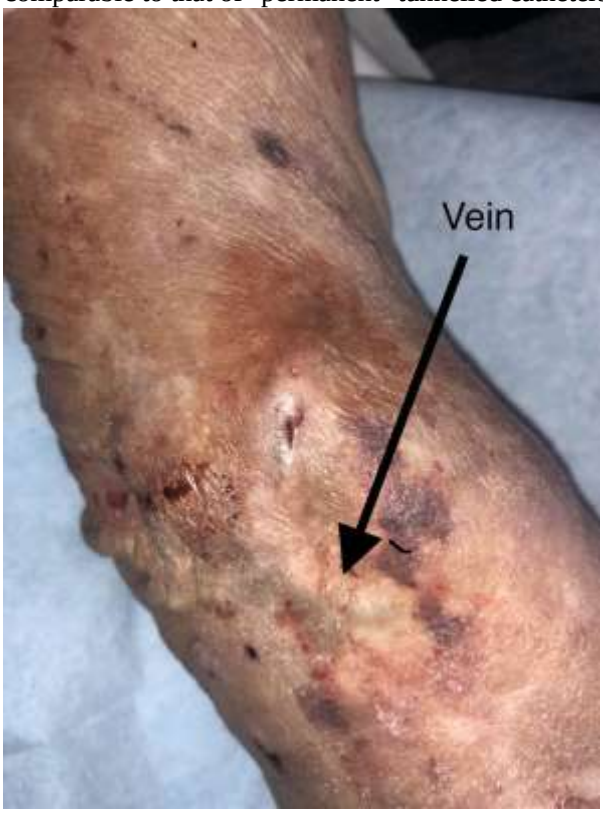

Fig. 2. Patient's peripheral (ulnar) vein for blood return

In spite of the guidelines and recommendations, we have a long-standing practice of using two "temporary", precurved, non-tunnelled, single-lumen catheters inserted into the same jugular vein as permanent vascular access in selected patients not eligible for an AVF, AVG, or a kidney transplant. The recently published observational study has shown that tunnelled and precurved, non-tunneled, jugular catheters are comparable in terms of reaching the combined endpoint of catheter-related infections and catheter malfunction [8]. The observations in recent and our own studies [9-12,22] are of considerable significance, as the most recent KDOQI guidelines [6] recommend the use of tunnelled catheters in the event that central venous catheters are used for more than 1 week. This guideline [6] is of an older age (2006) and does not mention the use of precurved catheters. The recommendations are based on older literature and experience in which non-tunnelled catheters were all straight. This can explain the preference for and advantage of tunnelled over non-tunnelled catheters. At our Center, we have excellent experience with the use of two precurved, non-tunnelled, single-lumen, predominantly jugular catheters for vascular access in patients with acute renal failure [9], and we successfully use "temporary", precurved hemodialysis catheters as long-term vascular access in selected chronic hemo-

dialysis patients [10]. The use of antimicrobial citrate-locking solution in combination with a precurved, non-tunnelled, jugular catheter may have a special advantage. Antibiotic ointment at the exit site may have a lubricating effect on the exit site, avoiding skin damage and enabling maturation of the exit site in addition to the antimicrobial effect. The advantage of using precurved, non-tunnelled, single-lumen jugular catheters is in that the replacement of a malfunctioning catheter is a much less traumatic and easier procedure than the replacement of a much thicker, double-lumen, tunnelled catheter [9]. "Temporary", precurved, jugular catheters could be an optimal permanent vascular access in very elderly patients when the placing of an AVF is not feasible [11]. Two precurved, single-lumen, hemodialysis catheters in the same jugular vein, locked with 30% trisodium citrate, seem to be a safe and long-lasting form of vascular access for hemodialysis and apheresis in selected patients [12].

We have demonstrated that "temporary", precurved, non-tunnelled, single-lumen jugular catheters (one or two), with trisodium citrate as locking solution, can be successfully used as long-term vascular access in chronic hemodialysis patients, with a complication rate (malfunction and infection) comparable to tunnelled, cuffed catheters, and having the important advantage of much easier insertion, exchange and removal. The main reasons for catheter exchange or removal were malfunction and mechanical damage [10]. In the case of thrombosis, catheter thrombolysis is an efficient procedure, otherwise catheter exchange over a guide-wire can be performed [9].

Much effort has been devoted in past years to improving function and reducing complications when using "temporary" hemodialysis catheters, which are much easier to handle and can easily be removed or replaced by guide-wire at the bedside [10]. In contrast, the insertion, removal or exchange of tunnelled catheters can only be performed by a physician with surgical skills. The design of precurved, "temporary", single-lumen jugular catheters, which are bent over the clavicle and fixed to the chest wall, restrain movement of the catheter at the exit site, thus reducing microinjury of the adjacent tissue and consequently improving resistance of the tissue against infection [13]. Locking solutions preserve the patency of the catheter in the interdialytic period, and prevent the formation of intraluminal biofilm and bacterial colonization within the lumen. Until some years ago, tunnelled silastic catheters were the only catheters used for permanent vascular access [15]. The main reason for their use was the significant reduction of bacteremia compared to straight, non-tunnelled catheters [16]. New evidence has shown that thrombosis rates of the "temporary" hemodialysis catheter inserted into the right internal jugular vein was higher with straight versus precurved "temporary" hemodialysis catheter, both during catheter dwell-time and after catheter removal [17]. The newly designed, precurved, "temporary" jugular catheters were comparable to the tunnelled ones, or were even better in terms of incidence of exit site infections and bacteremias. Previously, we presented our excellent results with "temporary", precurved, jugular catheters: only 0.2 exit site infections and 0.2 bacteremias per 1000 catheter days [10]. The bacteremia rate was much lower even when compared with tunnelled, cuffed catheters (1.6–5.5 bacteremias/ 1000 catheter days) [18-20]. The low incidence of infection observed in this study is probably not due solely to the new design of the catheter, but also its locking with 30% trisodium citrate and the routine use of antibiotic ointment at the exit site (mupirocin or a mixture of gramicidin, bacitracin and polymixin B). Excellent results with precurved, "temporary" jugular catheters have also been observed by others [8,13]. For more than 10 years, a 30% trisodium citrate solution has been used as locking solution for hemodialysis catheters at our Dialysis Center. Besides acting as a local anticoagulant, it has also exerted antimicrobial activity and presumably contributed to the lower infection rate related to catheters. No significant side-effects have been observed so far, which can also be attributed to the smaller priming volume (around 1 ml) of the catheter compared to the tunnelled ones. Nevertheless, careful priming is recommended and we never exceeded the priming volume of the catheter [21].

The nephrologists at our Center have taken care of all kinds of vascular access: the creation and reconstruction of AVF and AVG, and insertions of hemodialysis catheters. As a busy dialysis and apheresis unit and a referral center for vascular access, we performed 822 hemodialysis catheter insertions in 2018.

Conclusion

Long-term hemodialysis survivors, such as the patient described above, are living evidence of the remarkable accomplishment, but also limitations, of hemodialysis therapy. The case of our patient suggests that "temporary", precurved, non-tunelled, jugular hemodialysis catheters with trisodium citrate locking and routine use of antibiotic ointment at the exit site can serve as a chronic, long-term access for hemodialysis in patients not eligible for AVF or AVG creation, even those with severe diabetic complications. Returning blood to the peripheral vein can enable double-needle hemodialy sis, together with the use of this type of catheter. In our patient, who has been dialyzed in this way for 18 years,the peripheral veins of the legs and arms "matured" like a fistula vein, both in vein diameter (about 4.2 mm) (Figure 3) and wall thickness (about 1

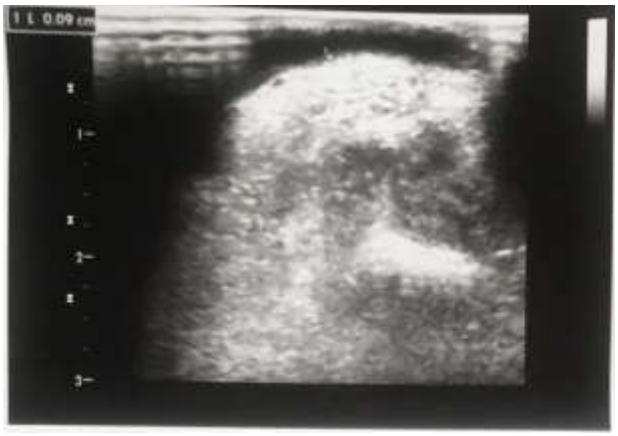

Fig. 3. Patient's peripheral (ulnar) vein 'matured ' like a fistula vein in diameter (about 4.2 mm)

mm) (Figure 4 and 5). During these 18 years she had two catheter-related infections and single occurrence of catheter adherence to the right atrium wall with using a "temporary"catheter (the first being with Ash Split catheter). However, new evidence has revealed that tunnelled and precurved, non-tunnelled jugular catheters are comparable in terms of reaching the combined endpoint of
catheter-related infections and malfunction. Regardless of the possible complications, such hemodialysis catheters can enable the long-term survival of a dialysis patient through proper medical care and teamwork between the patient and dialysis personel.

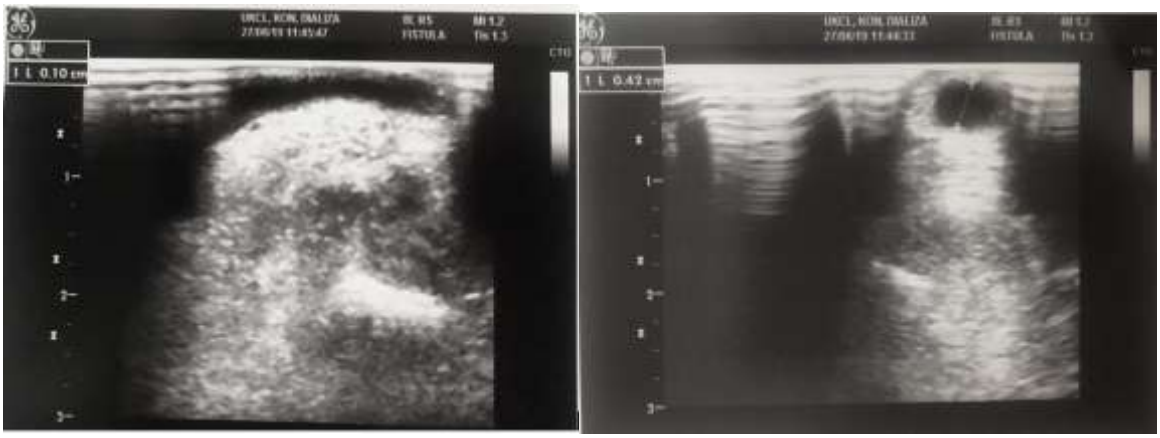

Fig. 4 and 5. Same ulnar vein 'matured' in wall thickness (about 1 mm)

Conflict of interest statement. None declared.

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