Effect of plasmapheresis on panel reactive antibodies in kidney transplant candidates

Sahban Al Mallah¹, Kais Hasan Abd Altaee², Shawqi Watheq Mohammed Ali Altareehee³*

Abstract

Panel reactive antibody (PRA) is one of the tests used to assess the degree of sensitization before kidney transplant, and plasmapheresis is one of the measures used for desensitization. In this study we assessed the role of plasmapheresis as a desensitizing measure. This study was conducted in the medical city complex in Baghdad, Iraq; for the whole year 2012, enrolling patients attending the lab. of the nephrology and kidney transplant centre recording their PRA readings before and after plasmapheresis (for those with PRA >=20%); in addition to date, age, sex, blood group and whether or not they had been previously transplanted. Data were analyzed using chi square and pair T-test. A 179 patients enrolled, 35 (19.6%) of them had positive PRA test, 12 reading were 20% or more; and were rechecked after plasmapheresis with no significant difference. A part from history of previous transplant; none of the other factors showed significant association with PRA levels. In conclusion, no significant effect of plasmapheresis on the levels of PRA.

Keywords: Desensitization; HLA antibodies; Kidney transplant; Panel reactive antibodies; Plasmapheresis B

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Introduction

Owing to the limited supply of organs available for kidney transplantation, the number of patients waiting for a compatible donor has gradually increased worldwide, especially for human leucocyte antigen (HLA)-sensitized patients with an elevated panel-reactive antibody (PRA) level [1, 2, 3, 4]. It is widely accepted that pre-transplant positive PRAs are related to increased incidence of hyperacute/acute rejection, chronic rejection, and early/latent graft loss [5]. There is no universal consensus among nephrologists on the usefulness of PRA in pre-transplant work-up and many centers do not perform PRA for renal transplantations. This is largely due to lack of agreement about its usefulness and also partly on account of the
economic constraints [6]. Our immune response has evolved to counteract foreign antigens, such as viruses and bacteria. Both cellular and humoral antibody responses can be triggered as a result of infection. However, the immune system may undesirably react to non-infectious agents such as food, pollen (allergies) allograft (transplant rejection) and respond to hidden or unhidde

t or self-antigens (auto-immunity) [9]. When exposed to tissues of another person, the immune system mounts a reaction, mainly to the polymorphic (variables or different) antigens of that individual. This is called priming of the immune system to the antigen(s). For example transfusion of platelets from an individual with a different class I antigens (constitutively expressed on platelets) will result in priming both arms of the immune response (cellular and humoral) against class I antigens. Organ transplanttation for such an individual may result in hyperacute rejection [9].

Priming of the immune response to HLA class I and / or II can occur as a result of pregnancy, blood transfusions or rejection of a previous transplant [7]. However, there are individuals who have HLA antibodies with no history of exposure to the above factors; this can be explained by the presence of cross-reacting antibodies, usually of IgM class [9]. Leukodepleted blood has been used to reduce the risk of all immunization.

However, a randomized trial [8], found that filtration of leukocyte still results in post transfusion alloimmunization, merely because filtration cannot deplete all leucocytes. Anti-HLA antibodies are usually referred to as panel reactive antibody (PRA) test that sometimes is referred to as percent reactive antibody, since the result is expressed as a percentage. Anti-HLA antibodies vary with time in one individual patient and may be influenced by many factors. Therefore, it is recommended to screen patients for PRA in specified intervals. This should not be more than 3 months. In case of blood transfusion, a sample should be sent to the laboratory between 14 to 28 days post transfusion [9]. An allograft is the most important cause for development of antibodies, but transfusions, infections, and pregnancy can also stimulate antibody formation; the degree of sensitization is stronger and more prolonged when different causes act together in the same person [10]. Women with ESRD are disproportionately sensitized compared with men; approximately 60%–80% of highly sensitized patients are women [11]. Currently, 17% of patients on the waiting list have PRA titers between 10% and 79%, with 8% having PRA titers >80%. Workers
have differed in the cut-off levels of PRA for labeling a patient as sensitized (6). In 2000, only 2.8% of all kidney transplants were performed in sensitized patients, even though this population represents approximately 20% of the waiting list.1 Thus, the highly sensitized patient is destined to wait extended periods of time on dialysis, a factor known to increase morbidity and mortality [11]. In addition, transplant outcomes in highly sensitized patients are inferior to those in non-sensitized patients [12].

**How is PRA detected?**

1. **Complement dependent cytotoxicity (CDC)**

Complement dependent cytotoxicity (CDC) methods have formed the basis of anti-HLA antibody detection, since it was first introduced by Patel and Terasaki in 1969 [13]. The widespread use of CDC cross-match has resulted in the almost complete elimination of hyperacute rejection in the modern era. In this test, the serum of the recipient is incubated with lymphocytes from the donor, and a complement is added to determine if the recipient has antibodies that bind to donor cells, activate the complement and the membrane attack complex, and result in cell death. The sensitivity of the cytotoxic assay can be enhanced with the use of an antihuman globulin (AHG) antibody as a secondary reagent (AHG-enhanced cross-match). A positive cytotoxic T-cell immunoglobulin G (IgG) cross-match is an absolute contraindication to transplantation [14].

**Complement dependent cytotoxicity (CDC)** has many drawbacks, (i) it is limited by the cell panel used, (ii) it depends on the quality of lymphocytes and rabbit complement, (iii) it detects non-HLA antigens and (iv), it only detects complement fixing antibodies. Therefore, patients cannot be tagged as sensitized on the basis of this test. Results are expressed as percentage of cells reacted with the tested serum [9].

2. **ELISA**

The enzyme-linked immunosorbent assay (ELISA) employs the use of HLA antigens, this removes the need for complement, live cells and excludes the non-HLA specific antibodies [15, 16]. There are two types of commercially available kits, one detects presence or absence of PRA, the other detects antibody specificity. PRA detected by ELISA is more sensitive than the CDC assay [17,18].

3. **Flow cytometry**

This assay depends on the availability of a flow cytometer in the laboratory. Two different methods can be employed (i) an in house method in which whole lymphocytes
are used as the antigens [19-23] and (ii) commercially available kits which employ beads coated with specific HLA-antigens [24] HLA-A, B, Cw, DR, DQ, and DP coated beads have been used and shown to be much more sensitive than the CDC method [17].

Advances in this methodology result in the introduction of single antigen coated beads. This method helps identifying the specificity of PRA in highly sensitized patients and improves the definition of acceptable mismatches [25].

Various strategies enable desensitization for renal transplant recipients who are highly sensitized. Therapeutic strategies for desensitization include various combinations of the following with different outcome [11, 26, 27].

- intravenous (IV) Ig
- removal of antibodies by plasmapheresis (PP) or immunoadsorption (IA)
- rituximab (anti-CD20)
- splenectomy
- Thoracic duct drainage

**Plasmapheresis:**

Plasmapheresis has been used in two different contexts in renal transplantation. It has been used to remove anti-HLA antibodies as a pretreatment for transplantation, and it has also been utilized to treat humoral or antibody-mediated rejection. The pretreatment model has combined antibody reduction by plasmapheresis with immunosuppression to prevent antibody resynthesis. A specific anti-B cell agent, cyclophosphamide, was used in combination with azathioprine and prednisolone. This work was done by Taube and his colleagues in London [28, 29]. This cross-sectional study is designed to investigate the effect of plasmapheresis and other confounding factors on the levels of PRA when preparing sensitized kidney transplant candidates in the centre of Nephrology and Kidney Transplant in The Medical City Complex in Baghdad; Iraq.

**Patients and methods**

The study was conducted in the hospital of surgical specialties / centre of nephrology and kidney transplant in The Medical City Complex in Baghdad; Iraq. This is a cross-sectional study by which we studied 179 patients (67 female and 112 male) attending the laboratory of the transplant centre for assessment of their PRA; from January 2012 to January 2013. The results were obtained using CDC technique, and were then dated and recorded in addition to their sex, age, blood group and whether they are going to receive the transplant for the first time (first transplant) or are previously transplanted (second transplant). The data were recorded by the laboratory team.
Those patients whose tests yielded a significant positive result of 20% or more were arranged to undergo plasmapheresis twice weekly up to a total of 12 sessions (different researches used variable cut-off for PRA levels to be taken as "significant" from 10% to 50% [6, 26, 28]; also they used variable number of plasmapheresis sessions [1, 11, 26]).

Plasmapheresis sessions were done using 2-types of machines: Spectra Optia Apheresis System and Haemonetics MCS+; both of which work by centrifugal method and use Ringer and normal saline as replacement fluids. After completion of their plasmapheresis sessions; they were sent to repeat their PRA testing to see their new sensitization levels. Some patient who remained positive after the plasmapheresis required another course sometimes more than once. The patients PRA levels were then compared before and after plasmapheresis to check for statistically significant effect.

Other comparisons were made for other recorded parameters (age, sex, blood group, distribution through the year-seasonality- and whether or not the patient has a second transplant). Statistical analysis was done by using SPSS version 20 in which we use chi square test for categorical data and paired T-test for measurement data before and after readings. We set P value <0.05 as significant.

Results

Of 179 patients enrolled in the study; 36 patients (19.6%) were shown to be sensitized (positive PRA) and those significantly positive (>= 20%) were sent to undergo plasmapheresis protocol; 12 such reading were obtained with its corresponding post-plasmapheresis reading (Data not shown). Analysis of these readings revealed no significant effect of plasmapheresis on levels of PRA. (See table-1)

<table>
<thead>
<tr>
<th>Table-1.</th>
<th>Mean PRA level before and after plasmapheresis (PP) for patients with (PRA&gt;=0.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Std. Error Mean</td>
</tr>
<tr>
<td>Before PP</td>
<td>0.5083</td>
</tr>
<tr>
<td>After PP</td>
<td>0.4500</td>
</tr>
</tbody>
</table>
Distribution of positive (sensitized) and negative (non-sensitized) patients across the year of the study are shown (see table-2) with the highest number and percent of positivity being in June.

**Table-2.**

Distribution of patients across the year of the study

<table>
<thead>
<tr>
<th>Months</th>
<th>Positive</th>
<th>Negative</th>
<th>Percent of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>3</td>
<td>16</td>
<td>8.6</td>
</tr>
<tr>
<td>February</td>
<td>3</td>
<td>23</td>
<td>8.6</td>
</tr>
<tr>
<td>March</td>
<td>3</td>
<td>12</td>
<td>8.6</td>
</tr>
<tr>
<td>April</td>
<td>1</td>
<td>12</td>
<td>2.8</td>
</tr>
<tr>
<td>May</td>
<td>1</td>
<td>24</td>
<td>2.8</td>
</tr>
<tr>
<td>June</td>
<td>8</td>
<td>9</td>
<td>22.8</td>
</tr>
<tr>
<td>July</td>
<td>4</td>
<td>18</td>
<td>11.4</td>
</tr>
<tr>
<td>August</td>
<td>3</td>
<td>7</td>
<td>8.6</td>
</tr>
<tr>
<td>September</td>
<td>6</td>
<td>13</td>
<td>17.2</td>
</tr>
<tr>
<td>October</td>
<td>2</td>
<td>5</td>
<td>5.8</td>
</tr>
<tr>
<td>November</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>1</td>
<td>4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Categorization of the study group according to their blood groups and relating them to the positivity of their PRA levels (see table-3) didn't show a statistically significant association.

**Table-3.**

Distribution of positive cases according to blood groups

<table>
<thead>
<tr>
<th>blood group</th>
<th>Positive</th>
<th>Negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6(17.1%)</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>9(25.7%)</td>
<td>33</td>
<td>0.142</td>
</tr>
<tr>
<td>AB</td>
<td>5(14.3%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>15(42.9%)</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>

The distribution of the study group between different age groups and comparing them to their PRA levels revealed no statistically significant association (see table-4).
Table-4.
Distribution of positive cases according to age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Positive</th>
<th>Negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-15Y</td>
<td>1 (2.8%)</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>16-45Y</td>
<td>25 (71.5%)</td>
<td>84</td>
<td>0.707</td>
</tr>
<tr>
<td>46-60Y</td>
<td>8 (22.9%)</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>&gt;60Y</td>
<td>1 (2.8%)</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

When we studied the effect of gender on PRA levels; no statistically significant association was shown (see table-5).

Table-5.
Distribution of positive cases between males and females

<table>
<thead>
<tr>
<th>Sex</th>
<th>Positive</th>
<th>Negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>19 (54.3%)</td>
<td>93</td>
<td>0.258</td>
</tr>
<tr>
<td>Female</td>
<td>16 (45.7%)</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>144</td>
<td></td>
</tr>
</tbody>
</table>

When we compared between those with history of previous kidney transplant (second transplant) and those without (first transplant), regarding the positivity of PRA (see table-6); a statistically very significant association was shown.

Table-6.
Distribution of positive cases between first and second transplant candidates.

<table>
<thead>
<tr>
<th>Transplant</th>
<th>Positive</th>
<th>Negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>22 (62.9%)</td>
<td>128</td>
<td>0.0001</td>
</tr>
<tr>
<td>Second</td>
<td>13 (37.1%)</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Many studies accomplished about the role of plasmapheresis in sensitized patients addressed the controversy or ineffectiveness of plasmapheresis in this respect when it is used alone [11, 26]. This study also revealed that the use of plasmapheresis alone has no statistically significant effect at reducing PRA levels (table-1). The next parameter evaluated was the distribution through the year which revealed variable results regarding percent of positivity of PRA levels across the year (table-2) with the highest percent being in June (8 positive cases out of 17 total patient in that month and 22.8% of total positive cases in the study interval). This variability may need to be further assessed in a separate study designed for this purpose. A statistically significant correlation didn't exist between the blood group of the patients and their PRA levels (table-3); although the ABO incompatibility does have an adverse effect on the outcome of the transplant [1, 9]. The study revealed that neither the age nor the sex of the patient has a statistically significant effect on the positivity of PRA level (table 4, 5) despite the high percentage found in the group of (16-45) year (22.9% of the age group and 71.4% of the total positive cases in the study) possibly because it includes the vast majority of patients enrolled in the study. The absence of effect of gender on PRA levels in the study group; despite being more common in females as shown in other studies [6, 11] may be explained by the fact that most uremic females have much less chance of being pregnant especially if they are poorly managed; moreover; they may have short time waiting the transplant, making them less liable to receive blood transfusion; reducing the chance of exposure to these two important sensitizing factors (pregnancy and blood transfusion). At last the study found a very significantly higher number of positive cases in those with previous transplant (44.8% out of patients with history of previous transplant and 59% out of total positive cases in the study) when compared to those candidates of kidney transplant for the first time (table-6). It is well known that exposure to organ transplant renders the person highly sensitized for the future [9, 11, 26].

Conclusion

Plasmapheresis has no significant effect as a desensitizing measure for sensitized candidates of kidney transplant. Only history of previous transplant; among other variables studied; showed statistically
significant association with positive PRA levels.

Recommendations

- An alternative approach for desensitization should be adopted (e.g. adding IVIG, use of rituximab, etc).
- To use donor specific antibody (DSA) for better detecting the sensitized patient.
- Assessing for possible effect of certain interval in the year on PRA positivity i.e. seasonality of PRA.

References

1. Gabriel M. Danovitch, Option for Patients with End-Stage Renal Disease; Handbook of Kidney Transplant; Fifth Edition, p 1; Gabriel M. Danovitch; A Lippincott Williams & Wilkins; 2010.


