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Risk factors of sensitization to human leukocyte antigen in end-stage renal disease patients

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ABSTRACT

Background: Pre-sensitization to human leukocyte antigen (HLA) is closely related to the prognosis of renal transplantation. Concerning the risk factors for HLA sensitization, most studies focused only on selected transplant candidates.
Methods: All patients with end-stage renal disease (ESRD) in a single teaching hospital and a group of healthy subjects were enrolled for the tests of panel-reactive antibodies (PRA).
Results: A total of 1177 subjects were recruited, including 289 ESRD patients (140 hemodialysis, 98 peritoneal dialysis, and 51 pre-dialysis) and 888 healthy volunteers. The prevalence of PRA positivity (for either type I or II HLA) for ESRD patients was higher than for healthy subjects (23.2% vs. 12.8%, $p = 0.000$). Only pregnancy and transfusion showed independent correlations with PRA positivity, and not ESRD itself. The PRA-positive ESRD patients were prone to be female, have histories of pregnancy, transfusion, no hepatitis B, and use of graft shunt for dialysis. Multivariate analyses showed that pregnancy and time interval of the latest transfusion had independent correlations with PRA positivity. The time interval of less than 1 year had the highest odds ratio 10.06 ($p = 0.000$).
Conclusions: Pregnancy and recent transfusion, not ESRD itself or dialysis modality, remain the independent risk factors for HLA sensitization.

1. Introduction

The process of individuals having developed antibodies against human leukocyte antigen (HLA) is called "sensitization". Sensitization to HLA has been reported to be independent risk factors for delayed graft function, early graft failure, reduced time to graft failure, and chronic rejection of renal transplant recipients [1]. High levels of anti-HLA antibodies presenting at the time of transplantation frequently result in active humoral rejection and early graft loss [2]. Even in the post-transplant stage, the presence of anti-HLA antibodies confers a risk for graft loss before a noticeable decline in renal function [3]. Testing for panel-reactive antibodies (PRA) is commonly accepted as a routine method for detecting sensitization to HLA in transplant candidates. PRA levels have been found to have predictive value for kidney graft survival and the occurrence of rejection episodes [4]. Increasing PRA levels are also associated with increasing risk of graft failure; even many transplantations have been avoided using crossmatch tests to detecting donor-specific HLA antibodies in the transplant candidates [5,6]. Furthermore, the probability of receiving a deceased donor kidney transplant has been demonstrated to be inversely related to the level of PRA, a higher risk for not receiving a kidney transplantation becomes evident with a PRA > 20% [7].

Abbreviations: ESRD, end-stage renal disease; PRA, panel-reactive antibodies; rHuEPO, recombinant human erythropoietin; HD, hemodialysis; PD, peritoneal dialysis; CKD, chronic kidney disease.

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Anti-HLA antibodies normally are not present in the general population. Earlier reports have demonstrated that the factors affecting PRA levels include previous transplantation history, number of pregnancies, female gender, and number of blood transfusions [8–11]. The developments of anti-HLA antibodies are more likely after exposure to alloantigen in females with pregnancy and in allograft recipients. Contaminated leukocytes in red blood cells are considered responsible for transfusion-related HLA sensitization [12]. After the introduction of recombinant human erythropoietin (rHuEPO) in 1989, a significant decrease in the requirements for blood transfusion among patients awaiting transplantation has been demonstrated. It has also been associated with a significant reduction in transfusion-related HLA sensitization and the waiting time for transplantation in kidney transplant candidates [13]. In this rHuEPO era, however, the 1998 study of Sezer et al. still showed that 65 (33.7%) out of their 193 hemodialysis (HD) patients had a PRA level >30% [14]. Another study conducted by Pour-Reza-Gholi et al. in 2005 also found 22 (21.4%) in 98 HD patients demonstrate positive results of PRA test [15].

The clinical risk factors for HLA sensitization in this time marked by widespread use of rHuEPO are still controversial. Sezer et al. found statistical correlation between PRA positivity with either HD duration or previous transplantation history, but not for age, gender, blood group, or number of blood transfusions [14]. In contrast, Pour-Reza-Gholi et al. showed that only age and history of kidney transplantation have close correlations with high PRA levels [15]. Although Heise et al. have shown that HLA phenotypes are the risk factors for kidney transplant recipients, some HLA class II-linked genes have been observed to modulate the PRA response in a significant manner [11]. However, this is an inheritable factor that cannot be modified by clinical efforts.

Dialysis modality has been shown to affect renal graft function. As compared with peritoneal dialysis (PD), HD shows a stronger association with delayed graft function [16]. Fitzgerald et al. also demonstrated that decreased allograft survival was most pronounced in patients who were on HD before transplantation [17]. Moreover, Pour-Reza-Gholi et al. observed that PRA levels after a dialysis session were significantly higher than those before dialysis [15]. HD therapy itself might activate some immune reaction and also the PRA response, and then contribute to worsening graft survival. Nonetheless, the relationship between dialysis modality and the PRA response has yet to be investigated.

Concerning the studies on the risk factors of HLA sensitization, most investigated PRA response in transplant recipients or the patients on the waiting list for transplantation [8–11]. However, since these subjects have been carefully selected as good candidates for transplantation, they obviously had a favorable clinical condition and cannot serve to represent the population of end-stage renal disease (ESRD) patients. For this reason, it is doubtable that the risk factors found in these studies are applicable to all ESRD patients. Few studies have actually focused on a cohort of regular, non-selected HD patients [14,15], and no studies concerning PD and pre-dialysis ESRD patients have been conducted. In this present study, we thus try to determine the risk factors of HLA sensitization in a cohort of ESRD patients including HD, PD and pre-dialysis patients. A group of volunteer blood donors, regarded as healthy subjects, was also taken for the comparison with ESRD patients.

2. Materials and methods

This is a single center, cross-sectional study. A cohort of ESRD patients including HD, PD and pre-dialysis patients at E-DA hospital, in the south of Taiwan, were enrolled to this study. This study was approved by the Institutional Review Board on Human Research of the E-DA hospital (EMRP-098–079).

The inclusion criteria included adult (>20-year-old) patients on regular HD thrice weekly for more than 3 months, on regular PD therapy for more than 3 months, or never receiving any dialysis therapy but having chronic kidney disease (CKD) with MDRD-estimated glomerular filtration rate <10 ml/min for more than 3 months. The exclusion criteria were patients ever receiving solid organ or bone marrow transplantations, having fever or clinical evidences of infectious disease within 1 month before entry, or receiving agents that have been suggested to affect PRA response within 1 month before entry, including 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (lovastatin, simvastatin, pravastatin, atovastatin and fluvastatin), hydralazine, procaine, and alfa-methyldopa [18,19].

All patients gave informed consents and received detailed history reviews by the staffs in charge, including underlying etiology of ESRD, comorbidites including diabetes, hypertension, hepatitis B (positive hepatitis B surface antigen) and hepatitis C (positive anti-hepatitis C antibody), duration of dialysis, blood group, number of pregnancies, episodes and amount of transfusions received (including packed red blood cell and whole blood). The transfusion information was obtained from the patient's recall, the records of dialysis unit and blood bank of the hospital. Fasting blood samples for all patients (mid-week predialysis for HD patients) were obtained for the PRA test. The time intervals between study entry and the latest pregnancy or blood transfusion were calculated and recorded. A decoding group of 888 volunteer blood donors taken from the Kaohsiung Blood Center was also enrolled for PRA testing. However, only limited data were available for this group including gender, number of pregnancies, and history of transfusions.

For screening PRA, serum samples were analyzed for HLA antibodies by Quikscreen (GTI Diagnostics, Waukesha, WI), a solid phase enzyme-linked immunoabsorbent assays (ELISA) utilizing pooled HLA class I or class II antigens with the cut-offs for borderline or positive results set according to the manufacturer's instructions [20]. Samples with borderline results were reanalyzed by FlowPRA™ Screening Test (FL12–60; One Lambda, Canoga Park, CA). The results were regarded as positive when $\geqslant$20% of class I or class II beads exhibited fluorescence above the negative control serum.

For statistical analysis, the parameters between patients with negative and positive PRA were tested for significance by Student's t test, Mann–Whitney U test and Chi-square test. Associations between two parametric variables were evaluated with the Pearson correlation test. Binary logistic regression tests with univariate and multivariate analyses were performed to determine the relationships to positivity of PRA for the single significant parameter and the multiple significant parameters, respectively. General data are described with a mean ± SD. A p value of less than 0.05 is considered statistically significant. The software SPSS 16.0 for Windows was used for statistical analysis.

3. Results

Among the approximately 400 ESRD patients at our hospital, a total of 289 patients (137 females and 152 males) were finally recruited after giving informed consent, including 140 chronic HD patients, 98 chronic PD patients, and 51 pre-dialysis patients. Their mean age was 58.6 ± 12.3 years, and the average dialysis duration of HD and PD patients was 3.0 ± 2.6 years. A total of 67 (23.2%) patients showed positive PRA test (PRA level $\geqslant$ 20%) either for type I or type II HLA. Among them, 57 (57/289 = 19.7%) showed positive PRA for type I HLA, 37 (37/289 = 12.8%) showed positive PRA for type II HLA; and 27 (27/289 = 9.3%) showed positive PRA for both type I and type II HLA. The presence of positive PRA for type I HLA was highly correlated to the presence for type II HLA (p = 0.000).

The prevalence of positive PRA test (either for type I or type II HLA) in the 888 healthy subjects (200 males and 688 females) was