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Osteopontin level correlates with acute cellular renal allograft rejection

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ABSTRACT

Background: Osteopontin (OPN) is a potent proinflammatory cytokine that is upregulated in cell-mediated immunity and various inflammatory states of the kidney. However, the relationship between OPN levels plasma/urine and acute renal allograft rejection is still unknown. Therefore, we assessed the relationship between OPN levels in plasma/urine and acute cellular rejection post-renal transplantation.

Materials and methods: Clinical data and biologic samples of renal transplant recipients were analyzed retrospectively. Patients with biopsy-proved acute cellular rejection (ACR) ($n = 22$), protocol biopsy-proved non-rejection (non-R) ($n = 16$), and living related donors as healthy control (HC) ($n = 10$) were involved in this study. OPN level in plasma and urine was detected using the human OPN enzyme-linked immunosorbent assay kit. Type and grade of ACR were diagnosed based on Banff' 03 classification criteria of renal allograft pathology. No prisoners or organs from prisoners were used in this study.

Results: Compared with non-R patients and HC, plasma and urine OPN levels in ACR patients were significantly increased ($P < 0.05$), whereas there was no significant difference between non-R patients and HC ($P > 0.05$). In ACR patients, plasma OPN level was positively correlated with Banff grading of acute rejection, and a cut-off value of 24.20 ng/mL was further demonstrated a good clinical value in receiver operation characteristic curve.

Conclusions: The data obtained suggested that assessment of OPN levels in plasma and urine, especially in plasma, should be useful in predicting and evaluating the severity of ACR in renal transplant recipients.

1. Introduction

Osteopontin (OPN), an arginine-glycine-aspartate domain-containing extracellular matrix protein, can be produced by various tissues and cells in many conditions. As known as early T lymphocyte activation-1, it plays multifunctional roles in immune response by influencing cell survival, migration, and differentiation, especially on T cells [1]. OPN is also identified as an amplifier of the Th-1 immune response and contributes to the development of Th-1 related disease [2,3]. In many inflammatory diseases, such as rheumatoid arthritis and multiple sclerosis, elevated OPN expressions were

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reported and subsequently demonstrated to be facilitative for these diseases [4–8]. In a recent study of graft-*versus*-host disease (GVHD), a major immune rejection response after hematopoietic stem cell transplantation, elevated serum OPN level was found positively co-related with the pathogenesis of the disease. Furthermore, it was demonstrated to be critical for the disease initiation and persistence [4]. All these studies suggest a strong tie between OPN and immune-related diseases, and OPN level, especially in body fluids, may be predictable for these diseases.

Acute renal allograft rejection is actually a kind of inflammatory response with significant accumulation and activation of monocytes, especially lymphocytes in the graft. Acute rejection can also be characterized as a Th1-directed immune response. In rats, OPN upregulation has been observed in several models of acute or chronic renal injury [9], with OPN displaying a peculiar expression pattern. One study suggested that inducible expression of OPN in the tubular epithelium may have a pathogenic role in acute renal allograft rejection by mediating interstitial monocyte infiltration and possibly tubular regeneration [10].

However, the direct relationship between OPN level and acute cellular rejection (ACR) of renal allografts is still not clear. Thus, we sought to characterize the predictive interaction between OPN and ACR by examining the level of OPN in body fluids (peripheral blood and urine) post-transplantation. In this study, we found that plasma OPN and urine OPN levels in patients with non-R were similar as in health control (HC) ($P > 0.05$), but significantly lower compared with ACR group ($P < 0.01$). The plasma OPN level was positively correlated with Banff grading of acute rejection ($P < 0.05$). Moreover, the receiver operating characteristic (ROC) curve demonstrated a plasma OPN cut-off value of 24.20 ng/mL, which was accepted as the discriminative level between patients with and without AR.

2. Materials and methods

2.1. Selection and description of participants

Twenty-two patients with biopsy-proved acute cellular rejection (ACR), 16 patients with stable renal function and protocol biopsy-proved non-rejection (non-R), and 10 living related donors as HC were involved in this study. Patients with fever of undetermined origin, pneumonia, and other causes of renal graft dysfunction (e.g., infection, pyelonephritis, CNI toxicity) were excluded from this study. Peripheral blood and first urine in the morning (urina sanguinis) were collected from these patients and living related donors. All these living related donor renal transplantations and this study were approved by the Ethics Committee of Zhongshan Hospital, Fudan University (Shanghai, China). Procedures in this study were in accordance with the Helsinki Declaration of 1975. No prisoners or organs from prisoners were used in this study. Informed consents were obtained from these patients and living related donors.

All recipients started receiving combined immunosuppression from the first day after transplantation. Immunosuppressive agents in our center included mycophenolate mofetil (MMF), prednisone (Pred), tacrolimus, or cyclosporine A.

2.2. Samples and reagents

All peripheral blood and urina sanguinis samples tested in this study were collected before renal allograft biopsies or unilateral nephrectomy, so as to exclude the influence of biopsy and nephrectomy on the OPN levels. Plasma samples were separated from peripheral blood and frozen at −80°C until further analysis. Urina sanguinis samples were also frozen at −80°C until analysis. Type and grade of ACR were diagnosed based on Banff 03 classification criteria of renal allograft pathology.

OPN was assayed in duplicate using a sandwich enzyme-linked immunosorbent assay (Quantikine kit for Human Osteopontin Immunoassay; R&D Systems Inc., Minneapolis, MN, USA). The sample preparation and procedure were performed according to the manufacturer's instructions.

2.3. Statistical analysis

Student *t*-test was used to test the difference between two groups. ANOVA was used for statistics among multi-groups, and post hoc comparisons were then performed by Scheffe test. Spearman rank correlation coefficient and receiver operating characteristic (ROC) curve were also used to analyze results. Differences were considered statistically significant if the *P* value was less than 0.05.

3. Results

3.1. Characteristics of the transplant recipients

Table 1 shows the clinical profile of transplant recipients with ACR *versus* non-R group. There was no significant difference between the two groups with regards to age and the time of biopsy ($P > 0.05$). There was a significant difference of serum

Table 1 – Baseline characteristics of transplant recipients.

	ACR ($n = 22$)	Non-R ($n = 16$)	*P* value
Age (y)	44.00 ± 14.36	45.06 ± 17.35	>0.05
Sex			
Male	13	13	
Female	9	3	
Biopsy time (mo)	36.64 ± 8.32	32.13 ± 6.25	>0.05
Scr at biopsy (μmol/L)	202.59 ± 58.36	119.23 ± 43.04	<0.05
Banff category			
IA	8	0	
IB	3	0	
IIA	3	0	
IIB	8	0	
Immunosuppression			
MMF + CsA + Pred	8	6	
MMF + Tac + Pred	14	10	

Tac = tacrolimus; CsA = cyclosporine A.