Association of soluble human leukocyte antigen-G with acute tubular necrosis in kidney transplant recipients

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Summary

Background: **Human leukocyte antigen (HLA)-G is a nonclassical HLA class I molecule that displays strong immune-inhibitory properties and has been associated with allograft acceptance. However, there are conflicting data on the correlation of soluble HLA-G (sHLA-G) and acute rejection and no data on the correlation with acute tubular necrosis in kidney transplantation.**

Objective: **To evaluate the association of sHLA-G level in early post-transplant period and allograft rejection/ and acute tubular necrosis (ATN) in kidney transplant recipients.**

Methods: **The sera procured before transplantation and serially on day 3 and day 7 after transplantation from 76 kidney transplant recipients were analyzed for the level of sHLA-G by enzyme-linked immunosorbent assay.**

Results: **The levels of sHLA-G from three serial sera did not differ between patients with acute rejection and patients without rejection. However, the sHLA-G levels on day 3 post-transplant and day 7 post-transplant in patients with ATN were significantly higher than that in patients without ATN (16.3 vs 9.85 U/ml, p = 0.018, for day 3 post-transplant and 12.47 vs 5.42 U/ml, p = 0.044, for day 7 post-transplant). In addition, the ROC analysis of sHLA-G for identifying patients with ATN showed that the area under curve was 0.67 (95% confidence interval 0.54-0.80).**

Conclusion: **There was no significant difference for sHLA-G levels between patients with acute rejection and without rejection. Interestingly, high levels of sHLA-G in day 3 and day 7 after transplantation were associated with acute tubular necrosis. Our findings raise the question whether the increased levels of sHLA-G in patients with acute tubular necrosis after transplantation might be a result of ischemia and reperfusion injury.** ***(Asian Pac J Allergy Immunol 2015;33:117-22)***

Keywords: *acute kidney injury, acute tubular necrosis, graft rejection, kidney transplantation, soluble HLA-G*

Introduction

Human leukocyte antigen-G (HLA-G) is a nonclassical HLA class I antigen which is far less polymorphic than the classical HLA antigens. An immune tolerance role of HLA-G was discovered from the study of its expression on trophoblast cells, where it contributes to maternal-fetal tolerance.[1-2] There are seven isoforms of HLA-G, including membrane-bound isoforms (HLA-G1, -G2, -G3, and HLA-G4) and soluble isoforms (HLA-G5, -G6, and HLA-G7).[3] Both membrane-bound and soluble isoforms possess similar immunosuppressive properties by binding to inhibitory receptors such as immunoglobulin-like transcript 2 (ILT2), ILT4, and KIR2DL4.[4] Through these receptors, HLA-G can modulate the activity of natural killer (NK) cells and dendritic cells, inhibit cytotoxic T cell-mediated cytolysis and activate regulatory T cells.[5]

The expression of HLA-G is restricted in normal conditions but it is upregulated in response to transplantation, malignancies and inflammation. Clinical studies have demonstrated that high levels of soluble HLA-G (sHLA-G) were associated with better graft acceptance after heart, liver, and combined kidney-liver transplantation.[6-8] However, data regarding the correlation of sHLA-G levels and allograft rejection in kidney transplantation alone remains controversial. Previous studies in kidney

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transplant recipients demonstrated that patients without rejection had significantly higher sHLA-G levels than patients with rejection.[9-10] On the other hand, a study in pediatric kidney transplant recipients found no correlation between sHLA-G levels and rejection episodes.[11]

The role of HLA-G in several inflammatory pathologies has been extensively investigated.[12-14] Nevertheless, the association of sHLA-G levels and acute tubular necrosis (ATN) after kidney transplantation has not been reported yet. Therefore the aims of this study were to investigate the association of sHLA-G level with allograft rejection and with acute tubular necrosis in kidney transplant recipients.

Methods

Patient Population

All 76 consecutive kidney transplant recipients at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand between January 2010 and December 2010 were recruited. The medical records and the pathological reports of allograft biopsies of the study patients were reviewed to obtain their baseline characteristic and clinical outcomes. This study was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

The initial immunosuppressive treatment was categorized into two groups: standard immune-suppression and other protocols. Standard immune-suppression consisted of a calcineurin inhibitor (tacrolimus or cyclosporin), and antiproliferative (mycophenolate mofetil or azathioprine) and prednisolone. Other protocols included the use of combined immunosuppressive drugs other than the standard immunosuppression.

Soluble HLA-G enzyme-linked immunosorbent assay

After informed consent, clotted blood samples of the patients were obtained before transplantation and serially on days 3 and 7 after transplantation. A total of 228 blood samples were collected. After collection, the samples were subjected to centrifugation at 1200 g for 10 mins. The sera were collected and stored at -30^{o}C until used. Soluble HLA-G concentration (units (U)/ml) was determined by enzyme-linked immunosorbent assay (ELISA) using the sHLA-G kit (BioVendor, Modrice, Czech Republic) which measures sHLA-G1 and HLA-G5. The procedures were performed according to the manufacturer's instruction and the detection limit was 3 U/ml.

Definition of clinical outcomes

Biopsy-proven acute rejection was defined as a rejection episode which occurred within 6 months after kidney transplantation. Biopsy specimens were evaluated by light microscope and scored according to the Banff schema. C4d staining was performed using the immunoperoxidase technique in all biopsies. The diagnosis of acute rejection was based upon the Banff 09 classification.[15]

Statistical analysis

Continuous variables were described as means (standard deviations, SD) and medians (ranges) for data with normal distribution and non-normal distribution respectively. Categorical variables were described as proportions. Quartile regression and Chi-square test were used to compare the differences between groups for continuous and categorical data respectively. Receiver operating characteristic (ROC) curve analysis was performed in order to determine the optimal cutoff and the diagnostic performances of the sHLA-G level. All analyses were performed using Stata statistical software, version 11.0 (Stata Corp., Collage station, Tx). P values < 0.05 were considered statistically significant.

Results

Patient characteristics

A total of 76 kidney transplant recipients transplanted between January and December 2010 at our center were included in the study. Among the 76 patients, 70 patients (91.5%) had no acute rejection episodes and 6 patients (7.9%) experienced biopsy-proven acute rejection (AR) within 6 months after transplantation. The demographic profiles of kidney transplanted recipients with and without rejection have been detailed in Table 1. There were no statistical differences between these two groups with regard to age at transplantation, gender, donor type, panel reactive antibody levels, HLA mismatch, induction therapy, or immunosuppressive treatment.

Soluble HLA-G levels in patients with and without acute rejection

Before transplantation, the median of sHLA-G levels were not significantly different among patients with and without rejection (12.44 [10.41, 26.3] vs. 18.33 [9.57, 26.13] U/ml, $p = 0.758$) (Figure 1). The median of sHLA-G levels at day 3 and day 7 post-transplantation were also not

Table 1. Patient characteristics

Variable	No rejection (n = 70)	Acute rejection (n = 6)	*p*-value
Age at transplant (y, median (IQR))	46 (33, 54)	54.5 (39, 61)	0.083
Gender			0.999
Male	45 (64.29%)	4 (66.67%)	
Female	25 (35.71%)	2 (33.33%)	
Deceased donors	30 (42.86%)	2 (33.33%)	0.999
Time on dialysis (y, median (IQR))	2 (1, 6)	2.38 (0.5, 5)	0.741
First transplant	68 (97.1%)	5 (83.33%)	0.221
Cr before transplant (mg/dl, median (IQR))	7.2 (5.8, 9.9)	6.75 (4.3, 7.9)	0.397
PRA			0.118
< 10%	64 (91.43%)	4 (66.67%)	
≥ 10%	6 (8.57%)	2 (33.33%)	
HLA mismatch, median (IQR)	3 (2, 3)	4 (2, 5)	0.107
Induction therapy			0.999
Yes	25 (35.71%)	2 (33.33%)	
No	45 (64.29%)	4 (66.67%)	
Immunosuppression			0.068
Standard regimens	66 (94.28%)	4 (66.67%)	
Other	4 (5.72%)	2 (33.33%)	
Cold ischemic time (min, median (IQR))	53 (25, 1189)	36 (23, 59)	0.488

Cr, creatinine, IQR, interquartile range; PRA, panel reactive antibodies

significantly different between the two groups (12.41 [11.61, 20.72] vs. 10.57 [3.62, 19.96] U/ml, p = 0.355, for day 3 post-transplant and 7.04 [4.99, 10.59] vs. 8.45 [0.5, 17.22] U/ml, p = 0.969, for day 7 post-transplant).

Association of sHLA-G levels with acute tubular necrosis (ATN)

In order to investigate the association between the sHLA-G levels and ATN, 70 patients without acute rejection episodes were stratified into two groups: 44 patients with stable graft function and 26 patients who experienced ATN within the first month post-transplantation. Among the twenty-six patients with ATN, 25 patients were biopsy-proven and one patient was clinically diagnosed after excluding acute rejection, vascular and urinary tract complications as causes of delayed graft function.

There was no difference in baseline sHLA-G levels between the stable graft function group and the ATN group (17.00 [5.33, 23.70] vs. 20.83 [15.39, 27.58], p = 0.118) (Table 2). However, at day 3 and day 7 post-transplant, the median of sHLA-G levels in the stable function group was significantly lower than that in the ATN group (9.85 [2.08, 16.09] vs. 16.3 [6.32, 24.01], p = 0.018, for

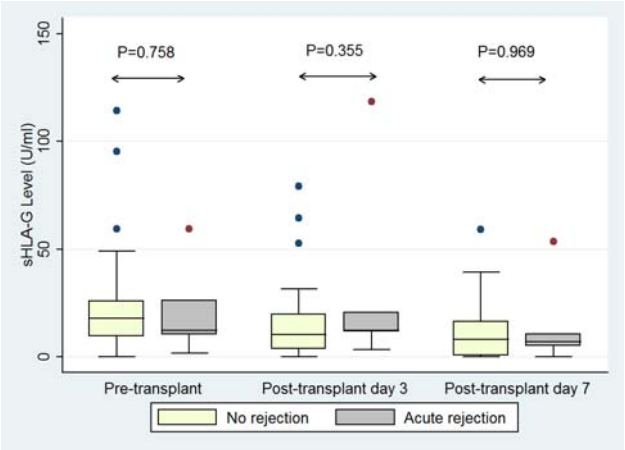

Figure 1. Comparison of soluble HLA-G (sHLA-G) levels between patients without rejection (n = 70) and patients with rejection (n= 6) before transplantation, on day 3 and day 7 after transplantation. The solid line in each box represents the median.

Table 2. Comparison of soluble HLA-G (sHLA-G) levels at three time points between patients with stable graft function and patients with acute tubular necrosis

sHLA-G levels (U/ml) at	Stable graft function (n = 44)	ATN (n= 26)	*p*-value
Pre-tx, median (IQR)	17.00 (5.33, 23.7)	20.83 (15.39, 27.58)	0.118
Post-tx day 3, median (IQR)	9.85 (2.08, 16.09)	16.3 (6.32, 24.01)	0.018
Post-tx day 7, median (IQR)	5.42 (0.12, 11.59)	12.47 (4.33, 21.0)	0.044

Tx, transplantation; ATN, acute tubular necrosis

day 3 post-transplant and 5.42 [0.12, 11.59] vs. 12.47 [4.33, 21.0], p = 0.044, for day 7 post-transplant).

ROC analysis of sHLA-G level for acute tubular necrosis

To analyze the diagnostic performance of the sHLA-G level at post-transplant day 7 for acute tubular necrosis, the receiver operating characteristic (ROC) curve analysis was performed. The analysis showed that the area under the curve (AUC) was 0.67 (95% confidence interval 0.54 - 0.80) (Figure 2). The optimal cut-off value of 11.19 U/ml resulted in 69.23% sensitivity and 65.91% specificity.

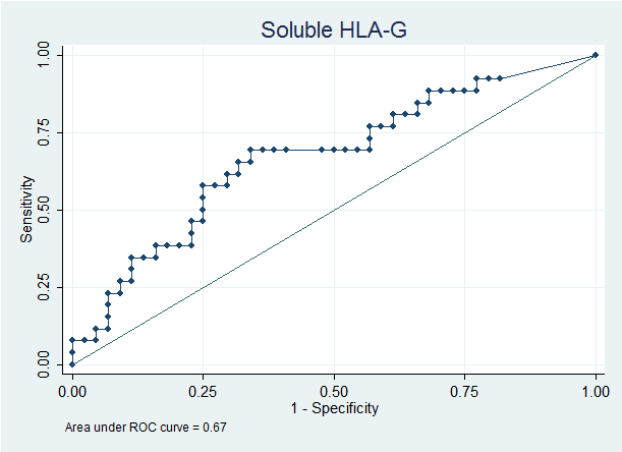

Figure 2. Receiver operating characteristic (ROC) curve analysis of sHLA-G levels (U/ml) for distinguishing patients with acute tubular necrosis. Area under the curve (AUC) was 0.67 (95% CI 0.54-0.80).

Discussion

An immunoregulatory role of soluble HLA-G and intragraft HLA-G expression in allograft acceptance had been previously demonstrated[9,16] suggesting that sHLA-G might be useful as a biomarker for predicting the allograft acceptance. In the current study, we did not detect significant differences in sHLA-G levels in patients with and without acute rejection. Our result was in accordance with the observations by Zarkhin V et al who demonstrated no differences of sHLA-G levels between pediatric kidney transplant recipients with and without acute rejection episodes.[11] In contrast, other investigators have shown that kidney transplant recipients with biopsy-proven rejection had lower sHLA-G levels in sera compared with recipients without rejection.[9-10, 17]

The discrepancy in these results is possibly explained by the variation in collection times of blood samples. A study by Zheng J et al.[10] obtained blood samples on the day when a biopsy was performed and the other study collected the sera several weeks after transplantation. On the other hand, the blood samples of recipients in our study were collected within the first week after transplantation.[17] Since several patients in our study developed acute rejection episodes weeks after transplantation, the sHLA-G levels measured in the first week of these patients might not reflect the conditions that developed later. Another explanation for the lack of association between sHLA-G levels and acute rejection demonstrated in our study was the very small sample size in the acute rejection group. The small sample size could lead to insufficient power to determine small differences of sHLA-G levels between the patients with acute rejection and without rejection.

The main finding in this study was that high levels of sHLA-G after kidney transplantation were associated with acute tubular necrosis. To our knowledge, this is the first report to suggest an association of sHLA-G with acute tubular necrosis after transplantation. The study by Racca et al. comparing expression levels of HLA-G1 in the biopsies between patients with acute rejection and with ATN had shown that the patients with acute rejection had higher HLA-G1 levels than those with

ATN.[16] However, a comparison of expression levels of HLA-G1 between the patients with ATN and with stable graft function has not been reported. In our study, the sHLA-G levels before transplantation in the patients with stable graft function was similar to that in the patients with ATN. The sHLA-G levels then dropped after transplantation in both groups, very likely in response to immunosuppressive treatment. However, despite immunosuppressive therapy, the sHLA-G levels remained higher in patients with ATN than in patients with stable graft function.

Acute kidney injury (AKI) of allograft is a result of ischemia and reperfusion injury in the recipient after transplantation. Ischemia and reperfusion injury triggers kidney dysfunction mainly by inducing oxidative stress, augmenting intrarenal hypoxia and activating inflammation.[18] Inflammation-induced leukocyte-endothelium interactions lead to a distortion of the homeostatic balance between oxygen, nitric oxide (NO) and reactive oxygen species (ROS). *In vitro* study demonstrated that increased oxidative stress results in upregulated expression of endothelial nitric oxide synthase (eNOS), leading to increased nitric oxide production.[19]

It has been shown that nitric oxide enhanced proteolytic shedding of HLA-G and did not affect the HLA-G immune suppressive properties.[20] This suggested that nitric oxide could participate in the availability of soluble HLA-G molecules in increased oxidative stress. Nitric oxide-promoted HLA-G shedding is a mechanism to extend immuneregulation to the surrounding tissue under stressful conditions. This mechanism may provide an explanation for our finding of the association between increased sHLA-G levels and acute tubular necrosis after transplantation.

Although this study demonstrated the correlation of sHLA-G levels and acute tubular necrosis in kidney transplanted patients, the area under the ROC curve in patients with acute tubular necrosis was 0.67 suggesting that sHLA-G levels was not an excellent diagnostic tool for acute tubular necrosis. Further studies with larger sample size and more various time points of the monitoring are needed to establish firm conclusion.

In summary, this study demonstrated for the first time that high levels of serum HLA-G in patients after transplantation were associated with acute tubular necrosis. Taking into account the promoted HLA-G shedding by nitric oxide in the ischemia and reperfusion injury, our findings raise the question of whether the elevated sHLA-G levels post-transplant in the patients with ATN is a consequence of acute kidney graft injury. More studies are needed to further explore the role of sHLA-G in the pathogenesis of ischemia and reperfusion injury in kidney grafts.

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