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Difference in Severity of Acute Rejection Grading between Superfical Cortex and Deep Cortex in Renal Allograft Biopsies

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= Abstract =

Twenty-six renal allograft biopsies which showed acute rejection and had renal capsule and medulla in the same specimen were selected in order to compare the severity of acute rejection between superficial cortex, deep cortex and medulla. Disregarding the mid cortical region, the superficial cortex was considered as being one-third of the distance from the renal capsule to the medulla and the deep cortex as being that one-third of the cortex which was adjacent to the medulla. Using semiquantitative histologic analysis the following parameters were compared in superficial cortex, deep cortex, and medulla: interstitial inflammation, edema, tubulitis, and acute tubulointerstitial rejection grade. Also, the presence of lymphocyte activation and polymorphonuclear leukocytes was evaluated.

Significantly greater histologic changes of acute rejection were found in the deep cortex vs. superficial cortex for the following parameters: interstitial inflammation(P=0.013), edema (P=0.023) and tubulointerstitial rejection grade(P=0.016). These findings support the view that biopsies in which deep cortex is not included may result in underestimation of the severity of renal allograft rejection. **(J Korean Soc Pediatr Nephrol 2007;11:152-160)**

Key Words : Graft rejection, Kidney transplantation

INTRODUCTION

Percutaneous renal allograft biopsy has been useful in the management of renal allograft recipients; a biopsy can help to differentiate between various causes of elevated creatinine levels, to quantitate the severity of acute rejection episodes, to predict whether a given acute rejection episode will be reversible, and to prognosticate graft outcome[1-3]. Thus, biopsy severity grade of even a single rejection episode is predictive of graft survival and graft loss due to chronic rejection. We had occasionally noticed striking differences in the degree of the pathologic change between superficial cortex and deep cortex in some patients with acute rejection. The current study was performed in order to determine if these anecdotal observations could be confirmed and to estimate the magnitude of potential sampling errors.

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MATERIALS AND METHODS

We reviewed 596 renal allograft biopsy specimens in order to select 26 specimens which

showed acute rejection by Banff criteria[5] and had renal capsule and medulla in the same specimen. Renal biopsies were performed using the Vim-Silverman needle and standard techniques. The light microscopic slides were reviewed by two authors at the same time.

Disregarding the immediately subcapsular ischemic area, the superficial cortex was defined as being one-third of the distance from the renal capsule to the medulla and the deep cortex as being that one-third of the cortex adjacent to the medulla(Fig. 1). The middle portion of the cortex was disregarded as it was considered likely to represent a blend of the superficial and deep cortex and thus unlikely to be significantly different from these other two cortical regions.

Using a semiquantitative histologic analysis representing a modification of the Banff criteria [5](Table1), the following parameters in superficial cortex, deep cortex and medulla adjacent to the cortex were compared: interstitial inflammation(0-3+), edema(0-3+), tubulitis(0-3+) and

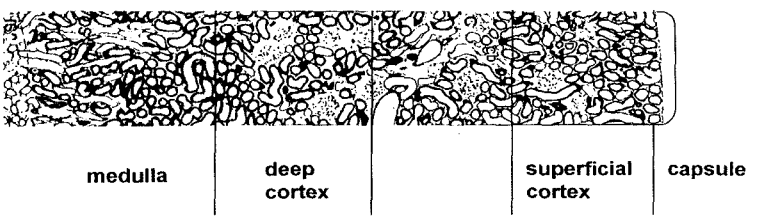

Fig. 1. Schematic diagram of the renal allograft biopsy tissue.

Table 1. Semiquantitative Histologic Rejection Criteria

Interstitial Inflammation	
0	No interstitial inflammation
1	One focus of interstitial inflammation
2	2 or more foci of interstitial inflammation but not diffuse
3	Diffuse interstitial inflammation
Interstitial Edema	
0	No edema
1	Edema in less than one high power field
2	Edema in more than one high power field but not diffuse
3	Diffuse edema
Tubulitis	
0	No Tubulitis
1	Occasional lymphocytes in isolated tubulitis
2	Lymphocytes in adjacent tubules with foci of inflammation separated by uninflamed parenchyma
3	Most or all tubules affected
Acute tubulointerstitial rejection(TIR) grading	
0	No acute rejection
1	Overall tubulointerstitial change approximately grade 1
2	Overall tubulointerstitial change approximately grade 2
3	At least 2 of 3 categories including interstitial inflammation, Interstitial edema or tubulitis scored as grade 3

acute tubulointerstitial rejection grade(0-3+) (Table 1). The presence of the lymphocyte activation, polymorphonuclear leukocytes and eosinophils were also evaluated. Vasculitis and perivenular infiltration were not assessed since these structures are more numerous in deep cortex.

Statistical Analysis

The Wilcoxon signed rank test for nonparametric data was used and values for P=0.05 were considered as statistically significant.

RESULTS

The histopathologic data in superficial cortex

Table 2. Histhologic Data in Superficial Cortex and Deep Cortex

Patient	Glomerular Number		Interstitial Inflammation		Activation		Edema		PMNs		Tubulitis		TIR grade	
Number	S	D	S	D	S	D	S	D	S	D	S	D	S	D
1	10	10	1	2	1	1	1	2	1	1	1	2	0.5	1.5*
2	7	4	1	2	1	1	0	1	0	0	1	2	0.5	1*
3	10	1	1	1	1	1	1	1	0	0	1	1	1	0.5*
4	6	7	1	3	1	1	1	3	0	1	2	2	2	3
5	9	3	2	3	1	1	2	2	1	0	3	2	2.5	3
6	6	3	3	2	1	1	3	3	0	0	3	3	3	3
7	8	7	1	2	1	1	1	2	0	0	1	2	1	2
8	8	3	2	2	1	1	2	2	1	1	1	1	1.5	2
9	8	10	1	1	1	1	1	1	0	0	1	1	1	1
10	7	4	1	2	1	1	1	1	1	0	1	2	1	2
11	11	13	0	1	0	1	0	1	0	0	0	1	0	1*
12	6	3	1	1	1	1	1	1	1	1	1	0	1	1
13	5	7	1	3	1	1	1	3	0	1	0	2	1	3
14	9	7	2	2	1	1	2	2	1	0	2	1	2	2
15	4	6	2	2	1	1	2	2	0	0	0.5	0.5	2	2
16	8	11	1	1	1	1	1	0.5	0	1	0	0	1	1
17	8	11	1	1	1	1	1	0.5	0	0	0	0	1	1
18	8	4	3	3	1	1	3	3	1	1	2.5	2.5	2	2
19	7	8	3	1	1	1	1	0.5	1	0	2	0	2	1
20	9	24	0.5	1	1	1	0.5	0.5	0	0	1	1	1	1
21	8	10	2	3	1	1	2	3	0	1	1	3	2	3
22	6	10	1	3	1	1	2	3	0	0	2	3	2	3
23	11	17	1	1	1	0	1	1	0	0	1	1	1	0.5*
24	20	11	1	2	1	1	1	1	0	1	1	2	1	2
25	11	9	0.5	2.5	0	0	0	1.5	0	0	0	2	0	1.5*
26	7	9	3	2.5	1	1	3	2	1	1	1	1	3	2
Median			1	2	1	1	1	1.75	0	0	1	1	1	2
P			0.019		NS		0.023		NS		NS		0.019	

*Cases where diagnosis of acute rejection might not have been made had only a partial sample been availble. In 4 cases, TIR grade was greater in D and in 2 cases, greater in S.
Abbreviation : PMN, polymorphonuclear leukocyte; TIR, tublointerstitial rejection; S, superficial cortex; D, deep cortex

and deep cortex in the 26 cases are shown in Table 2. There was significantly greater severity of acute rejection changes in the deep vs. the superficial cortex for the following parameters: interstitial inflammation(P=0.019), edema(P=0.023), tubulointerstitial rejection grade (P=0.019). Fig. 2 and 3 illustrate the differences between the superficial and deep cortex in patients #13 and #25.

In 6 cases(Table 2), including patient #25 illustrated in Fig. 3, the diagnosis of acute rejection might not have been made if only a

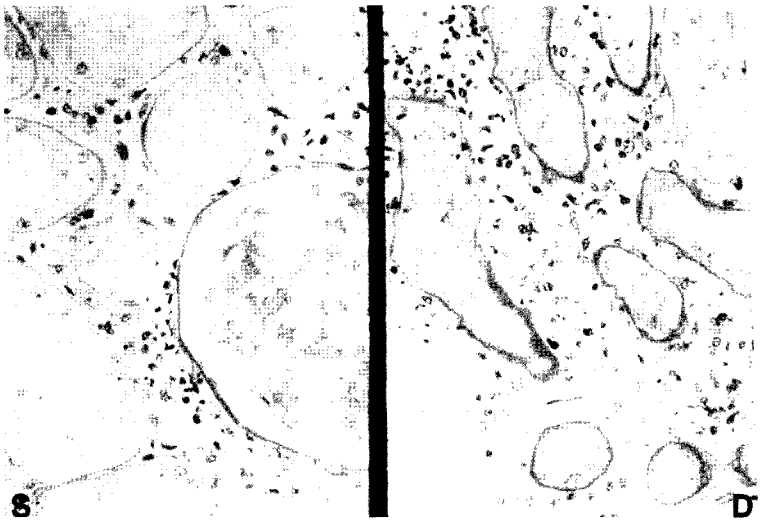

Fig. 2. Patient number 13 showing interstitial infiltrate, tubulitis and edema in D(deep cortex) with minimal interstitial infiltrate and peritubular capillary margination of leukocytes but no tubulitis in S(superficial cortex).

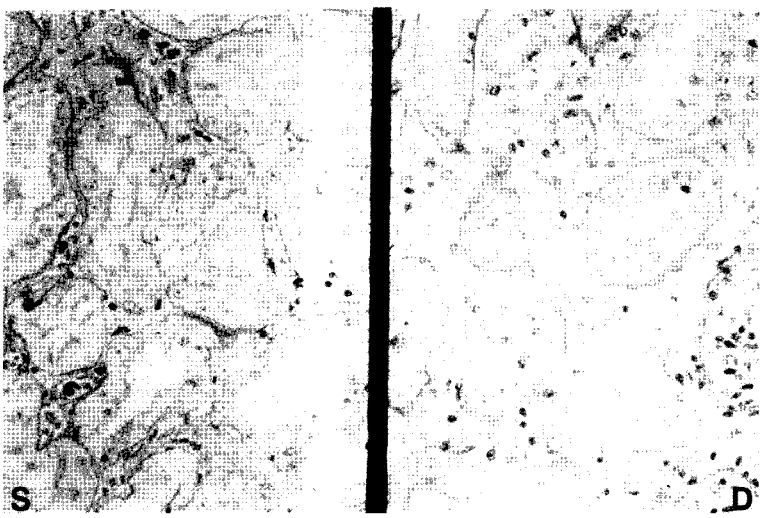

Fig. 3. Patient number 25 showing tubulitis, interstitial infiltrate and edema in D(deep cortex) with minimal interstitial infiltrate and peritubular capillary margination of leukocytes but no tubulitis in S(superficial cortex).

partial sample(superficial or deep cortex) had been available. In 4 cases, tubulointerstitial rejection grade was greater in deep cortex and in 2 cases, it was greater in superficial cortex. Furthermore, the degree of rejection was greater in the deep cortex as compared to the superficial cortex in 9 cases; conversely, in only 2 cases was the degree of rejection greater in the superficial than in the deep cortex. In 9 cases the degree of rejection was identical in the superficial cortex and deep cortex for any other parameters.

There were no significant differences in severity grade for the various histologic parameters between superficial or deep cortex and medulla. In 4 out of 24 cases(17%), in which the medullary portions were adequate for evaluation, the diagnosis of acute rejection would not have been made if only medullary tissue had been available(Patient; 9, 23-25, Table 2: data not shown).

DISCUSSION

The usefulness of percutaneous allograft biopsy in the management of the renal transplantation recipient is well accepted[1-3]. Renal allograft biopsy can frequently provide a definitive answer in a confusing clinical situation. Thus, biopsy has been used to differentiate rejection from other causes of decreased renal transplant function, to document the type of rejection, to grade the severity of rejection, and to predict whether a given acute rejection episode will be reversible[1-3]. Although the renal biopsy represents the "gold standard" for differential diagnosis of parenchymal dysfunction in the renal transplant, the procedure has potential sources of error. One of these, sampling variability, is addressed here. Thus, rejection injury may not be distributed uniformly throughout the kidney[6]. Although two studies have shown that serious sampling errors in percutaneous renal allograft biopsies are infrequent[7, 8], such errors can occur and should be taken into account in biopsy interpretation.

This current study included only patients with accepted histologic criteria for acute renal allograft rejection[5], whose biopsy sample in cluded superficial and deep cortex and medullary tissue in the same core in order to address the question of the distribution patterns of acute rejection. Criteria for acute rejection applied in this study were similar to the Banff criteria[5], and similar to those used in our previous studies which had demonstrated important relationships of this classification system to graft outcome [2, 4]. However, modification of published criteria was necessary because the cortex was divided into 3 parts, so the individual segments were too small to be evaluated by percentage of injured area in this study.

The present study demonstrated significantly greater histologic changes of acute rejection in the deep cortex than in the superficial cortex for parameters including interstitial inflammation, edema, and tubulointerstitial rejection grade. In 6 of 26 cases(23%), the diagnosis of acute rejection might not have been made had only a partial(superficial or deep cortex) sample been available. Tubulointerstitial rejection grade was greater in deep cortex in 4 and in the superficial cortex in 2 of these 6 cases.

There were no statistically significant differences in the above histologic parameters between superficial or deep cortex and medulla.

However, in 83% of cases, the diagnosis of acute rejection could have been made with medullary tissue only, but in the fifth of cases acute rejection would have been missed if only medullary tissue were evaluated. Hongwei et al. reported similar results[9].

Sorof et al.[10] recently argued that two cores should be obtained for the evaluation of acute renal allograft rejection. When analysis of two cores resulted in an overall diagnosis of moderate or severe acute rejection, examination of only one of these two cores would have missed this diagnosis in 25.6% of cases[10]. It is uncertain whether their results were due to the volume of the biopsied tissue or the distribution pattern of rejection areas. We used the 14 gauge Vim-Silverman biopsy needle. On the other hand, many clinicians use biopsy guns or TruCut needles with smaller gauges and the role of this variable in the accuracy of renal allograft biopsy diagnosis has yet to be determined. Two cores may also be more likely to include corticomedullary junction than one core, perhaps accounting for some of the results of Sorof et al.[10]. It would be preferable to obtain a single core containing superficial cortex, deep cortex and medulla together. A further study evaluating the relationship between the biopsy core size and diagnosis rate of acute rejection is necessary.

Other tests have been sought to make the diagnosis of acute rejection in a less-invasive manner. Fine needle aspiration biopsy(FNAB) involves the examination of cellular specimens aspirated from the allograft using a small-gauge needle. The procedure is safe and can be done repeatedly even by individuals with relatively little experience[11, 12]. However, FNAB cannot determine rejection severity or the presence of vascular rejection, both important in estimating prognosis and selecting therapy[12, 13]. Duplex Doppler ultrasound can estimate resistance to diastolic flow(resistive index). However, there are significant overlaps between the resistive indices of rejection, cyclosporine toxicity and acute tubular necrosis limiting their clinical usefulness[16]. Magnetic resonance imaging (MRI) cannot differentiate between acute rejection and other causes of graft dysfunction[17]. Phenotypic monitoring of peripheral blood cells cannot make the diagnosis of rejection with high specificity and sensitivity. CD4/CD8 ratio [18, 19], T-cell activation antigens, IL-2 receptor[20, 21], Ta1 antigens[19], and soluble IL-2 receptors[22] have been reported to be elevated in states of rejection, infection, and clinical quiescence. Urinary cytologic diagnosis of rejection[23-25], like FNAB, cannot grade severity or rejection type. Thus renal biopsy remains the gold standard for the diagnosis of acute allograft rejection and allograft dysfunction not caused by obvious obstructive, vascular or nephrotoxic factors.

As suggested above, no indirect test provides diagnostic and prognostic information. Matas et al.[2] reported that approximately 40% of biopsies lead to a decision not to treat for rejection either because another diagnosis was made or because the rejection changes were considered irreversible. Thus, one of the most important values of the allograft biopsy is that it can prevent unnecessary antirejection treatment. For example, the renal allograft biopsy can be very helpful in differentiating CsA nephrotoxicity[26] recurrent[27-29] or de novo renal disease[29] from rejection.

The utility of the renal allograft biopsy has been improved by the adoption of standardized criteria, such as the Banff classification[5]. In this scheme the criteria for rejection are defined, with classification of pathological changes in grades I-III for acute rejection and chronic transplant pathology. A numerical coding system has been developed for the scoring of histological changes in glomeruli, tubules, interstitium, and vessels, together with standards for specimen adequacy and staining techniques. The Banff classification provides an internationally accepted definition of histological terms and delineates histological criteria resulting in greater uniformity in the diagnosis of acute rejection. Nonetheless concerns have been expressed as to the possibility that the Banff criteria are too strict and may underdiagnose "clinical" rejection[30].

Based upon the results of the present study, it is recommended that the biopsy core be examined at the bedside using a dissecting microscope. If the core includes capsule and corticomedullary junction and the gauge of the needle is similar to that used here, we suggest that the biopsy specimen be regarded as adequate. The biopsy core which includes deep cortex and corticomedullary junction without superficial cortex is probably adequate given that our results suggest an error rate of less than 10%. Thus the risk benefit ratio for taking more tissue is questionable. If the biopsy tissue has only medullary tissue, we recommend taking another core since the error rate is nearly 20%. When the core contains only superficial cortex or no possibility of determining the depth of the cortical tissue, the decision regarding an additional core should be made depending on the difficulty of doing the biopsy and the patients risk factor. When blood pressure control is adequate, coagulation status and platelet counts are normal, and when the initial core was obtained with relative ease, then a second core should be obtained, especially if the renal dysfunction is quite subtle. Otherwise the initial biopsy core should be examined microscopically; if it does not support the diagnosis of rejection and does not provide an alternative explanation for the renal dysfunction, or if it is not a fully adequate specimen, the biopsy should be repeated.

한 글 요 약

목 적 : 이식신 생검은 이식신 기능 이상의 원인, 거부반응의 정도, 예후 등을 확인하는데 도움이 된다. 그러나 이식신의 조직학적 변화가 신피질에 고르게 분포하지 않는 경우를 흔히 보게 된다. 따라서 본 연구는 이러한 이식신 생검에서의 잠재적인 표본추출의 오류를 평가하기 위하여 시행 되었다.

방 법 : 569개의 이식신 생검 표본 중에서 Banff criteria에 준하는 급성 거부반응을 보이고 있으며, 신피막부터 수질까지의 전 층을 포함하고 있는 신생검 표본 26개를 조사하였다. Banff criteria를 변형하여 조직의 변화를 간질성 염증(0-3+), 부종(0-3+), 요세관간질염(0-3+)으로 구분하여 급성 거부반응의 등급을 표면 피질, 깊은 피질과 피질에 근접한 수질층 각각을 비교하여 조직학적 분석을 시행 하였다.

결 과 : 간질성 염증(P=0.019), 부종(P=0.023), 요세관 거부등급(P=0.019)에서 깊은 피질에서 표면 피질에 비해 급성 거부반응의 정도가 심하였다.

결 론 : 이식신의 급성 거부반응을 진단하기 위하여 신생검을 실시 할 경우 깊은 피질이 포함되지 않으면 급성 거부반응을 과소 평가 할 수 있으

므로 주의해야 할 것으로 사료된다.

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