Comparing the Serum Levels of Adipocytokines in the Renal Transplant Recipients and Healthy Individuals: A Case-Control Study

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Abstract

Background: Increasing evidence implies that Adipocytokines may result in cardiovascular disease (CVD) events and metabolic changes in the general population and also increase graft failure rate in the renal transplant recipient.

Objectives: To compare the serum levels of Adipocytokines and lipid profiles in renal transplant recipients with healthy individuals.

Methods: In a case-control study undertaken from the beginning of 2015 to December 2016; 30 renal transplant recipients, with stable conditions, whose renal transplant at least survived well over six months, were randomly selected to be the case group. Besides, 30 healthy individuals who referred to the transplantation clinic as the patients’ attendants were considered as the control group. The serum levels of IL-1, IL-6, TNF-α, Adiponectin, Visfatin, Leptin, and the Lipid profiles were measured after 12 hours of fasting and were compared between the two groups.

Results: The serum levels of Adipocytokines including IL-1, IL-6, TNF-α, Visfatin, and Leptin were significantly higher in renal transplant recipients than in healthy individuals (P < 0.001) and the serum level of adiponectin (P < 0.004) was significantly lower in the renal transplant recipients. Linear regression results indicated that in the renal transplant group, only the Visfatin had a significant negative correlation with age (Unstandardized Coefficient = -0.104, P = 0.002). There were significant positive correlations between Leptin with cholesterol (P = 0.005), triglyceride (P = 0.008), and LDL (P = 0.014) in the healthy individuals, however, these relationships were not found in the renal transplant recipients and only a significant negative correlation was found between Tumor necrosis factor alpha (TNF-α) and cholesterol levels (P = 0.01).

Conclusions: The findings of the present study demonstrated that renal transplant recipients suffer from inflammation and accompanying changes in levels of Adipocytokines in comparison with healthy controls.

Keywords: Adipocytokines, Adiponectin, Leptin, Visfatin, Renal Transplantation

1. Background

Nowadays, it is known that adipose tissue, as an endocrine organ, produces Adipocytokines that have the same function as hormones. Adipocytokines include Leptin, Visfatin, adiponectin, IL-6, IL-1, and TNF-α. Adipocytokines play an important role in homeostasis, glucose and lipid metabolism, insulin resistance, inflammation, and atherosclerosis (1-3). On the other hand, their production, secretion, and regulation are not limited to adipose tissue, however, other organs, including kidneys, are involved in this area. The kidney can alter the production and clearness of these Adipocytokines (4, 5). Increasing evidence implies that these Adipocytokines may result in CVD events and metabolic changes in the general population and may also increase mortality and graft failure rate in renal transplant recipients (6). Adipocytokines, particularly adiponectin and Leptin, have been implicated in the pathogenesis of CVD through a crosstalk between adipose tissue and blood vessels and they are suggested to be associated with inflammation and nitric oxide (NO) production (7). In addition, Leptin and adiponectin generally elicit opposing pro-inflammatory and anti-inflammatory effects (8, 9).

Among these adipokines, Leptin and adiponectin play...
an important role in creating the relationship among obesity, high blood pressure, and chronic nephropathy. Leptin causes high blood pressure by activating the sympathetic system (10). Moreover, decreased adiponectin causes high blood pressure (11).

Visfatin or nicotinamide phosphoribosyl pre Bcell enhancing (NAMPT) or Transfer factor 1 is another adipokine that increases the proliferation of neutrophils and also has insulin-like effects. Hyperlipidemia has been reported to be associated with endothelial function damage and more mortality rates in ESRD patients (12, 13).

2. Objectives

The aims of the present study were to compare the serum levels of Adipocytokines in renal transplant recipients with healthy individuals and also to investigate the relationship between Adipocytokines and lipid profiles in these patients.

3. Methods

3.1. Study Sample

In this hospital based case-control study, conducted in Modarres hospital in Tehran, which is the only governmental facility in north-western Tehran, from the beginning of 2015 to the end of December 2016, 30 renal transplant recipients, with stable conditions, whose renal transplant at least survived well over 6 months, were randomly selected to be the case group. We assessed the 50 renal transplant recipients for eligibility criteria and of the total, 30 patients were included in the study. The exclusion criteria for the case group included receiving a renal transplant from a cadaver (n = 5), undergoing peritoneal dialysis before renal transplantation (n = 1), not undergoing dialysis before a renal transplant (pre-emptive renal transplantation)(n = 1), having malnutrition (n = 2), applying drugs other than transplant drugs (composed of prednisolone, calcineurin inhibitor, cellcept ) (n = 1), having signs and symptoms of active infection (n = 0), having a history of transplant rejection in the last 3 months (n = 1), receiving the corticosteroid pulse therapy in the last 3 months (n = 1), a history of hospitalization in the last 3 months (n = 2), having serum creatinine greater than 1.7 mg/dL (n = 2), using ACE inhibitor or ARB drugs and statins (n = 3), smoking and having drug abuse (n = 0), and having abnormal liver tests (n = 1). In addition, 30 healthy individuals who referred to the transplantation clinic as the patients’ attendants were considered as the control group after receiving their informed consent and having the inclusion criteria. The inclusion criteria for the control group included no history of malignant disease and other systemic diseases, no daily intake of drugs, and no signs and symptoms of infection and inflammation. A frequency matching was also undertaken for BMI variable as a substantial confounder (14). The proposal of this study was reviewed and approved by the ethics committee of Shahid Beheshti University of Medical Sciences.

3.2. Sampling Strategy

We used a probabilistic sampling strategy for selecting the studied cases, and simple random sampling was considered by using a random digit table. Moreover, the controls were selected from the people who take a visit of patients (relatives) in the ward if they had the inclusion criteria’s and were accepted to participate in the study.

Basic information of individuals including age, gender, ESRD cause, and duration of the disease were recorded in the data collection form. After 12 hours of fasting, 5 ml of venous blood was taken from individuals and centrifuged for 10 minutes at 3000 g. The serum was isolated and kept at -30°C. Then, in addition to the serum levels of cholesterol, triglyceride, HDL, and LDL, the serum levels of IL-1, IL-6, TNF-α, Adiponectin, Visfatin, and Leptin were measured and recorded in the checklist.

This study was approved by the Ethical Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.921222/4).

3.3. Biochemical Assay

Venous blood samples were obtained from each participant after an overnight fasting and before and after garlic extract tablets or placebo intake. Samples were collected into standard plain and EDTA vacutainer tubes and allowed to stand at room temperature for 20 minutes to clot, then centrifuged. Serum aliquots prepared for storage at -20°C until future analysis.

Routine biochemical parameters were assayed in standard automated analyzer using commercial kits with standard methods. The serum immune assays were performed using a Human Enzyme-linked immunosorbent assay ELISA kit (IBL International GmbH, Germany). Samples were measured in duplicate manufacturer recommended wave length against a known standard curve depending on the specifications of the protocol. All assays were done in the Modarres hospital diagnostic laboratory. Furthermore, to prevent the information bias due to measurement error, all the devices are calibrated regularly.

3.4. Statistical Analysis

Clinical and demographic data were continuously presented as mean ± standard deviation and the grouped
data were as frequency and percentage. Chi-square or Fisher’s exact test was used to check the independence of the two categorical variables. The normality assumption of main variables distribution was explored using Kolmogorov-Smirnov test. In the case of normality of data, an independent t-test (or Mann-Whitney U test) was used to examine the mean difference between the two groups. Moreover, the relationship between Adipocytokines with age, duration of ESRD, and lipid profiles were investigated through multivariate linear regression. Since there is less than 3% missing data on main variables in each group, the last observation carried forward (LOCF) approach was applied in case of missing data. All analyses were performed using the Stata Software (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). The level of significance was less than 0.05.

4. Results

In the renal transplant group, 18 patients (60%) were male, and 12 (40%) were female. In the healthy individuals group, 15 (50%) were male and 15 (50%) were female; there was no significant difference between the two groups regarding gender (P = 0.436). The mean age of patients with renal transplantation was not significantly different from that of the healthy individuals group (respectively 49 ± 5 years and 50 ± 4 years, P = 0.124). The most common cause of ESRD in the renal transplant recipients was diabetic nephropathy (in 15 persons, 50%) and the next one was hypertension (in 6 persons, 20%). Other causes consisted of obstructive uropathy, chronic glomerulonephritis, and polycystic kidney disease. The Mean and SD of the age and length of time on dialysis prior to renal transplantation in these patients were 49.4 ± 4.6 years and 19.5 ± 4.6 months, respectively.

In Table 1, the serum levels of Adipocytokines between two groups of renal transplant recipients and healthy individuals are compared. The serum levels of IL-1, TNF-α, Visfatin, and Leptin in the renal transplant recipients were significantly higher than those of the healthy individuals (P < 0.001), whereas their Adiponectin was significantly lower than healthy subjects’ (P < 0.001). The serum level of IL-6 was not significantly different between the two groups (P = 0.166).

In Table 2, the serum levels of Adipocytokines in renal transplant recipients and healthy individuals are compared based on gender. In the renal transplant group, Leptin was significantly higher in females than in males (P = 0.001). For other variables, there was no significant difference between the two genders (P > 0.05). Furthermore, an additional analysis was done for comparing the serum Adipocytokines in the male and female population between the healthy and kidney transplanted groups using t-test or Mann-Whitney U tests. The results showed that there is a significant difference in all studied variables except IL-6 (P = 0.49) and adiponectin (P = 0.64) in male and female categories respectively (results are available upon request).

To explore the relationship between age and the duration of ESRD with serum levels of Adipocytokines in the renal transplant group, the results of linear regression with the simultaneous inclusion of age and duration of disease in the model showed that age was only statistically significant with the serum level of Visfatin (P = 0.006); no association was found between the other Adipocytokines with age. The relationship between age and the serum level of Visfatin results showed that the serum level of Visfatin would be reduced by a factor of -0.1 with each year added to the individual’s age. None of the Adipocytokines had a significant relationship with age in healthy individuals (P > 0.05).

In Tables 3 and 4, the correlation between the serum level of Adipocytokines and lipid profiles in healthy individuals and renal transplant recipients are depicted, respectively. There were significant positive correlations between Leptin and cholesterol, triglyceride, and LDL in healthy individuals (P < 0.05). These relationships were not found in the renal transplant recipients, and only a significant negative correlation was found between TNF-α and cholesterol levels in these patients.

5. Discussion

The findings of the present study revealed that the serum levels of IL-1, IL-6, TNF-α, and Leptin were significantly higher in renal transplant recipients and the serum level of adiponectin and Visfatin was significantly lower than those in the healthy group. In the renal transplant group, only Leptin was higher in females than in males. This comparison for healthy control group showed that the serum level of IL-6 was higher in male participants. Also, Visfatin was significantly correlated inversely with age in the case group. Although the correlation coefficients of all Adipocytokines were negative with the duration of ESRD, their relationship was not statistically significant. There were also significant positive correlations between Leptin and cholesterol as well as triglyceride and LDL in healthy subjects; however, these relationships did not found in renal transplant recipients. Only a significant negative correlation was found between the level of TNF-α and cholesterol in renal transplant recipients.

Since the production, secretion, and regulation of Adipocytokines are not limited to adipose tissue, and other...
Table 1. Comparison of Serum Adipocytokines Levels Between the Renal Transplant Patients and Healthy Participants (N = 30)\(^a\)

<table>
<thead>
<tr>
<th>Adipocytokines</th>
<th>Case, Kidney Transplant</th>
<th>Control, Normal Subject</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1, pg/mL(^b)</td>
<td>0.5 ± 1.11</td>
<td>0.23 ± 0.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-6, pg/mL(^b)</td>
<td>61.05 ± 37.19</td>
<td>26.65 ± 20.12</td>
<td>0.032</td>
</tr>
<tr>
<td>TNF-α, pg/mL(^b)</td>
<td>10.76 ± 13.63</td>
<td>0.89 ± 3.47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adiponectin, μg/mL(^c)</td>
<td>10.78 ± 22.7</td>
<td>7.04 ± 29.89</td>
<td>0.004</td>
</tr>
<tr>
<td>Visfatin, pg/mL(^b)</td>
<td>136 ± 6.1</td>
<td>357 ± 1.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Leptin, ng/mL(^b)</td>
<td>4582 ± 8470</td>
<td>2454 ± 3203</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

\(^a\)P < 0.05 was considered significant.
\(^b\)Mann-Whitney U test.
\(^c\)Independent t-test.

Table 2. Comparison of Serum Adipocytokines Levels Stratified by Sex and Groups (N = 30)\(^a\)

<table>
<thead>
<tr>
<th>Adipocytokines</th>
<th>Case, Kidney Transplant</th>
<th>Control, Normal Subject</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>P Value</td>
</tr>
<tr>
<td>IL-1, pg/mL(^b)</td>
<td>0.41 ± 1.22</td>
<td>0.58 ± 0.96</td>
<td>0.15</td>
</tr>
<tr>
<td>IL-6, pg/mL(^b)</td>
<td>38 ± 71.6</td>
<td>35.9 ± 43.5</td>
<td>0.88</td>
</tr>
<tr>
<td>TNF-α, pg/mL(^b)</td>
<td>12.86 ± 16.49</td>
<td>4.01 ± 9.34</td>
<td>0.074</td>
</tr>
<tr>
<td>Adiponectin, μg/mL(^b)</td>
<td>9.92 ± 20.21</td>
<td>11.36 ± 26.44</td>
<td>0.12</td>
</tr>
<tr>
<td>Visfatin, pg/mL(^b)</td>
<td>0.76 ± 6.08</td>
<td>1.62 ± 6.2</td>
<td>0.79</td>
</tr>
<tr>
<td>Leptin, ng/mL(^b)</td>
<td>4100 ± 6407</td>
<td>3458 ± 10564</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^a\)P < 0.05 was considered significant.
\(^b\)Independent t-test.
\(^c\)Mann-Whitney U test.

Table 3. Relationship Between Serum Adipocytokines Levels and Lipid Profile in Healthy Subjects\(^a\)

<table>
<thead>
<tr>
<th>Adipocytokines</th>
<th>Cholesterol</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>P Value</th>
<th>Cholesterol</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>P Value</th>
<th>Cholesterol</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation Coefficient(^b)</td>
<td>P Value</td>
<td>Correlation Coefficient</td>
<td>P Value</td>
<td>Correlation Coefficient</td>
<td>P Value</td>
<td>Correlation Coefficient</td>
<td>P Value</td>
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<td></td>
</tr>
<tr>
<td>IL-1, pg/mL(^c)</td>
<td>0.212</td>
<td>0.26</td>
<td>0.150</td>
<td>0.482</td>
<td>-0.170</td>
<td>0.370</td>
<td>0.259</td>
<td>0.467</td>
<td></td>
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</tr>
<tr>
<td>IL-6, pg/mL(^c)</td>
<td>0.209</td>
<td>0.245</td>
<td>0.086</td>
<td>0.650</td>
<td>0.021</td>
<td>0.93</td>
<td>0.301</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TNF-α, pg/mL(^c)</td>
<td>-0.010</td>
<td>0.959</td>
<td>0.068</td>
<td>0.722</td>
<td>-0.298</td>
<td>0.110</td>
<td>0.139</td>
<td>0.463</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin, μg/mL(^d)</td>
<td>-0.056</td>
<td>0.771</td>
<td>-0.055</td>
<td>0.412</td>
<td>-0.108</td>
<td>0.570</td>
<td>0.103</td>
<td>0.587</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visfatin, pg/mL(^c)</td>
<td>0.038</td>
<td>0.842</td>
<td>-0.036</td>
<td>0.934</td>
<td>0.186</td>
<td>0.326</td>
<td>0.096</td>
<td>0.644</td>
<td></td>
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<td></td>
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<tr>
<td>Leptin, ng/mL(^c)</td>
<td>0.503</td>
<td>0.005</td>
<td>0.472</td>
<td>0.008</td>
<td>-0.278</td>
<td>0.317</td>
<td>0.442</td>
<td>0.054</td>
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<td></td>
</tr>
</tbody>
</table>

\(^a\)P<0.05 was considered significant.
\(^b\)Pearson Correlation coefficient.
\(^c\)Mann-Whitney U test.
\(^d\)Independent t-test.

In recent years, there is growing attention to the relationship between Adipokines secreted by adipose tissue and renal dysfunctions. In-vitro studies have confirmed that adipokines, including Leptin and adiponectin, may possibly mediate pathological and functional alterations in renal parenchyma (16, 17). On the other hand, renal transplantation can lead to renal function recovery in patients. Therefore, many studies have been carried out on Adipocytokines after renal transplantation. Malgorzewicz et al.,...
evaluating Adipocytokine abnormalities including Leptin, adiponectin, and Visfatin in 80 kidney transplant recipients, indicated that Leptin had a direct correlation with time from transplantation and an inverse relationship with eGFR (18). In a review study in 2016, Nagy et al., examined the role of Adipocytokines in renal transplant recipients and suggested that Adipocytokines such as Leptin, adiponectin, and Visfatin may lead to cardiovascular events and metabolic changes in the general population, and may increase mortality rates and the rate of transplantation loss in renal transplant recipients (6). Shu et al., examined the serum levels of Adipokines in 280 renal transplant recipients with and without metabolic syndrome and reported that the serum levels of Leptin, adiponectin, and Visfatin in renal transplant recipients with metabolic syndrome were higher and there was a close interrelationship in the serum levels of these Adipokines (19).

Several studies have also been conducted on each of the Adipocytokines separately. For example, in the case of Leptin, Souza et al., compared the serum levels of Leptin in the first year post-replacement transplantation in 32 patients and 19 healthy individuals and suggested that during the first post-transplant year the serum Leptin levels significantly decreased; thus, it was different depending on post-transplant time (20). Nicoletto et al., reviewed Leptin and metabolic changes in 32 renal transplant recipients for 5 years and indicated that Leptin decreased immediately after transplantation and remained high up to one year later; however, 5 years after transplantation, Leptin and lipids had a profile similar to those in the pre-transplant period; this condition could indicate a higher incidence of cardiovascular diseases in this post-transplant period (21). Rafieian-Kopaei and Nasri reported a significant difference of serum Leptin between males and females in 72 renal transplant recipients; there was no relationship between serum Leptin with body mass index, age, and creatinine clearance, however, there was a negative relationship between Leptin and the duration of renal transplantation (22). Comparing the Visfatin serum levels in renal transplant recipients with healthy volunteers, some studies, reported that Visfatin was associated with inflammatory markers and may be able to justify the relationship between inflammation and levels of Adipocytokines in long-term renal transplant recipients (23, 24).

The present study discovered that the serum levels of IL-1, IL-6, TNF-α, and Leptin were significantly higher in renal transplant recipients. Since transplantation in the present study's patients lasted for six months, the high rates of these indicators, which have been described as inflammatory indicators in other studies, can indicate that inflammatory conditions continue to be present in these patients after transplantation. We found the relationship between Leptin and lipid profiles in healthy individuals that did not exist in transplanted individuals (25). Various studies have been done on the relationship between Adipocytokines and lipid profiles in CKD and renal transplant recipients. Nusken et al., stated that active Visfatin in hemodialysis patients compared to those in the control group increased independent of blood glucose, serum insulin, diabetes, and HDL (26). Furthermore, in hemodialysis patients, only HDL and insulin-treated diabetes mellitus were independently associated with active serum Visfatin (26). Following renal transplant patients concerning weight gain and serum levels of Adipocytokines for one year, Teplan et al., reported an increase of the levels of Leptin and Visfatin and a decrease of adiponectin in the pa-

<table>
<thead>
<tr>
<th>Adipocytokines</th>
<th>Cholesterol</th>
<th>Lipid Profiles</th>
<th>Correlation Coefficient</th>
<th>P Value</th>
<th>Correlation Coefficient</th>
<th>P Value</th>
<th>Correlation Coefficient</th>
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<tr>
<td></td>
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<tr>
<td>IL-1, pg/mL</td>
<td>-0.282</td>
<td>0.131</td>
<td>-0.005</td>
<td>0.980</td>
<td>0.002</td>
<td>0.992</td>
<td>-0.192</td>
<td>0.310</td>
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<tr>
<td>IL-6, pg/mL</td>
<td>0.230</td>
<td>0.221</td>
<td>0.355</td>
<td>0.478</td>
<td>0.215</td>
<td>0.255</td>
<td>-0.032</td>
<td>0.867</td>
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<tr>
<td>TNF-α, pg/mL</td>
<td>-0.461</td>
<td>0.010</td>
<td>-0.162</td>
<td>0.391</td>
<td>0.087</td>
<td>0.646</td>
<td>-0.304</td>
<td>0.102</td>
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<tr>
<td>Adiponectin, µg/mL</td>
<td>0.293</td>
<td>0.116</td>
<td>-0.192</td>
<td>0.003</td>
<td>0.008</td>
<td>0.968</td>
<td>0.273</td>
<td>0.444</td>
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<tr>
<td>Visfatin, pg/mL</td>
<td>-0.187</td>
<td>0.322</td>
<td>0.004</td>
<td>0.988</td>
<td>0.0167</td>
<td>0.378</td>
<td>-0.024</td>
<td>0.899</td>
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<tr>
<td>Leptin, ng/mL</td>
<td>0.282</td>
<td>0.131</td>
<td>0.042</td>
<td>0.827</td>
<td>0.168</td>
<td>0.376</td>
<td>0.071</td>
<td>0.701</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 was considered significant.

**Pearson Correlation coefficient.

*Mann withney U test.

Independent t-test.
tients that developed obesity (27). Another study showed a significant direct correlation with HDL in both the control group and patients, which could lead to an increased risk of cardiovascular diseases (28, 29). The present study also noted that high levels of Adipocytokines were observed in the renal transplant group compared with the healthy group; therefore, this indicated an inflammatory status in these patients.

According to the results of the current study, which were in line with those of other studies in this field, it was revealed that the level of Adipocytokines and their relationships with lipid profiles in renal transplant recipients were not similar to those of healthy individuals. Since this study was a case-control study, it was not possible to examine the course of changes and the relationships between Adipocytokines and transplantation outcomes. It is recommended that more extensive studies be conducted to determine the average levels of Adipocytokines in renal transplant recipients, the course of their changes after transplantation, and their relationships with transplantation outcomes. As another limitation of this study, although the study was conducted in a referral hospital, the results should be interpreted conservatively due to the generalizability of the data. We do not have demographic data of both groups, and this is another limitation of our study; however, we used a frequency matching technique in adjusting the effects of probable confounders such as body mass index and so on.

The level of Adipocytokines in the renal transplant recipients was not related to the duration of ESRD and did not have a significant relationship with lipid profiles. Therefore, the relationships of Adipocytokines with the mentioned variables in renal transplant recipients does not seem to be similar to those in healthy people, and further studies are needed to determine their natural rates, the course of their changes after renal transplantation, and their relationships with the implications of the transplant.

Footnotes

Conflict of Interest: The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Ethical Approval: The proposal of this research has been approved in the ethics committee of Shahid-Beheshti University of Medical Sciences, Tehran, IRAN.

Informed Consent: All the individuals had been informed of the purposes of the study and gave their oral informed consent.

References


