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Chapter

Role of Urine Examination in Renal Transplant Recipients

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Abstract

Kidney transplantation has emerged as a major advance of modern medicine, providing high-quality life years to patients with end-stage renal disease (ESRD). Post-transplant monitoring of the transplanted kidney is based on physical examination, urine volume, the assessment of albuminuria or proteinuria, serum creatinine, and glomerular filtration rate (GFR) estimation based on serum creatinine. Of these multiple investigations, serum creatinine and urine analysis is one of the most widely used and accepted tool to assess graft dysfunction as well as plan management. Various immunological (rejections-antibody, cellular) and non-immunological (polyoma virus nephropathy, mycosis, recurrent/de novo diseases) may affect the graft function. Changes in various parameters like urine osmolality, proteinuria, hematuria and presence of casts, crystals and other cellular constituents aids in diagnosis diseases of the allograft. This chapter thus highlights the importance of most frequent parameters that help in assessing the graft function. In addition to these parameters, a brief introduction of biomarkers is also included. Many studies have shown that these biomarkers have a promising role in diagnosis of allograft disease and thus avoiding interventional procedures like renal biopsy. Easy availability as well as low-cost of the urine examination makes it a promising tool for overall assessment of the graft dysfunction.

Keywords: renal transplant, proteinuria, hematura, rejection, tubular injury, biomarkers

1. Introduction

Kidney transplantation has emerged as a major advance of modern medicine, providing high-quality life years to patients with end-stage renal disease (ESRD) [1, 2]. The prevalence of end-stage renal disease requiring transplantation in India is estimated to be between 151 and 232 per million population [3]. Post-transplant monitoring of the transplanted kidney is based on physical examination, urine volume, the assessment of albuminuria or proteinuria, serum creatinine, and glomerular filtration rate (GFR) estimation based on serum creatinine [4]. Of these multiple investigations, serum creatinine and urine analysis is one of the most widely used and accepted tool to asses graft dysfunction [3]. Urine examination, known as "Uroscopy" in ancient time was considered as the mirror of medicine for several thousands of years. The physicians felt they could view the body's inner workings and get the insight of

the disease process by urine examination [5]. Urine examination aids diagnosis as well as management of both native as well as allograft kidney diseases [6].

Specific patterns in urinalysis provide information about graft function as well as renal diseases that can influence graft function [7]. It is a readily accessible, non-invasive tool, can be repeated anytime, cost effective as well as better tolerated than an invasive renal allograft biopsy. There are various causes for graft dysfunction, and these could be either acute or late. Urine analysis can help in diagnosis, follow-up and as well help in determining the graft outcome. Urinary abnormalities, such as hematuria or casts, are also useful in detecting and diagnosing allograft dysfunction [8, 9].

1.1 Causes of graft dysfunction

Before discussing about the role of urinalysis, it is important to determine the reasons for renal allograft dysfunction [9, 10]. Renal allograft dysfunction may be acute or late, the causes can broadly be classified as immunological or non-immunological. The immunological causes are usually acute and chronic rejections. The non-immunological causes include recurrence of a native disease, infections (bacterial, viral or fungal), acute tubular injury, drug toxicity, vascular complications, etc. [10]

Various parameters have been analyzed in urine of renal transplant recipients. These include determination of urine volume, urine osmolality, protein, glucose, blood and leucocytes. We conducted a pilot study in 310 renal transplant recipients who underwent renal allograft biopsy over a period of one year, where we analyzed the corresponding urinary findings which were compared with the morphological findings on renal allograft biopsy.

2. Urine osmolality

Osmolality marks the renal concentrating power, which depends on tubular function of the nephrons. Mazloum et al. in their study observed that altered osmoregulation performance, three months after transplantation is independently associated with allograft loss as well as reduced mGFR at 12 months [11]. When the graft suffers an ischemic lesion, the osmolality is lower as compared to that of a healthy kidney [12]. When we analyzed our set of patients, we found that the mean osmolality for patients with morphological evidence of rejection on RAB was 322.7 ± 141.3 mOsmol/l. This value was high as compared to patients having biopsy that were unremarkable for any immune or non-immune injury (mean urine osmolality: 116.2 ± 75.2 mOsmol/l). The osmolality of patients with biopsy features of acute tubular injury was 210 ± 82.2 mOsmol/l. Overall we recorded a higher value for urine osmolality in patients having acute rejection as compared to acute tubular injury or an unremarkable graft morphology.

Similar findings were also reported by Jenni et al. The receiver operator curve for osmolaluria to predict a rejection in the first 14 postoperative days showed an AUC (area under the curve) of 0.816 on day 2. The same study observed that if osmolaluria falls below 600 mOsmol/l, sensitivity and specificity for prediction of rejection is 66.7% and 89.5%, respectively [7]. Otto Schuck et al. examined early-morning urine osmolality in 104 transplant recipients (aged 21–76 years) and compared with findings of chronic renal allograft nephropathy by studying changes of interstitial fibrosis and tubular atrophy on biopsy. They postulated that the concentrating capacity of the graft kidney is decreased, however they did not report a significant correlation between concentrating function and tubulointerstitial histology findings with a mean urine

osmolality of 384 ± 120 mOsmol/l [13]. In our patients with chronic renal allograft nephropathy the mean osmolality was found to be 282.4 ± 137.1 mOsmol/L. In biopsies with morphological features of interstitial fibrosis and tubular atrophy the mean urine osmolality was 242.2 ± 114.4 mOsmol/l. We conclude that alone urine osmolality might not be a good variable for diagnosis. The values need to be interpreted with respect to clinical features as well as taking other findings in considerations.

3. Proteinuria

Proteinuria (including albuminuria) is an independent factor implicated in kidney damage in native as well as kidney allografts [14]. Recommendations are to perform urinalysis and urinary protein excretion to be assessed regularly in the post-transplant period. Most of the studies recommend that these investigations need to be performed at least every 2 to 3 months during the first post-transplant year and annually henceforth [8]. Many unique proteins, peptides, and other substances are excreted in urine in the patients who undergo renal transplant which could be useful to predict the outcome of the renal allograft [15, 16]. It is estimated that proteinuria is a common finding in post-transplant patients, the incidence being more than 40% kidney transplant per year. Various studies have found that even if proteinuria is low (<500 mg/day), there is still significant reduction in the graft function and reduced patient survival [17]. Even late onset proteinuria in post-transplant patients has been found to be associated with reduced graft and patient survival [18]. Proteinuria in the first year of transplant appears to be multi-factorial. Common causes of protein-uria implicated are residual proteinuria, glomerular diseases, effects of anti-HLA class II antibodies and drugs like mTOR inhibitors, tubulointerstitial disease of the graft, nephrosclerosis, renal vein thrombosis and reflux nephropathy [7, 17]. Causes for late onset proteinuria in renal transplant patients include: relapse or de novo glomerulonephritis, transplant glomerulopathy and chronic rejections. A proteinuria of >0.5 g/l and > 0.8 g/l have found to have a specificity of 80% and 90%, respectively, regarding prediction of rejection [7]. Studies have shown pre-transplant proteinuria (even of nephrotic range) considerable reduces in the first weeks, once a normal functioning kidney is transplanted [7, 17]. This happens due to reduction in the blood flow which occurs in native kidneys after transplant, if the graft is functioning normally. In a patient with poor graft function, the blood flow of native kidneys is maintained, which is the cause for persistent proteinuria in such patients. For patients with a normal functioning graft, the presence of proteinuria above 3000 mg/day, three weeks after the transplant should raise a suspicion for presence of a glomerular disease. This could either be a de novo or a recurrence of a primary glomerulonephritis in the graft.

Many studies have studied the causes for post-transplant proteinuria. Among the commonly used immunosuppressive agents, only Sirolimus has been impli-cated in development of post- transplantation proteinuria [19]. One of the studies documented that 58% of transplant patients with proteinuria (150 mg/day) did demonstrate transplant-specific lesions (allograft nephropathy, transplant glomeru-lopathy, or acute rejection) on biopsy as compared to 11% of patients that showed morphological evidence of glomerulonephritis on biopsy [20]. Shamseddin et al. in his meta-analysis stated that allograft nephropathy was documented in 8–54% of patients (average: 32%). Transplant glomerulopathy ranged from 0 to 39% (average: 17%) with an average prevalence of 37% as compared to glomerular disease [21].

Various methods have been implicated in estimation of urinary protein. Of all the methods available, urine dipstick testing is highly specific and most commonly used method used in most of the laboratories. Despite of false-positive or false-negative results that can be obtained in some situations, it is still the most preferred screening method for proteinuria. As urine dip-stick is not as sensitive as quantitative methods, a twenty-four-hour urine protein excretion stands as the gold standard for quantitative protein assessment. In cases where a twenty-four hour urine collection is problematic, urinary protein/creatinine (mg/mg) ratio can be assessed in a 'spot' urine. A UPCR acts as an excellent surrogate and is shown to have an excellent correlation with the protein content of a twenty-four-hour urine collection [22].

In our study the mean 24-hour urinary proteinuria was highest in cases which presented with recurrence of the native disease (4.9 ± 2.31 g) followed by patients with biopsies showing chronic allograft nephropathy (2.69 ± 1.96 g). Proteinuria was insignificant in biopsies with acute rejection (0.5 ± 1.46 g). Of the recurrent diseases maximum proteinuria was observed in biopsies showing focal and segmental glomerulosclerosis (5.7 ± 3.8 g), followed by those with IgA nephropathy (4.4 ± 3.6 g) and membranoproliferative glomerulonephritis (4.5 ± 1.7 g). Thus evaluation by 24-hour urine protein does help in diagnosis of recurrent diseases as well as chronic allograft nephropathy.

4. Glucosuria

Recurrence of diabetic nephropathy in renal allograft and post-transplant diabetes mellitus are the main reasons for glycosuria. Multiple factors come into play for the above stated diseases. These include transplant done in an old aged patient, high body mass index, presence of a family history of diabetes, use of immunosuppressive reagents (Prednisone, Tacrolimus) and concomitant history of hypertension. Other risk factors include polycystic kidney disease, episode of immune injury (acute rejection), hepatitis B virus infection and hepatitis C virus infection. However the KDIGO guidelines recommend determination of blood glucose levels and glycosylated hemoglobin for diagnosis of diabetes, the role for the measurement of glucosuria after renal transplantation is limited [7, 23]. In our study of one year, we did not come across any case of glycosuria or post-transplant new onset diabetic nephropathy.

5. Hematuria

Presence of at least five red blood cells/high power field (hpf) in three of three consecutive centrifuged specimens obtained at least seven days apart is defined as hematuria [24]. Haematuria may be present in 0.7–3% of the general population, and has a much higher prevalence in patients undergoing renal transplant. Hematuria, like proteinuria has been implicated as one of the factors for graft loss [25]. Increased bleeding tendency in renal allograft recipients could be possibly due to preexisting states of postrenal transplant patients, the use of antiplatelet agents for cardiovascular disease and platelet dysfunction. Additionally, post-transplant patients are susceptible to anemia which accentuates bleeding diathesis. This usually occurs as the circulating red blood cells displace platelets towards the vessel wall thus leading to contact with the subendothelial tissue at the site of injury. Also red blood cells release

adenosine diphosphate which inactivates prostacyclin and enhances platelet function [25, 26]. Although mechanisms of hematuria are many, following causes are main causes for hematuria in renal transplant patients:

5.1 Infections

Immunosuppressants are mainstay for graft stability, however use of these agents predisposes patients to urinary tract infections, which can be heralded by the sign of haematuria. Rivera-Sanchez conducted a prospective study on post-renal transplant patients with hematuria. They reported nearly 37% of the renal transplant recipients with hematuria have urinary tract infection, of which 13.4% had history of recurrent infections [27]. Certain predisposing factors have been implicated in causing recurrent acute graft pyelonephritis. These include presence of anatomical abnormalities like strictures at the ureterovesical junction or neurogenic bladder. Vesicoureteral reflux in these patients also contribute to recurrent infections [24]. As these patients are immunosuppressed, a higher index of suspicion for mycobacterial, fungal, and viral infection has to be kept in mind. Hematuria can occur secondary to cystitis, sparing the kidneys and can be associated with bacteria, fungus or viruses. Fungal organisms associated with hemorrhagic cystitis include *Candida albicans*, Cryptococcus, Aspergillus fumigates and mucormycosis whereas viruses implicated include BK virus, adenovirus, Cytomegalovirus, and herpes virus [28, 29].

5.2 Malignancy

Patients undergoing renal transplant are at risk of developing certain malignancies, in particular those cancers that are associated with viral infections. Common viruses include human papillomavirus (HPV) for cutaneous malignancies and Epstein–Barr virus (EBV) which are associated with post-transplant lymphoproliferative diseases.

Incidence of urological malignancies in these patients is less common. However some malignancies that can occur in these patients and present as hematuria include, renal cell carcinoma and cancers of the urinary bladder. Risk factors implicated in development of renal cell carcinoma are: prior history of renal cell carcinoma, polycystic kidney disease (PKD), duration of dialysis pre-transplant and tuberous sclerosis [30]. Larcom et al. showed that there is an estimated twofold increase for development of prostate carcinoma in the first 3 years after transplantation [31].

5.3 Rejections

Chronic rejection of the transplanted kidney typically presents with microscopic haematuria. Isolated case reports of patients with rejection presenting with gross haematuria have been documented [32].

5.4 Disease recurrences

Haematuria is a common manifestation of recurrence of glomerulonephritis. Those glomerulonephritis which present with a primarily nephritic picture present with hematuria predominantly. These commonly include Goodpasture's syndrome, systemic lupus erythematosus, and Ig A nephropathy. Acute syndromes that present with hematuria and lead to acute progressive renal failure with proteinuria

and anemia include anti-neutrophil cytoplasmic autoantibodies (ANCA) and anti-glomerular basement membrane (GBM) glomerulonephritis [33].

Another remote cause for hematuria is development of a pseudoaneurysm in renal transplant recipient. A pseudoaneurysm is defined as arterial dilation accompanied with disruption of the one or more layers of the arterial wall. This lesion may be present at the site of puncture as a complication of procedures like arterial catheterization or as a complication of percutaneous nephrolithotomy (PCN). This procedure is done in a native or a transplanted kidney following a urinary tract obstruction and an infected hydronephrosis. Other reasons for doing a PCN include: urinary leakage, to remove calculi or a foreign body, chemotherapy and for urinary diversion due to hemorrhagic cystitis [34].

In our pilot study we observed hematuria in 35 (11.2%) patients. Of these 15 (4.8%) of a total 43 patients with active antibody mediated rejection (AMR) presented with hematuria, i.e. 34.8% patients with active AMR showed RBCs in their urine. Of 15 (4.8%) patients having recurrent glomerulonephritis, 9 (60%) presented with hematuria, five had IgA nephropathy and two each of C3 glomerulopathy and systemic lupus nephritis. None of the patients with acute tubular injury or chronic rejections or cellular rejection pr with patients with biopsy reported as unremarkable presented with hematuria. Thus, hematuria if present does indicate a disease process of graft.

5.5 Urinary tract infection (UTI)

Patients undergoing renal transplant have a suppressed immune response and hence have poor resistance to infection. Thus, infections in these group of patients is quite a common leading to morbidity and mortality post-transplantation [35]. Infections are the second most common cause for causing death in patients with renal transplant. The most common cause for predisposition of these patients to infection is that they are immunocompromised. Infection of the urinary tract is the most common infection affecting these subsets of patients, with an estimated incidence between 10 and 98% and is implicated for a longer hospital stay as well as increased health care cost [36–38].

Urine examination plays an indispensable role in diagnosis of urinary tract infection. Significant quantitative bacterial count (of $\geq$105 CFU/mL) in an appropriately collected urine sample aids the diagnosis of UTI in patients showing signs and symptoms of urinary tract infection [37]. Urinalysis in adjunct with urine culture studies are essential in determination of the causative organism of pyuria [39]. Presence of leucocytes in urine is an indicator of acute pyelonephritis and urinary tract infection [36, 37].

UTI can have enormous consequences on the lives of kidney recipients. For instance, it is the most common source of bloodstream infection among recipients, especially when it occurs during the first three months after transplantation [40]. Evaluations of UTI effects on renal parenchyma have shown how infections of the urinary system may result in prolonged inflammation and potential renal scarring [40, 41], which can lead to impaired renal function [42].

6. Role of novel biomarkers in renal transplant recipients

With advances in the field of renal transplantation, newer modalities for monitoring graft function have been developed. Determination of novel biomarkers in urine,

plasma, serum and tissue have been implicated in monitoring renal allograft function. According to WHO, a novel biomarker is defined as a "alteration occurring at cellular, biochemical or molecular level in cells, tissues or body fluid which can be measured and evaluated to indicate the normal biological or a pathogenic processes, or a pharmacological response to a therapeutic intervention [43, 44]. Serum creatinine level, is the most commonly used biochemical parameter to assess the renal allograft function, but is not an affective marker to detect early renal dysfunction. This happens as creatinine concentration in serum is greatly influenced even by various non-renal factors (factors influencing serum creatinine levels: body weight, race, age, gender, total body volume, drugs, muscle metabolism, protein intake) [4, 43]. Additionally, it is not able to predict or evaluate the progression of chronic injury and making it a non-specific or non-predictive marker for graft dysfunction. Alternatively, this makes the histological examination through renal allograft biopsy the gold standard to determine the immunological or non-immunological cause for graft dysfunction [4, 11]. Therefore, these biomarkers, can be used for diagnosis of patients with a disease or an abnormal organ function and also to know the severity and prognosis of a disease, as well as monitor response to a medical procedure [4]. Thus, it is predicted that estimation of these novel biomarkers could possibly help in early recognition of allograft disease as well as help in monitoring disease activity. In addition to this, it is predicted that the novel marker estimation would optimizing the need for an invasive biopsy [45–47].

However, biopsy being an invasive procedure, may not be straightforward to perform and can be complicated by major bleeding. Other drawbacks associated are: risk of potential sampling errors, the inter-observer variability in assigning Banff scores and associated cost of the procedure. Hence it is not only impractical, it is also cumbersome and economically not feasible to monitor graft function by renal biopsy. Urine, on the other hand are readily available and direct product of the allograft and have minimal influence from systemic inflammation, making it a more desirable source for biomarkers [48].

An ideal biomarker is supposed to have certain characteristics. These include readily availability, accuracy, low cost, should be easy to standardized, produce repeatable results and be non-invasive. Overall such a biomarker should be useful to reduce the necessity for performing a renal allograft biopsy and help the clinician for early management [43, 44, 48].

6.1 Classification of novel biomarkers for renal allografts

Biomarkers used to monitor renal allografts can be grouped under two broad headings [44]:

- Immunologic biomarkers: Immunologic biomarkers are those characterizing immune dysfunction ranging from subclinical to overt rejection. These include following:

- Chemokines: Cystine-X-Cystine (C-X-C) motif chemokines 9 and 10, Plasma-derived fractalkine, IFN-γ, and interferon gamma-induced protein 10, cluster of differentiation thirty (CD30).

- Free micro ribonucleic acid: specific serum microRNAs miR-15B, miR-103A, and miR-106A, miR-223-3p, miR-424-3p, miR-145-5p.

- Leukocyte subclasses: donor-reactive memory B cells (mBCs), Donor-specific memory CD4 T cells.

- Gene expression profiles: Kidney Solid Organ Response Test (17 gene set), TruGraf® Molecular diagnostic test.

- Donor-derived cell-free deoxyribonucleic acid:

- Non-immunological biomarkers: Biomarkers that demonstrate adverse transplant outcomes, where immune dysfunction is not the only sole aberration implicated in the disease process, e.g., delayed graft function, cardiovascular events, infection, malignancy.

- Graft quality: The first and foremost important step in kidney transplantation is appropriate allocation of the organs and to predict the future outcome of transplanted organ. The biomarkers in this category include neutrophil gelatinase-associated lipocalin (NGAL) and liver fatty acid binding protein, KIM-1.

- Delayed graft function: It's a type of acute kidney injury, occurring in the first week after transplantation making renal replacement therapy essential for management. This group includes determination of NGAL.

6.2 Neutrophil gelatinase-associated lipocalin (NGAL) (Aka: uterocalin/ lipocalin-2, 24p3/siderocalin)

This molecule is a member of lipocalin superfamily with molecular weight of is a 21 kD [49]. NGAL is secreted by neutrophils, acting as an acute-phase proteins [44]. First discovered as a complex protein with human neutrophil gelatinase in 1993 [49]. NGAL molecule is found in 3 isoforms: monomeric (25 kDa), dimeric (45 kDa), and as heterodimeric (135 kDa—complexed with gelatinase) [49]. The gene for this protein is located on chromosome 9 and this molecule is expressed in renal, liver, endothelial, smooth muscle cells, neurons, and cells of immune system (macrophages and dendritic cells) [50–52]. NGAL molecule expresses its action via a primary ligand, siderophore and metalloproteinase 9 (MMP-9) and is present in plasma as well as urine [4, 49]. Why is NGAL considered to be a biomarker of choice? The reason is that this biomarker is quite efficient and accurate in detecting kidney injury, very early in the post-transplant period. It is observed that there occurs a rapid rise of NGAL in urine, which is detectable even within few hours after the initial insult, whereas rise in serum creatinine occurs hours later [53]. Following AKI, the glomerular filtration rate (GFR) is also reduced which in turn causes the levels of NGAL to rise. A study showed that in patients with acute kidney injury, the levels of NGAL in blood and urine increase by 300-fold (0.1–30 µg/ml) and 1000-fold (0.04–40 mg/ml), respectively [52]. In severe cases of acute tubular injury large quantities of NGAL is excreted into urine, reaching almost up to 1000-fold. This happens due to induction of NGAL mRNA and protein in the renal epithelium as this molecule is expressed in the renal epithelium. Many studies have postulated that patients with higher urinary NGAL values in the early posttransplant phases are more prone to develop delayed graft dysfunction [53]. It has been observed that increase in serum creatinine happens several hours after renal cell destruction, but increase in urine/blood levels of NGAL can be

observed as early as two hours of inception of injury. Thus, it is suggested that NGAL can be used to assess transplant status as early as a few hours post-transplantation [4].

6.3 Kidney injury molecule: 1 (KIM-1)

This protein is a type-1 transmembrane glycoprotein. KIM-1 comprises of two domains, viz.: six-cysteine immunoglobulin-like domain and a mucin domain (extracellular) [54]. KIM-1 also known as HAVCR/TIM-1 is a protein of 104 kD and the gene for this protein is located on chromosome 5q33.2 [4, 55]. KIM-1 (designated as Kim-1 in rodents, KIM-1 in humans) mRNA was identified using techniques of representational difference analysis (which is a PCR-based technique). This technique, which was carried out to find genes, the expression of which was found to be markedly upregulated 24–48 hours after ischaemia in the rat [56]. KIM-1 is expressed in the kidney, liver, and spleen and uninjured kidney tissue. Urine expresses very low or undetectable levels of KIM-1. Studies have shown that KIM-1 plays different roles via various molecular targets in immune diseases and kidney injury. This molecule is expressed on the apical membrane surface of proximal tubular epithelial cells of the kidney (in the S3 segment) and readily responds to hypoxia and renal tubular injury. The extracellular domain of KIM-1 molecule is a quantitative marker of kidney injury and is detached by metalloproteinases and then secreted into the urine. KIM-1 is also an important marker for kidney transplant rejection [52–55]. Various studies including one by Jin et al. reported that serum KIM-1 might be a marker for the prediction of early kidney transplant rejection. They also predicted that this molecule could possibly be helpful in monitoring renal graft function in transplant recipients, and thus might contribute in early diagnosis of organ rejection [57].

6.4 C-X-C motif chemokine 10 (CXCL-10)

This molecule is an interferon-γ-inducible protein-10 (IP-10), a chemokine belonging to the CXC subfamily. This molecule consists of two cysteines that are located at the N-terminus. These two cysteines are separated by a single amino acid which can be variable [58]. The gene for this protein is located on chromosome 4. This chemokine is excreted from all the leukocytes, viz. neutrophils, eosinophils, monocytes and epithelial, endothelial, as well as stromal cells and keratinocytes. The chemokine is secreted as a response to several proinflammatory factors, like interferon-γ (IFN-γ) [58, 59]. CXCL-10 is secreted by leukocytes in the transplanted kidney and is a marker for inflammation. According to the observations of Elkman et al. CXCL9 and CXCL10, which are induced by IFNγ are supposedly to be the most studied as well as promising protein biomarkers for predicting acute renal rejection. Both CXCL9 and CXCL10 bind with CXCR3, that are expressed on activated T-cell which in turn recruit T-cells to the inflammatory site [45]. Schaub et al. demonstrated that the sensitivity and specificity of urinary CXCL-10 (uCXCL-10) exceeded those of serum creatinine levels. Various studies have been performed to determine the role of CXCL10 molecule in allogenic kidney transplant rejection. Study by Ciftci et al. which was performed on living donor related transplant recipients to assess the efficacy of CXCL10, showed that urine levels of CXCL-10 correlates well with serum creatinine level is patients having acute cellular rejection [60–62]. On the other hand, Rabant et al. studied 244 renal allotransplant recipients and monitored urinary CXCL-10 and serum creatinine levels. They further determined the ratio of CXCL10 and serum creatinine and proposed that the ratio can effectively determine the risk of antibody-dependent transplant rejection [63].

Blydt-Hansen et al. also reported similar observation for the CXCL-10 to creatinine ratio in pediatric renal transplant recipients and concluded CXCL-10 to be a promising biomarker of acute cellular rejection [64]. Matz et al. in his study reported that CXCL-10 chemokine levels may predict the development of acute cell-type rejection [65]. Watson et al. demonstrated that high pretransplant serum CXCL-10 levels may indicate a high risk of severe rejection and transplant failure and it would be appropriate to determine the CXCL-10 levels pre-transplantation [66]. Jackson et al. found that urine CXCL-10 levels can increase in acute transplant rejection as well as in patients suffering from polyoma virus nephropathy, however this chemokine cannot be used to differentiate between these two conditions [67].

6.5 Calreticulin (CRT)

CRT is a major calcium 2+ (Ca2+) binding (storage) protein. This protein is present in the lumen of the endoplasmic reticulum with a molecular weight of 46 kDa, having 400 amino acid residues. This protein is basically a major Ca2+ binding chaperon. Calreticulin has three distinct structural domains: the amino-terminal N-domain, middle P-domain, and the terminal carboxyl-C-domain along with a cleavable amino acid signal sequence. This amino acid signal sequence is present at the beginning of the N-terminal, which helps in directing CRT to the endoplasmic reticulum. The C-terminal functions for ER retention/retrieval signal. Two main functions have been implicated to this protein in the ER: One as a chaperon and other as a Ca2+ binding and storage protein. It can be identified at several other sub-cellular locations like cell surface, cytoplasm, and the extracellular matrix [68].

6.6 Cystatin C (CysC)

CysC is an endogenous proteinase inhibitor with a molecular weight of ~13.4 kD. This molecule is a member of cystatin superfamily of cysteine protease inhibitors. The main function of the protein is to inhibit cathepsins, namely cathepsin L, B, and H [69, 70]. CysC is composed of polypeptide chain having 120 amino acids and the chromosome 20 harbors the gene for this protein [71]. CysC has a role in intracellular catabolism of proteins and peptides. Another advantage of this protein is that concentration of CysC does not depend on factors like gender, age, or muscle mass, making it more suitable to determine the dynamics of GFR changes as compared to serum creatinine [72]. Krishnamurthy et al. concluded CysC as an additional diagnostic parameter in assessing the function of a transplanted organ, which additionally might be helpful and serve to tailor immunosuppressive treatment [73]. Changes in the glomerular filtration rate secondary to a deteriorating transplant function and thus an increased risk of rejection, can be detected by the determination of cystatin C according to, according to Taghizadeh Afshari et al. Study by A. Taghizadeh-Afshari showed that at 14 days post-transplant, levels of CysC exceeds the sensitivity and specificity of serum creatinine [74]. Similar observations were also made by Le Bricon et al. According to him CysC is a more accurate marker than serum creatinine. He additionally postulated role of Cystatin C in assessing the toxic effects of treatment [75].

6.7 Osteopontin (OPN):0020

Osteopontin, also known as bone sialoprotein 1 (BSP-1) or secreted phosphoprotein 1 (SPP1) and also as early T-lymphocyte activation-1 (ETA-1). This protein is

an extracellular matrix protein with a molecular weight of approximately 35 kD. It is composed of a polypeptide chain comprising of 314 amino acids. The polypeptide chain contains sequence of arginine-glycine-asparagine binding integrin [76–78]. This molecule is encoded by a single-copy gene which is mapped on the human chromosome 4 (4q13). This molecule is expressed on intestinal epithelial cells, bone, kidney, and immune cells, such as macrophages, dendritic cells, and the T lymphocytes [79, 80]. The serum osteopontin concentration in a normal individual is estimated to be around 23.56 ng/ml [80]. Osteopontin, in kidney, is produced at distal part of nephron. The function of this molecule is implicated in formation of renal vessels [81, 82].

6.8 Clusterin (CLU)

CLU is also called as apolipoprotein J (CLU). It is a glycosylated protein and is composed of two chains, the α-chain and β-chain. These both are linked via disulfide bonds and in human body it is present in two isoforms – secretory type and nuclear type. The mass of the secretory type is 80 kD, and is implicated in removing residues formed after apoptosis. The nuclear type isoform is 50 kD and has its role in DNA repair. The gene encoding for this protein is located on chromosome 8. Clusterin molecule has a role in apoptosis as well as in antiapoptotic pathway. CLU in human body is present in various organs, including kidney and is also detected in all biological fluids. The physiological concentrations of CLU in serum range from 35 to 105 μg/ml. In kidney, this molecule is present in the tubules and has numerous antiapoptotic functions, by mediating cell protection, recycling of lipids, attachment and aggregation of cells. Although the function or utility of CLU in renal transplant rejection is yet to be analyzed [83–85].

7. Conclusion

Multiple causes can affect the functioning of the renal allograft, and there are multiple modalities that are recommended in evaluation of the renal transplant. In the present era where most of the investigations fall under the category of molecular tests and genetics, immunohistochemistry, cytogenetics etc., urine examination still plays an indispensable role in management of the renal allograft. Overall certain parameters like urine osmolality, proteinuria, hematuria and urine microscopy along with the newer molecules (biomarkers) are a hit and help in monitoring of the renal allograft.

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