

CLINICAL STUDY

Correlation between Proteinuria Level and Renal Morphology with Special Reference to Electron Microscopy in Kidney Donors

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The aims of this study were to evaluate whether there is a correlation between protein level in urine and renal morphology in kidney transplant donors, as well as to detect the role of electron microscopy. For this purpose, kidney biopsies of 10 donors with urine protein levels were evaluated. Seven patients were female and three were male. Two had physiologic proteinuria (<150 mg/24h), four had non-significant proteinuria (150–300 mg/24h), and three had significant (> 300 mg/24h) proteinuria. Serum creatinine levels were in normal ranges in all patients except for one who had a slight increase (1.76 mg/dL). Seven cases were reported to have normal or nonspecific light microscopic findings. Two of those seven cases had physiologic proteinuria, three had non-significant proteinuria, and two had significant proteinuria. One case had IgA nephropathy with significant proteinuria. One donor had early stage focal segmental glomerulosclerosis with non-significant proteinuria, and one donor had focal interstitial fibrosis with normal urine protein level. There was no statistically significant difference between score means of ultrastructural morphology of the six patients with same patients' light microscopic results and score means of light microscopic results with urine protein levels of all patients. However, there was a significant difference between score means of ultrastructural morphology with urine protein levels of those six patients. In conclusion, urine protein levels and

light microscopic findings did not always reflect the detailed morphology alone and together. Therefore, combining with electron microscopic examination could be more beneficial in relieving problems occurring in long-term prognoses.

Keywords kidney donor, electron microscopy, correlation, light microscopy

INTRODUCTION

Many factors affect the outcomes of renal transplantations, including donor quality, coexisting diseases, and immunosuppression. For example, the initial condition of the organ as assessed mainly by urine samples and biopsies was reported to play important role in chronic allograft nephropathy development.^[1] Initial condition evaluation is mostly performed by light microscopic examination, but ultrastructural details could also carry future clues for possible problems. Proteinuria is also an indicator of renal lesions and is reported to be a risk factor for subsequent renal function decline in most nephropathies.^[2,3] In addition, it is also found to be a trigger for the production of substances causing fibrogenesis and glomerulosclerosis.^[4,5]

The risk–benefit ratio for the recipient is low and favors transplantation, but it is hard to define for the donor because the non-medical issues mostly outweigh small medical risks. Therefore, because the donor's well-being

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is as important as the recipient's outcome, physically and financially disturbing invasive diagnostic interventions should be avoided as much as possible. For this purpose, we aimed to evaluate whether there was a correlation between urine protein and serum creatinine levels with renal morphology in kidney transplant donors, and to detect the role of electron microscopy in morphologic evaluation in this study.

MATERIAL AND METHODS

Kidney biopsies of 10 donors with urine protein and serum creatinine levels were evaluated.

Patients were asked to deliver a 24-hour urine collection to assess proteinuria (with the sulphosalicylic acid method), and serum creatinine levels were measured with standard methods.

All 10 donors underwent renal biopsies in Baskent University Medical Faculty Ankara Hospital between May 2006 and March 2007. Tissues obtained by direct puncture with a biopsy needle were immediately sent for light and electron microscopic evaluation. Because of technical problems such as insufficient biopsy material, only six of 10 donor kidney biopsy specimens were evaluated ultrastructurally in addition to light microscopy. All morphologic examinations were performed by the same pathologist and expressed as normal, non-specific, and disease state.

Morphologic scoring was done to obtain pure morphologic data without clinical approach in order to fully appreciate the role of detailed morphologic evaluation. Such an application was performed to obtain more data from biopsies and find correlations between urine protein levels and renal morphology.

Light microscopic scoring was based on the presence and severity of certain findings like glomerular sclerosis, glomerular congestion and edema, interstitial fibrosis and mesangiolysis, dilatation of capillary loops, tubular degeneration, and arterial and arteriolar pathology. While focal pathologies added one point, diffuse changes in glomeruli added two points. In addition, the presence of abnormal structures added one point to total score of each. Additional scores were also added if there was positive amyloid histochemistry and immunofluorescent staining.

Ultrastructural scoring was made according to basement membrane changes of both glomeruli and tubuli, podocyte morphology, tubular degeneration, and the presence of abnormal structures (deposits or abnormal mitochondria) in the tubular epithelium. Scores were summed, and two distinct morphology scores were obtained for each individual.

Urine protein levels were also divided into four groups: levels between 0–150 got 0 points; 151–300, 1 point; 301–600, 2 points; and 601 and higher, 3 points.

Statistical comparisons were made between ultrastructural morphology of the six patients with the same patients' light microscopic results, ultrastructural morphology with urine protein levels of those six patients, and light microscopic results with urine protein levels of all patients. The score means of the group's electron microscopy, light microscopy, and urine protein levels were compared using Wilcoxon Signed Ranks test. $p < 0.05$ was considered as statistically significant. Data were analyzed with SPSS 13.0 statistical package software (SPSS Inc, Chicago, Illinois, USA). Significance means that scores are unrelated. For example, while urine protein is in the normal range and light microscopy is nonspecific, ultrastructural examination could indicate an abnormality (i.e., higher scores of protein levels or light microscopy). The absence of significance means that scores are close and supports each other.

RESULTS

Seven patients were female and three were male, with ages ranging from 34 to 63 (mean $\pm$ standard error of mean was 47.8 ± 8.52 ; median was 45.5). Urine protein levels were in physiologic limits in two patients (<150 mg/24h), between 151–300 mg/24h in five patients, 301–600mg/24h in two patients, and greater than 600 mg/24h in one patient. Serum creatinine levels were in normal ranges in all patients except one having a slight increase (1.76 mg/dL).

Urine protein and light and electron microscopic scores were all shown in Table 1.

In light microscopic evaluation, seven cases were reported to have normal and nonspecific findings in initial

Table 1
Patient scores

Patient number	Urine protein score	Light microscopic score	Electron microscopic score
1	1.00	3.00	—
2	1.00	1.00	3.00
3	1.00	1.00	2.00
4	0.00	1.00	1.00
5	2.00	5.00	—
6	1.00	3.00	3.00
7	4.00	2.00	—
8	2.00	2.00	—
9	0.00	4.00	2.00
10	1.00	3.00	3.00

pathologic examination. One of those seven cases had physiologic proteinuria. Protein levels were between 151–300 mg/24h in four, 301–600 in one, and greater than 600 in one of those seven patients with normal and non-specific light microscopic findings. One case had IgA nephropathy with 340 mg/24h proteinuria, one donor had early stage focal segmental glomerulosclerosis with 172 mg/24h proteinuria, and one donor had focal interstitial fibrosis with 62 mg/24h physiologic proteinuria.

Four patients had positive immunofluorescent stainings ranging from ++ to +++ and got an additional one point each, regardless of antibody type. None of them had positive amyloid histochemistry. Ultrastructurally, in four cases with normal and nonspecific light microscopic findings, the dominant lesion in glomeruli was a widening of lamina rara interna with subendothelial accumulation of finely granular and fibrillary material (see Figure 1). One patient with normal light microscopic findings and 240 mg/24h proteinuria had diffuse vesicular electron-dense and lucent accumulations (see Figure 2). The same patient also had mitochondria with abnormal cristae organization (see Figure 3) in tubular epithelium.

There was no statistically significant difference between score means of ultrastructural morphology of the six patients with same patients' light microscopic results and score means of light microscopic results with urine protein levels of all patients. However, there was a significant difference between score means of ultrastructural morphology with the urine protein levels of those six patients.

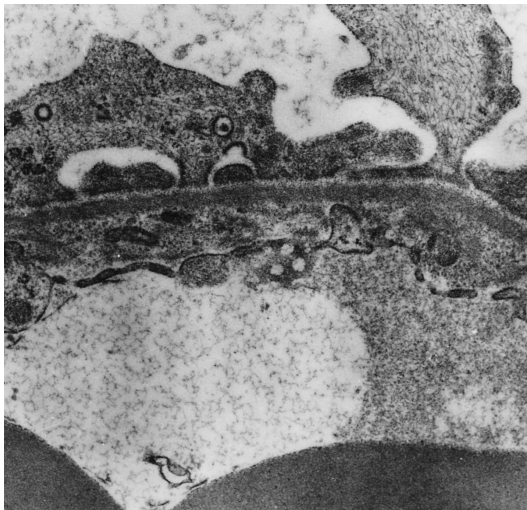


Figure 1. Note the widening of lamina rara interna with the subendothelial accumulation of finely granular and fibrillary material (*).

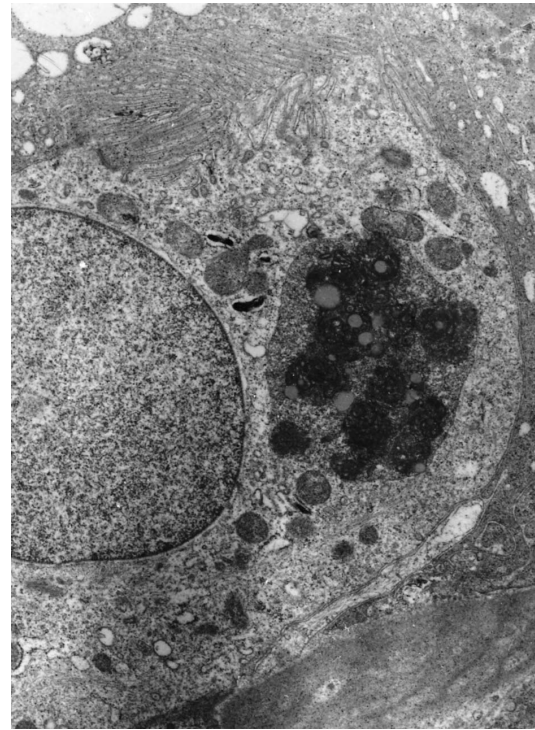


Figure 2. Diffuse vesicular electron-dense and lucent accumulations (→).

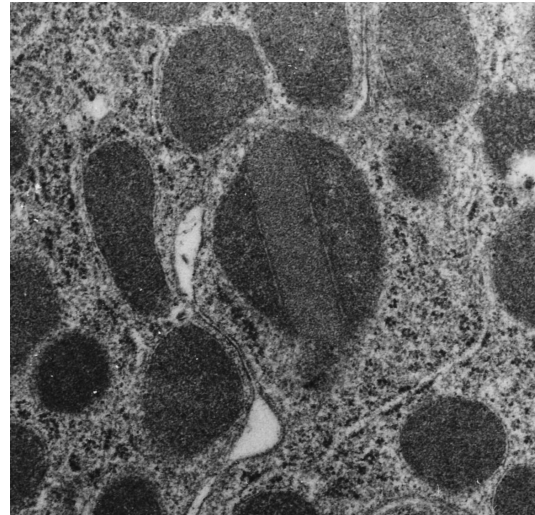


Figure 3. Mitochondria with abnormal cristae organization (→).

All seven donors that were reported to have normal and non-specific light microscopic findings were subjected to renal transplantation operation in 2007. The transplanted kidneys had no evident complication in 6–14 months follow-up.

DISCUSSION

This study appears to be the first to look for a correlation between proteinuria level and renal morphology in kidney donors according to our literature search. Although we had a limited number of patients, our data showed that while light microscopy supports urine protein levels, electron microscopy is not correlated with the degree of proteinuria. It means that ultrastructural findings could indicate a renal lesion, while light microscopy and urine protein levels are in correlation and show no signs of damage. In other words, lesions could not be in such a detectable form for light microscopy but provide several findings for proper diagnosis and follow-up ultrastructurally. Organelle-specific abnormalities (for example, unusual cristae organization in mitochondria or certain cytoplasmic accumulations) or still-unrealized fine details could be important in the prognosis of kidney disease, as the pathophysiology of transplant rejections is yet unexplained.

Many studies have focused on light microscopic results, be it conventional or immunohistochemical, in the kidney^[6] and other organ transplants, and compared morphological data with clinical progression. Demetris et al.^[7] and Haga et al.^[8] reported that conventional histologic assessment could be problematic and is supported with additional diagnostic applications like immunohistochemistry.

Sulikovski et al. examined kidney histopathologies of 92 patients and tried to detect a correlation between light microscopic morphology and graft function. Several pathologic entities, such as acute tubular necrosis, arterial hyalinization, arteritis, and tubular atrophy, were investigated to find an additional prognostic finding to improve graft survival.^[6] They also used some postoperative serum parameters like creatinine level in the evaluation of graft condition. Finally, they reported a correlation between certain morphologic entities and serum parameters with graft function.

Haas et al. also focused on intimal arteritis, a light microscopic entity, and examined the role of its subtypes on short- and long-term clinical outcomes.^[9] Additionally, Ibermon et al. used light microscopic data in his study in which they compared functional and structural donor parameters whether there is a relation or not. He reported that donor structural and functional parameters are independent.^[10]

Several other studies^[11–14] have investigated the correlation between morphologic data, serum parameters, and graft function. However, none of them used ultrastructure as a parameter in their comparisons. It could arise from following reasons:

- electron microscopic examinations are financially and practically coercing,

- tissue preparation methods carry several technical limitations that can cause tissue artefacts, leading to misinterpretations, and
- a very small portion of a tissue could be examined.

But on the other hand, renal transplantation carries so many clinically and theoretically unsolved problems that every method of examination should be performed to improve transplant quality. Therefore, fine details obtained in ultrastructural examinations could bring further expansions, resulting in a better pathophysiological understanding. In our study, the only significance was between our patients' electron microscopic scores and urine protein levels. However, the importance of this little significance is coming from the subject itself, renal transplantation. It means that if electron microscopy has a potential to reveal an unexplained point, no matter how small it is, it is worth performing in all cases when available. On the other hand, the absence of significance in other comparisons could be due to low patient number or short time of follow-up.

CONCLUSION

Living donation is the best choice for kidney transplantation. Although it is a life-saving action, transplantation carries several difficulties for both donor and recipient. Diagnostic interventions before and after transplantation, such as biopsies, also bring additional physical risks and financial loads. Therefore, because transplantation surgery itself brings important health risks with it, invasive diagnostic applications should be minimized. In the meantime, however, it should not be forgotten that mechanisms of post-transplant rejections are still poorly understood with current histologic methods. Thus, although we had a limited number of patients, our data suggested that serum parameters could routinely be confirmed with electron microscopy in addition to light microscopy in all transplantation donors, unless technically and/or financially not available. Because we have not been so successful in the prevention of organ rejections, electron microscopy could bring us new evidence for diagnosis and management.

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