Effect of Transplant on Platelet Function Markers (P-Selectin and Platelet Aggregation) and Adiponectin in Renal Transplant Patients

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Abstract

Objectives: In chronic kidney disease, both bleeding and thrombotic complications are observed, although with expected recovery after a successful transplant. Adiponectin has protective properties with respect to atherogenesis and inflammation. Plasma adiponectin levels are markedly elevated among patients with end-stage renal disease and are lower after kidney transplant. However, this topic is still debated in the literature. Here, we evaluated the effect of transplant on platelet function markers (P-selectin and platelet aggregation) and adiponectin in renal transplant patients.

Materials and Methods: Our study included 14 renal transplant patients. Preoperative and week 1, month 1, month 6, year 1, and year 2 samples after transplant were studied. In addition to plasma adiponectin, P-selectin levels, and platelet aggregation tests, biochemical tests and coagulation parameters were also studied.

Results: We observed a significant decrease in adiponectin levels 2 years after transplant. Platelet function tests with ADP and collagen were significantly improved, and no changes in P-selectin, ristocetin, and epinephrine levels were observed.

Conclusions: According to our findings, glomerular filtration rate has an important effect on platelet function, but adiponectin levels became normal only in the second year after transplant. Late improvement of low-density lipoprotein cholesterol and adiponectin after transplant suggested to us that patients with kidney transplant may still have risk of cardiovascular events, especially in the first years.

Key words: Adiponectin, Kidney transplant, Platelet aggregation, P-selectin

Introduction

In addition to traditional and nontraditional risk factors of cardiovascular morbidity, an antiatherogenic factor has been described: adiponectin, a secretory humoral protein mainly expressed in the adipose tissue.1 Adiponectin seems to inhibit the endothelial expression of adhesion molecules and to suppress the adhesion of the monocytes to the vascular endothelium, thereby countering the inflammatory response of the endothelium, which plays an important role in the development of uremic atherosclerosis.2-5

Adiponectin levels have been shown to be lower in male patients, those with obesity and insulin resistance, and those with type 2 diabetes mellitus, coronary artery disease, and essential hypertension.6,7 In contrast, adiponectin is elevated in end-stage kidney disease, with levels up to 3 times higher than in the normal population.8-10 Increased plasma adiponectin levels seem to rapidly normalize after kidney transplant.11-14 The rise in adiponectin with declining glomerular filtration rate (GFR) is hypothesized to be secondary to diminished renal elimination, although the exact role of the kidney in the biodegradation and excretion of adiponectin remains unclear. A close association between hypoadiponectinemia and endothelial dysfunction was reported.15
P-selectin, a member of the lectin family, is rapidly translocated from alpha-granules of platelets and Weibel-Palade bodies of endothelial cells to the cell surface on stimulation. P-selectin stabilizes initial platelet aggregates formed by glycoprotein GpIIb-IIIa-fibrinogen interactions, allowing the formation of large platelet aggregates. In a recently reported study, renal transplant was suggested to induce platelet activation without inducing platelet aggregation.

To our knowledge, there are no data in the literature regarding any relation between adiponectin, P-selectin levels, and platelet aggregation in kidney transplant patients. Therefore, in this study, we evaluated adiponectin levels associated with platelet function before and after transplant in patients with kidney transplant.

**Materials and Methods**

**Patients**

Our prospective study included 14 renal transplant patients. Preoperative, week 1, month 1, month 6, year 1, and year 2 samples were studied after transplant. In addition to plasma adiponectin, P-selectin levels, and platelet aggregation tests, biochemical tests and coagulation parameters were also studied.

To minimize confounding from comorbid conditions that could influence adiponectin and platelet aggregation, patients with known bleeding or other systemic disorders such as cardiovascular disease, hepatic failure and endocrine diseases, acute infections, autoimmune disorders, or cancer, a platelet count of less than 150 × 10^9/L or more than 450 × 10^9/L, and a hemoglobin level less than 10 g/dL were excluded. None of the patients received erythropoietin, IV iron, or active vitamin D, lipid lowering agents, or hormonal replacement therapy. The patients did not receive acetylsalicylic acid, ticlopidine, dipyridamole, or nonsteroidal anti-inflammatory drugs in the last 10 days before platelet aggregation studies, although the concomitant use of antihypertensive drugs was allowed. Glomerular filtration rate was assessed according to the Modification of Diet in Renal Disease formula.

All of the participants gave written informed consent, and the Ethical Committee of Eskisehir Osmangazi University Medical School (Eskisehir, Turkey) approved the study.

**Immunosuppressive therapy**

The immunosuppression regimen included tacrolimus and mycophenolate mofetil (2 × 1 g) or mycophenolic acids (2 × 720 mg) with maintenance doses of steroids. Tacrolimus concentrations were controlled according to standard protocols. Mycophenolate mofetil and mycophenolic acid doses were adjusted in cases of intolerance.

**Laboratory procedures and sample collection**

Samples for blood counts were drawn into Becton Dickinson anticoagulated tubes, and complete counts were made by Beckman Coulter Gen-S SM (Franklin Lakes, NJ, USA) automated blood counting device. Coagulation tests were performed on an ACL TOP Coagulation Analyzer (Instrumentation Laboratory, Bedford, MA, USA). Prothrombin time was measured by the HemosIL RecombiPlasTin kit (Instrumentation Laboratory), and activated partial thromboplastin time was measured by the HemosIL SynthASil kit (Instrumentation Laboratory). Fibrinogen was measured by the HemosIL Fibrinogen-C XL kit (Instrumentation Laboratory), and D-dimer was measured by the HemosIL D-Dimer kit (Instrumentation Laboratory). The normal ranges for these tests in our laboratory are 24 to 36 seconds for activated partial thromboplastin time, 8 to 13 seconds for prothrombin time, 200 to 400 mg/dL for fibrinogen, and 0 to 0.55 mg/L for D-dimer.

Blood samples for platelet aggregation studies were drawn into 4.5-mL vacutainers (Becton Dickinson) containing 3.8% trisodium citrate in a 9:1 blood anticoagulation ratio by direct vein puncture with 19-gauge needles. The specimens were kept at room temperature and processed within 1 hour of blood collection.

Platelet aggregation studies were performed in a whole blood lumi-aggregometer (model 560-Ca, Chrono-Log Corporation, Havertown, PA, USA) using optical methods according to manufacturer’s instructions. The whole blood specimen was centrifuged for 10 minutes at 200 g to obtain platelet-rich plasma. Platelet-poor plasma was obtained from the remaining specimen by recentrifugation at 200 g for 15 minutes. A platelet count was performed on the platelet-rich plasma and was adjusted to 300 × 10^3/μL with platelet-poor plasma. We transferred 450 μL of this platelet-rich plasma into cuvettes (No. P/N 312, Chrono-Log Corporation), each containing a disposable siliconized bar. After
agonist addition, platelet aggregation was measured over 6 minutes and expressed as a percentage of the maximal amplitude in platelet-rich plasma. The agonists used and their final concentrations were as follows: 5 μM ADP (using Chrono Par 384), 2 μg/mL collagen (using Chrono Par 385), 1.25 mg/mLristocetin (using Chrono Par 396), and 5 μM epinephrine (using Chrono Par 393).

Adiponectin and P-selectin measurements
A commercially available enzyme-linked immunosorbent assay method was used to determine adiponectin (Biovendor Human Adiponectin ELISA, Brno, Czech Republic), and P-selectin (BeBioscience Human P-Selectin Platinum ELISA, Vienna, Austria) in plasma and serum was prepared by centrifugation at 2500g at 4°C for 20 minutes and then stored in aliquots at −70°C until use. All analyses were performed in duplicate, and mean values were used for statistically calculations.

Statistical analyses
Statistical analyses were performed with SPSS software for Windows (SPSS: An IBM Company, version 21.0, IBM Corporation, Armonk, NY, USA). The distribution of variables was checked initially by the Shapiro-Wilks test. Parametric tests were applied to data having normal distribution, whereas nonparametric tests were applied to data having nonnormal distribution. Repeated-measures analysis of variance and Friedman 2-way analysis of variance (2-way analysis of variance on ranks) tests were applied to determine the differences between 6 different measures. In addition, post hoc tests were applied for checking the differences.

Results

Demographic data
Eight female and 6 male patients were included in this study. Mean age of the patients was 31.5 ± 9.5 years. Mean systolic and diastolic blood pressures before and after transplant were as follows: 130/85 mm Hg and 133/85 mm Hg. Causes of chronic renal failure were as follows: 3 patients had hypertension, 1 patient had glomerulonephritis, 1 patient had nephrolithiasis, and 9 patients had unknown causes. All of the patients received donations from living donors. The duration of dialysis before transplant was 29.28 months (range, 8-84 mo).

Body mass index evaluations were done according to the Quetelet index (weight in kg/height in m²). Mean body mass index value was 21.14 kg/m².

### Table 1. Laboratory Parameters of the Study Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Treatment</th>
<th>1st Week</th>
<th>1st Month</th>
<th>6th Month</th>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dL</td>
<td>11.05 ± 1.25b,c</td>
<td>10.1 ± 1.25b,c</td>
<td>11.7 ± 2.29</td>
<td>13.55 ± 1.79</td>
<td>13.91 ± 1.63</td>
<td>13.16 ± 1.31</td>
</tr>
<tr>
<td>Platelet, x10⁹/L</td>
<td>243.28 ± 69.8</td>
<td>316.6 ± 120.9d</td>
<td>296.4 ± 124.2</td>
<td>269.5 ± 117.52</td>
<td>271.3 ± 136.1</td>
<td>275.4 ± 93.2</td>
</tr>
<tr>
<td>Serum urea nitrogen, mg/dL</td>
<td>46.9 ± 18.55</td>
<td>19.07 ± 7.07</td>
<td>18.7 ± 4.75</td>
<td>19.4 ± 7.39</td>
<td>18.1 ± 7.47</td>
<td>16.56 ± 7.77</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>7.47 ± 2.78</td>
<td>1.09 ± 0.28</td>
<td>0.98 ± 0.27</td>
<td>1.2 ± 0.43</td>
<td>1.15 ± 0.38</td>
<td>1.13 ± 0.34</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>13.6 ± 10.1</td>
<td>76.53 ± 27.74</td>
<td>90.17 ± 33.11</td>
<td>76.5 ± 32.83</td>
<td>80.03 ± 21.6</td>
<td>81.04 ± 21.72</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.22 ± 0.79</td>
<td>3.68 ± 0.4a-c</td>
<td>4.33 ± 0.42</td>
<td>4.51 ± 0.5</td>
<td>4.55 ± 0.26</td>
<td>4.37 ± 0.39</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>9.13 ± 0.7ab</td>
<td>9.15 ± 0.6ab</td>
<td>9.45 ± 0.9</td>
<td>9.93 ± 0.6c</td>
<td>9.80 ± 0.5c</td>
<td>9.41 ± 0.4</td>
</tr>
<tr>
<td>Phosphorus, mg/dL</td>
<td>4.69 ± 1.95a</td>
<td>2.17 ± 0.82bc</td>
<td>2.65 ± 0.75</td>
<td>3.11 ± 0.92</td>
<td>3.23 ± 0.82</td>
<td>3.20 ± 0.46</td>
</tr>
<tr>
<td>Parathryoid hormone, pg/mL</td>
<td>503.5</td>
<td>263.5</td>
<td>136.0 ± 117.5</td>
<td>66.8</td>
<td>49.18</td>
<td></td>
</tr>
<tr>
<td>Sedimentation rate mm/h</td>
<td>425 (121.6-882.3)p-e</td>
<td>(121.6-882.3)p-e</td>
<td>10.5 (6.25-22.5)</td>
<td>135 (147.5-202.5)</td>
<td>(43.52-62.28)</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein, mg/dL</td>
<td>1.55 (0.77-2.67)p</td>
<td>1 (0.38-3.42)</td>
<td>0.33 (0.33-3.05)</td>
<td>0.04 (0.33-1.05)</td>
<td>0.34 (0.31-1.01)</td>
<td></td>
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<tr>
<td>Glucose, mg/dL</td>
<td>88.4 ± 26.8</td>
<td>93.8 ± 22.9</td>
<td>84.21 ± 17.15</td>
<td>90.5 ± 33.7</td>
<td>79.14 ± 13.7</td>
<td>74.9 ± 11.8</td>
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<tr>
<td>Total cholesterol, mg/dL</td>
<td>169.71 ± 41.8</td>
<td>155.7 ± 42.9</td>
<td>191.7 ± 52.7</td>
<td>178.2 ± 35.43</td>
<td>175.7 ± 25.84</td>
<td>178.4 ± 29</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>165.14 ± 50.7</td>
<td>202.2 ± 100.8</td>
<td>191.64 ± 109.6</td>
<td>187.74 ± 63.7</td>
<td>167.1 ± 544</td>
<td>164.78 ± 68.4</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>41.0 ± 8.55a</td>
<td>49 ± 14.3a</td>
<td>60.2 ± 16.02</td>
<td>49.3 ± 15.0</td>
<td>55.3 ± 13.4</td>
<td>55.0 ± 16.34</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>127.4 ± 43.7a</td>
<td>76.9 ± 18.4a-p</td>
<td>102.62 ± 30.63</td>
<td>94.85 ± 33.91</td>
<td>100.92 ± 20.32</td>
<td>100.42 ± 27.07</td>
</tr>
<tr>
<td>Steroid dose, mg/d</td>
<td>0.55 ± 0.07</td>
<td>0.53 ± 0.07</td>
<td>0.14 ± 0.04</td>
<td>0.13 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein
Data are expressed as means ± standard deviation or median with interquartile range.

P < .001 compared with data at 6th month.
P < .01 compared with data at 1st month.
P < .05 compared with data at year 1.
P < .001 compared with data at year 2.
P < .05 compared with data at 1st month.
P < .05 compared with data at year 2.
P < .01 compared with all of the postransplant data.
before and 22.18 kg/m² at 24 months after transplant. Patient biochemistry results, complete blood counts, and tacrolimus levels are shown in Table 1.

**Adiponectin, P-selectin assays, and platelet aggregation**

Table 2 and Figure 1 show results of platelet aggregation induced by 5 μM ADP, 5 mM epinephrine, 1.25 mg/mL ristocetin, and 2 μg/mL collagen and results of adiponectin and P-selectin and prothrombin time, partial thromboplastin time, international normalized ratio, and D-dimer levels.

![Figure 1. Adiponectin Levels in Renal Transplant Patients Before Treatment to 24 Months After Treatment](image)

**Discussion**

In chronic kidney disease, both bleeding and thrombotic complications are observed, although with expected recovery after a successful transplant. However, posttransplant cardiovascular mortality is still of concern in renal transplant patients. Coagulation abnormalities and arteriosclerosis seem to play key roles in cardiovascular disease risk observed in transplant recipients.

Adiponectin is an anti-inflammatory and anti-atherogenic adipose tissue-derived hormone. In the normal population, elevated adiponectin levels are associated with cardioprotective and anti-atherosclerotic properties. Recently, studies in normal human populations have reported that higher adiponectin levels are associated with a lower risk for coronary artery disease, including those with type 2 diabetes mellitus. This association has not been consistent in populations with renal failure. Renal clearance is an important factor in determining serum adiponectin levels, and those with chronic kidney disease exhibit significantly higher levels of adiponectin. The high levels of adiponectin in those receiving hemodialysis decline after renal transplant, but they do not return to normal levels. These findings are similar to those shown in our study, in which a statistically significant decrease in adiponectin levels was shown after transplant.

A few studies that investigated the change in adiponectin concentrations after successful transplant also support our results that adiponectin levels decrease after successful renal transplant, suggesting that kidneys assist in the biodegradation and elimination of adiponectin. There are several possible explanations for the elevated adiponectin levels in kidney transplant recipients. Hyperadiponectinemia may be the result of a compensatory

| Table 2. Results of Adiponectin, Platelet Function, and Coagulation Tests |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | Before Treatment| 1st Week        | 1st Month       | 6th Month       | Year 1          | Year 2          |
| Adiponectin, ng/mL             | 50.5 ± 9.84a    | 47.53 ± 12.3    | 51.47 ± 10.39a  | 47.72 ± 10.5    | 50.06 ± 9.3a    | 40.89 ± 9.1     |
| P-selectin, ng/mL              | 107.04 ± 3.3    | 109.33 ± 4.01   | 108.9 ± 5.8     | 109.8 ± 4.9     | 110.1 ± 6.4     | 110.4 ± 5.17    |
| Platelet aggregation           |                |                |                |                |
| ADP, ohm                       | 99.5 ± 12.9c    | 109 ± 14.8      | 101.5 ± 14.7c   | 110 ± 15.5      | 110 ± 5.8       | 111 ± 7.8       |
| Epinephrine, ohm               | 96 ± 14.4       | 106 ± 16.2      | 93.5 ± 22.3     | 106.5 ± 28.6    | 104.5 ± 23.6    | 102.5 ± 37.2    |
| Ristocetin, ohm                | 91 ± 27.7       | 103 ± 18.0      | 93 ± 24.0       | 101.5 ± 29.1    | 99.5 ± 28.5     | 98.5 ± 27.4     |
| Collagen, ohm                  | 95.5 ± 16.0b,d | 107.5 ± 17.6    | 98.5 ± 14.7b,d  | 109.5 ± 15.5    | 102.5 ± 6.3     | 110 ± 15.9      |
| Coagulation tests              |                |                |                |
| Prothrombin time, s            | 11.7 ± 0.9      | 11.0 ± 0.6      | 10.8 ± 0.85     | 12.6 ± 5.5      | 11.49 ± 0.7     | 11.51 ± 0.8     |
| Partial thromboplastin time, s | 29.9 ± 4.16     | 27.58 ± 5.63    | 24.35 ± 2.44    | 29.55 ± 4.98    | 28.34 ± 2.75    | 27.7 ± 3.07     |
| International normalized ratio | 1.05 ± 0.084q   | 0.97 ± 0.055e   | 0.96 ± 0.08a    | 1.14 ± 0.01q    | 1.00 ± 0.02     | 1.06 ± 0.05     |
| Fibrinogen, mg/dL              | 388.4 ± 95.9b   | 330.3 ± 91.9    | 297.4 ± 104.9b  | 321.2 ± 117.3b  | 301.1 ± 66.3b   | 397.3 ± 110.3b  |
| D-dimer, mg/L                  | 4.0 (1.98-6.98)b,1 | 6.31 (0.07-10.5)d,e | 2.94 (1.39-5.07)d,1 | 0.6 (0.4-1.1)d  | 0.38 (0.3-1.1)d | 0.32 (0.26-0.46) |
Adiponectin may indeed be beneficial in lessening atherosclerotic lesions, such as opposing the expression of endothelial adhesion, whereas the adverse risk would be mediated by the underlying arteriosclerotic or inflammatory processes. This effect seems unrelated to the direct effect of adiponectin; rather, it may be due to a process of protein-energy wasting.23,24 Other studies that investigated changes in adiponectin concentrations after successful transplant include Chudek and associates,12 Adamczak and associates,25 and Yilmaz and associates,11 all reporting that adiponectin levels decrease after successful renal transplant, which also suggests that kidneys assist in the biodegradation and elimination of adiponectin. However, no correlation was shown between creatinine and plasma adiponectin levels in these studies, consistent with our results. Unlike these studies, Taherimahmoudi and associates26 found unaltered plasma adiponectin levels after transplant. These findings demonstrated that factors other than decreased renal function may also contribute to elevated adiponectin levels in chronic kidney disease.27 Although chronic kidney disease was shown to be associated with high plasma adiponectin levels, the reason for this elevation was not clear. Adiponectin has vasculoprotective properties,10,28 with Menon and associates demonstrating that high rather than low levels of adiponectin predict poor outcome.28 As suggested by Menon and associates, this unexpected finding may be because of the differences in the ratio of high versus low molecular weight adiponectin. It is also known that residual renal function, inflammation, wasting, and urinary protein excretion may alter the balance of monomeric versus multimeric adiponectin levels in the uremic milieu.29

Our data showed a decrease of serum low-density lipoprotein cholesterol levels and an increase of serum high-density lipoprotein levels at 2-year follow-up. These findings may indicate a lipid profile recovery after transplant, also suggesting a protective situation for atherosclerosis. With the known increase in adiponectin levels due to atherosclerosis and in this situation the fall in adiponectin levels after transplant, these may be the result of recovery in atherosclerosis.

Another important result of our study is the reduction in sedimentation and C-reactive protein levels after transplant. These may be supportive data for the fall in adiponectin levels due to recovery of inflammation. Because our year 2 data showed the best results for adiponectin, it can also be postulated that the fall in adiponectin levels may be independent of the increase in GFR. Chronic inflammation in patients with end-stage renal disease may cause increased adiponectin levels, and, as shown in our year 2 follow-up results, transplant may cause normalization of adiponectin levels. All of these findings indicate the multifactorial effects of adiponectin levels in patients with chronic kidney disease.

In our study, we evaluated platelet aggregation with ADP, ristocetin, epinephrine, and collagen. We found a statistically significant improvement in platelet aggregation tests with ADP and collagen after transplant, but no significant results were shown with ristocetin and epinephrine. It has also been shown that increased P-selectin levels predict adverse thromboembolic events such as stroke and myocardial infarction.30 Contrary to this, we found an increase in P-selectin levels before and after transplant, but this was not statistically significant. These findings suggested that, with increased GFRs, adiponectin levels begin to fall, probably due to the excretion of uremic toxins, and platelet aggregation improves. In addition, improvements in D-dimer and partial thromboplastin time values of patients before and after transplant suggest that transplant has a significant restorative effect on platelet aggregation and coagulation functions independent of platelet activation, as evaluated with P-selectin levels.

We have some limitations in this study. First, it is not clear that the fall in adiponectin levels were due to the endothelial dysfunction, atherosclerosis, or secondary to the increase in GFR. This point may need further investigation. However, the improved platelet function observed early after transplant suggested to us that the fall in adiponectin at year 2 after transplant may be secondary to the improvement in endothelial function.

In conclusion, transplant is the most important and effective renal replacement treatment. In our study, due to decreases in uremic toxins and GFR increases, platelet function was improved soon after transplant, but adiponectin levels became normal only in the second year after transplant. This is probably due to the late recovery of atherosclerosis after transplant. Late improvement of low-density lipoprotein cholesterol and adiponectin after
transplant suggested to us that kidney transplant recipients may still have risk of cardiovascular events, especially in the first years.

References


