Cortical and medullary vascularity in renal allograft biopsies

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Aim: To evaluate the relation between cortical and medullary peritubular capillaries (PTCs) and scarring. There are presently no studies about medullary PTCs in renal allograft biopsies.

Materials and methods: Nonprotocol allograft biopsies were evaluated and 41 with adequate medullary and cortical tissues were selected. Vascular structures were counted separately at the medulla and cortex on anti-CD34 stained sections. Other histopathological and clinical findings were retrieved from the patient files.

Results: A weak but positive correlation was found between the number of cortical vascular structures (nVC) and medullary vascular structures (nVM). The nVC and nVM were decreased in cases with increased renal scarring; however, this was not statistically significant. A moderate negative correlation was found between nVC and blood creatinine (Cr) at the time of biopsy (P = 0.045, r = –0.32), but not between nVM and Cr. A moderate negative correlation was detected between both nVC and nVM and the most recent Cr (P = 0.01, r = –0.54 and P = 0.03, r = –0.51).

Conclusion: nVC might be a valuable prognostic marker, as described previously. The relation between the latest Cr and nVM suggests that nVM might also be of value, a novel finding requiring further investigation. The weak correlation between nVC and nVM, which is described here for the first time, may point to unequal responses of the cortex and medulla to injurious factors.

Key words: Peritubular capillaries, medulla, renal allograft

Renal allograft biyopsilerde kortikal ve medüller damarlanma

Amaç: Renal allograft biyopsilerde medüler peritübüler kapillerleri (PTK)’i tanımlayan çalışma yoktur. Bu çalışmada, kortikal ve medüler PTK ile skarlanma arasındaki ilişki değerlendirildi.

Yöntem ve gereç: Protokol dışı allograft biyopsiler değerlendirilerek, medüler ve kortikal doku açısından yeterli 36 biyopsi seçildi. Anti-CD34 boyalı kesitlerde medüllar ve korteksteki ayrı ayrı olmak üzere damar yapıları sayıldı. Diğer histopatolojik bulgular ve klinik veriler hasta dosyalarından elde edildi.

Bulgular: Kortikal damar sayısı (KDS) ve medüler damar sayısı (MDS) arasında zayıf pozitif korelasyon saptandı. İstatistiksel olarak anlamlı olmamakla birlikte ileri skarlanma gösteren olgularada KDS ve MDS’nin azalığı izlendi. Biyopsi sonrası kan kreatinin (Krá) düzeyi ile KDS arasında orta derecede negatif korelasyon saptanırken (P = 0.045, r = –0.32), MDS ile korelasyon saptanmadı. KDS ve MDS ile son Kr düzeyleri arasında da orta derecede negatif korelasyon saptandı (P = 0.01, r = –0.54 ve P = 0.03, r = –0.51).

Sonuç: KDS daha önce de tanımlandığı gibi pronostik bir gösterge olabilir. Bu seride saptanan son kreatinin düzeyi ve MDS arasındaki ilişki, MDS’nin de önemli bir veri olabileceğini düşündüğümüz araştırılamayla değer yeni bir bulgudur. İlk kez bu çalışmada tanımlanan KDS ve MDS arasındaki zayıf korelasyon, korteks ve medullanın zedelenmelere aynı yarits vermedikini düşündürmektedir.

Anahtar sözcükler: Peritübüler kapillerler, medulla, renal allograft
Introduction

Peritubular capillaries (PTCs) are essential for the maintenance and normal functioning of renal tubules. A considerable degree of attention has been recently focused on PTCs due to the recognition of antibody-mediated rejection and late or chronic antibody-mediated rejection (CAMR).

The multilayering of PTCs, a nondiagnostic feature of CAMR, might be followed by a capillary loss (1-3). The changes in PTCs are not only observed in antibody-mediated rejection. Decreased PTCs are expected in other conditions leading to interstitial fibrosis (4-6). There are a few articles about cortical vascularization, but the medulla is not described in allograft biopsies. Although we previously described how medullary and cortical scarring are correlated in allograft biopsies (7), as it is suggested that the medulla is more prone to hypoxic injury (8), it is not possible to make a presumption about the medullary findings related to PTCs by examining the cortical area. In this study, the cortical and medullary vascular structures are evaluated in a series of allograft biopsies by immunohistochemistry (IHC) with an endothelial marker (CD34) in order to highlight the relation between these 2 regions, as well as scarring and renal function. To the best of our knowledge, these medullary changes were not evaluated previously.

Materials and methods

From 77 renal allograft recipients, 105 formalin-fixed, paraffin-embedded, nonprotocol posttransplant biopsies were evaluated by light microscopy. The patient selection criterion was the availability of a minimum of 3 high-power field area tissues of the cortex and medulla. Accordingly, 41 (39%) cases were included in the study. Each case was evaluated by light microscopy with hematoxylin and eosin (H&E), periodic acid-Schiff, and periodic acid-methanamine silver, as well as immunofluorescence for IgG, IgA, IgM, C3, C1q, kappa, and lambda during previous evaluation of the biopsies for patient diagnosis. C4d results were not available for all cases. The clinical data of the patients were retrieved. The cases were scored according to the Banff classification. Medullary tissues were reviewed for rejection-related lesions, except intimal arteritis. Interstitial inflammation and tubulitis in the medulla were scored according to the Banff guidelines. Atrophy in the medullary tubules was defined as tubules with thickened basement membranes and was scored semiquantitatively, as provided for in the Banff criteria (7).

Evaluation of IHC for anti-CD34

Anti-CD34 IHC was performed in order to identify the vascular structures as described previously (3). Immunohistochemical staining was carried out on formalin-fixed, paraffin-embedded tissue sections using antibodies to CD34 (Ab-1, Clone QBEnd/10, NeoMarkers, Fremont, CA, USA; dilution of 1/200) by applying the biotin-streptavidin immunoperoxidase technique with an autoimmunostainer (Lab Vision Autostainer Universal Staining System, Lab Vision, Horsholm, Denmark).

The manual counting of vascular structures, excluding arteries, was performed on digitalized images (each image was 15,878 μm²) using image analysis software (Pro200, Babsoft, Ankara, Turkey). Every available cortical and medullary area was counted. The mean number of tested cortical and medullary images was 6.2 and 4.4, respectively. The mean numbers of cortical vascular structures (nVC) and medullary vascular structures (nVM) for the test area were determined (Figure 1).

Statistical analysis

The data were analyzed with the Kruskal-Wallis test and Pearson’s correlation test using SPSS 15. P < 0.05 was considered statistically significant.

Results

The biopsies were taken at 4 to 3650 (mean: 368.37 + 728.58) days following transplantation. The latest Cr level was evaluated 90 to 3740 (927.83 + 771.19) days following the biopsy. The ages of the patients ranged from 14 to 57 years (mean: 37). Ten patients (24.4%) had acute rejection and 12 (29%) had borderline changes. Ten (24.4%) had chronic allograft nephropathy (CAN). Features consistent with cyclosporine toxicity were noted in 8 cases (19.5%). The mean nVC and nVM were 55.92 + 13.88 (minimum: 17.4, maximum: 79.0) and 77.30 + 23.86 (minimum: 31.25, maximum: 136.60), respectively. When all cases were considered, a weak positive
correlation between nVM and nVC (P = 0.006 and r = 0.42) was observed. A moderate negative correlation was found between nVC and Cr at the time of biopsy (P = 0.045, r = −0.32), but not between nVM and Cr. A moderate negative correlation was detected between nVC and nVM with the most recent Cr (P = 0.01, r = −0.54 and P = 0.03, r = −0.51). There was no relationship between nVM and nVC with donor or recipient age or time interval between transplantation and biopsy.

According to the Banff tubular atrophy categories, the mean nVC for cases with a score of 0 was 53.98 ± 13.68 (5 cases), for cases with a score of 1 was 59.39 ± 11.66 (18 cases), for cases with a score of 2 was 51.55 ± 14.31 (11 cases), and for cases with a score of 3 was 46.13 ± 24.88 (3 cases) (Kruskal-Wallis test, P > 0.05) (Figure 2). Regarding the medullary tubular atrophy scores, the mean nVM for cases with a score of 0 was 81.49 ± 14.79 (10 cases), for cases with a score of 1 was 78.53 ± 24.28 (18 cases), for cases with a score of 2 was 75.84 ± 32.41 (5 cases), and for cases with a score of 3 was 72.18 ± 36.01 (4 cases) (Kruskal-Wallis test, P > 0.05) (Figure 3). According to the Banff interstitial fibrosis categories, the mean nVC for scores of 0 was 54.27 ± 16.07 (7 cases), for scores of 1 was 60.56 ± 9.91 (15 cases), for scores of 2 was 52.15 ± 15.35 (8 cases), and for scores of 3 was 48.42 ± 16.60 (7 cases) (Kruskal-Wallis test, P > 0.05) (Figure 4). For the medullary interstitial fibrosis scores, the mean nVM for scores of 0 was 77.89 ± 17.52 (10 cases), for scores of 1 was 81.26 ± 23.26 (17 cases), for scores of 2 was 89.05 ± 29.82 (4 cases), and for scores of 3 was 64.64 ± 30.66 (6 cases) (Kruskal-Wallis test, P > 0.05) (Figure 5).

Discussion

Changes in the renal medulla during the lifespan of the allograft are yet to be described in detail. However, the cortex has attracted considerable attention. We previously identified a positive weak correlation between the renal cortical and the medullary atrophic area (7). In this study, we focused on vascularity in the medulla and cortex. Just as with the findings of our previous study of the renal atrophic cortical and medullary area, in this study, we found a weak yet positive correlation between nVM and nVC, a novel finding. According to the results of this study and the previous one, it seems that the damaging influence on renal tissue affects both the cortex and the medulla, but not evenly.
Vascularity in renal allograft biopsies

Previously, Bishop et al. (4) suggested that PTCs might be the major target of the acute rejection response. Choi et al. (5) evaluated only the cortical area and demonstrated that the decreased density of PTCs was associated with an increased interstitial volume and decreased tubular area in tubulointerstitial injury. The nVC and nVM were evaluated with tubular atrophy and interstitial fibrosis scores in this series, and, as the previous findings have shown, there was a tendency toward a decrease in the number of vessels with increasing scores of atrophy and fibrosis. No statistical significance was found, which might be due to the small number of cases.

Ishii et al. (6) described the decrease of PTCs in CAN cases in allografts and also described a negative correlation with renal function at the time of the biopsy. We also found a weak negative correlation between nVC and Cr at the time of the biopsy and a moderate negative correlation of both nVC and nVM with the latest Cr measurement available.

It is well known that cortical tubular atrophy and interstitial fibrosis are very important predictors of renal function. The results of this study suggest that cortical vascularity might be another valuable feature, as Ishii et al. (6) described. The relation of the latest Cr level and nVM suggests that nVM might also be of value, which was not described previously.

References


