THE CORRELATION BETWEEN SERUM TEF3 AND SERUM SFRP5, KLOTHO, GALECTIN-3, AND NESFATIN-1 IN PATIENTS WITH DIABETIC NEPHROPATHY

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ABSTRACT

Objective: To explore the correlation between serum trefoil factor 3 (TEF3) and serum-secreted frizzled-related protein 5 (SFRP5), Klotho, galectin-3, and new satiety molecular protein 1 (nesfatin-1) in patients with diabetic nephropathy.

Methods: First, 56 adult patients hospitalized with diabetic nephropathy in the Department of Endocrinology of the Third Central Hospital of Tianjin between April 2017 and June 2019 were recruited as the diabetic nephropathy group. Meanwhile, 56 adult patients undergoing physical examination in the hospital during the same period were recruited as the control group. The fasting venous blood of each group was centrifuged and the supernatant was collected for testing. The serum levels of TEF3, SFRP5, Klotho, galectin-3, nesfatin-1, interleukin-6 (IL-6), IL-18, and high-sensitivity C-reactive protein (hs-CRP) were compared and their correlations were analyzed.

Results: The TEF3, IL-18, IL-6, and hs-CRP levels of the diabetic nephropathy group were significantly higher than those of the control group, whereas the SFRP5, Klotho, serum galectin-3, and nesfatin-1 levels were significantly lower (P<.05). We analyzed the correlation, taking TEF3 as the dependent variable and SFRP5, Klotho, galectin-3, nesfatin-1, IL-18, IL-6, hs-CRP as the independent variables. We found that TEF3 was negatively correlated with SFRP5, Klotho, galectin-3, and nesfatin-1 (P<.05) and positively correlated with IL-18, IL-6, and hs-CRP (P<.05).

Conclusion: The serum of patients with diabetic nephropathy had lower levels of TEF3, SFRP5, Klotho, galectin-3, and nesfatin-1 and higher levels of related inflammatory factors. The expression level of TEF3 was negatively correlated with SFRP5, Klotho, galectin-3, and nesfatin-1 (P<.05) and positively correlated with IL-18, IL-6, and hs-CRP (P<.05). This indicates that TEF3 can be used as an important indicator in the clinical monitoring of patients with diabetic nephropathy.

Keywords: Diabetic nephropathy, TEF3, SFRP5, Klotho, galectin-3, nesfatin-1.

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Introduction

In recent years, diabetes has become one of the world’s most pressing public health issues. Approximately 392 million adults suffer from diabetes worldwide, with China accounting for 131 million of those patients(1). Diabetes can cause many complications, including kidney disease and diabetic gangrene, that can severely impact patients’ physical and mental health(2). Diabetic nephropathy is a complication of type 2 diabetes and can lead to kidney failure, disability, and, in severe cases, death(3). Therefore, effectively diagnosing and treating diabetic nephropathy is of great importance. Serum Trefoil factor 3 (TEF3) can activate the downstream inflammatory mediator Interleukin-6 (IL-6) and other factors, accelerating the onset and progression of diabetic nephropathy(4). Secretory curl-associated protein 5 (SFRP5) is associated with obesity and abnormal changes in glucose metabolism and can be an independent risk factor for type 2 diabetes(5). Klotho is a factor related to aging that, according to
some studies, plays a role in antioxidant stress and anti-inflammatory reactions\(^6\).

Studies have also found that SFRP5 and Klotho are abnormally expressed in patients with diabetic nephropathy\(^7\). Galectin-3 plays an important role in apoptosis, tumor differentiation, and angiogenesis\(^8\), while nesfatin-1 is a neuropeptide that was discovered in 2006. At present, clinical studies mainly use nesfatin-1 as a food suppressor, and there are some controversies about its role in diabetes\(^9\).

In this study, we tested the levels of TEF3, SFRP5, Klotho, galectin-3, and nesfatin-1 in the serum of diabetic patients, and analyzed their correlation.

**Materials and methods**

**General information**

We identified 56 patients with diabetic nephropathy who were hospitalized in the Department of Endocrinology of the Third Central Hospital of Tianjin from April 2017 to June 2019 as the diabetic nephropathy group. This included 36 male and 20 female patients, with an average age of (62.44±1.40) years.

We included patients who:
- Had recently received their first diagnosis of diabetic nephropathy;
- Had symptoms that were consistent with the diagnostic criteria for diabetic nephropathy contained in the 2007 edition of the Guidelines for Prevention and Treatment of Type 2 Diabetes in China;
- Had primary type-2 diabetes;
- Were aged 18 or older;
- Agreed to participate in the study and signed an informed consent form, or whose family members had agreed to their participation;
- Were approved for participation by the ethics committee of our hospital.

We excluded patients who:
- Had parenchymal lung disease, primary or secondary kidney disease, or systemic inflammatory diseases such as pneumonia; or
- Had used drugs that affect kidney function within the previous 6 months.

We also recruited a control group of 56 adults who had undergone physical examination in the hospital during the same period. The group included 30 male and 26 female patients, with an average age of (60.47±9.94) years. There was no statistically significant difference between the two groups in terms of gender, age, and other general characteristics (P>0.05).

**Method**

We extracted 6 ml of fasting venous blood from patients in both groups. For the diabetic nephropathy group, this occurred in the morning following their admission, and for the control group, this occurred on the day of their physical examinations.

All the samples were then placed in a BD10ml EDTA anticoagulant tube (produced by Shanghai Keyuan Biotechnology Co., Ltd.), treated using a Hettich ROTOFIX32A centrifuge (produced by Xiang Technology Co., Ltd.), then tested for the relevant factors.

**Observation indicators**

First, we took the supernatant of each group and used an ELISA kit (produced by Jiangxi Jianglan Pure Biological Reagent Co., Ltd.) to detect the levels of SFRP5 and Klotho.

Next, we used the Mindray automatic biochemical analyzer BS-280 (produced by Nanjing Beiden Medical Co., Ltd.) to detect the levels of TEF3, galectin-3, nesfatin-1, IL-18, IL-6, and hypersensitivity in the serum of each group, as well as the levels of C-reactive protein (Hypersensitive C-reactive protein, hs-CRP).

**Statistical methods**

We conducted the data analysis using SPSS19.0. The measurement data (i.e., the serum levels of the patients) were expressed as (\(\bar{x}±s\)).

We compared the two groups using a t-test, then used the Pearson correlation coefficient to calculate the correlation between the serum levels of TEF3 and galectin-3, nesfatin-1, IL-18, IL-6, and hs-CRP levels in patients with diabetic nephropathy. P<.05 was considered to be statistically significant.

**Results**

Comparison of serum TEF3, SFRP5, and Klotho levels between the two groups

As shown in Table 1, the levels of TEF3 of the diabetic nephropathy group were significantly higher than those of the control group, whereas their SFRP5 and Klotho levels were significantly lower (P<.05).

Comparison of serum galectin-3 and nesfatin-1 levels between the two groups

As shown in Table 2, the serum galectin-3 and nesfatin-1 levels of the diabetic nephropathy group were significantly lower than those of the control group (P<.05).
The correlation between serum TEF3 and serum SFRP5, Klotho, galectin-3, and nesfatin-1 in patients with diabetic nephropathy

Comparison of serum inflammatory factor levels between the two groups

As shown in Table 3, the levels of IL-18, IL-6, and hs-CRP of the diabetic nephropathy group were significantly higher than those of the control group (P<.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TEF3 (μg/L)</th>
<th>SFRP5 (μg/L)</th>
<th>Klotho (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>56</td>
<td>8.13±1.46</td>
<td>8.37±1.08</td>
<td>15.60±2.24</td>
</tr>
<tr>
<td>Diabetic nephropathy group</td>
<td>56</td>
<td>15.97±2.45</td>
<td>5.25±0.69</td>
<td>10.74±1.86</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>20.571</td>
<td>18.218</td>
<td>12.491</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 1: Comparison of serum TEF3, SFRP5, and Klotho levels between the two groups (x̅±s).

Table 2: Comparison of serum galectin-3 and nesfatin-1 levels between the two groups (x̅±s).

Correlation analysis of serum TEF3 levels and SFRP5, Klotho, galectin-3, nesfatin-1, and inflammatory factor levels

In our correlation analysis, we took TEF3 as the dependent variable and SFRP5, Klotho, galectin-3, nesfatin-1, IL-18, IL-6, and hs-CRP as the independent variables.

As shown in Table 4, TEF3 was negatively correlated with SFRP5, Klotho, galectin-3, and nesfatin-1 (P<.05), while it was positively correlated with IL-18, IL-6, and hs-CRP (P<.05).

<table>
<thead>
<tr>
<th>Index</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-18</td>
<td>0.476</td>
<td>0.023</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.409</td>
<td>0.038</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.424</td>
<td>0.029</td>
</tr>
<tr>
<td>SFRP5</td>
<td>-0.604</td>
<td>0.039</td>
</tr>
<tr>
<td>Klotho</td>
<td>-0.638</td>
<td>0.046</td>
</tr>
<tr>
<td>galectin-3</td>
<td>-0.705</td>
<td>0.015</td>
</tr>
<tr>
<td>nesfatin-1</td>
<td>-0.648</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Table 4: Correlation analysis of serum TEF3 level and SFRP5, Klotho, galectin-3, nesfatin-1, and inflammatory factor levels.

Discussion

The incidence rate of diabetic nephropathy is currently about 0.005%, but its prevalence has increased gradually in recent years. In the long term, diabetic nephropathy can seriously threaten patients’ quality of life and lead to adverse clinical outcomes. Clinical studies have shown that changes in molecular-related biological factors can lead to glomerular or renal basement membrane damage, and eventually to the onset and progression of diabetic nephropathy. Therefore, identifying the pathogenic factors related to diabetic nephropathy can reveal its pathogenesis and support the clinical prevention and treatment of the disease.

Several studies have shown that TEF3 plays an important role in nephron ischemic injury and self-repair disorder by stimulating downstream inflammatory mediators such as IL-6 and IL-10. For example, a study of 83 patients with diabetic nephropathy found that their serum TEF3 content was elevated by more than 25%, with worse renal compensatory function leading to a greater increase. Notwithstanding these previous studies, there has been little research on the relationship between TEF3 and SFRP5, Klotho, galectin-3, and nesfatin-1. Previous studies have relied on small clinical samples and biased data selection, leading to low reliability.

The down-regulation of Sfrp5 and Klotho expression levels may also play an important role in the progression of diabetes. SFRP5 is specifically released by adipocytes, which can block inflammatory cell infiltration and the secretion of inflammatory mediators. Clinical reports have shown that the circulating expression of SFRP5 is closely related to insulin resistance. Klotho is an
anti-aging protein that is produced in the kidneys and plays an important role in the metabolism of calcium and phosphorus. One previous study tested the expression levels of SFRP5 and Klotho in the serum of 60 patients with type 2 diabetes, 60 patients with impaired glucose tolerance, and 60 healthy people, and found that patients with type 2 diabetes and impaired glucose tolerance had significantly lower levels of SFRP5 and Klotho than healthy people. Moreover, the serum levels of SFRP5 and Klotho in patients with type 2 diabetes are lower than those with impaired glucose tolerance, indicating that such levels are negatively correlated with glucose tolerance\(^{(16)}\). Galectin-3 is expressed in activated macrophages and non-large cells, and can also contribute to heart and kidney fibrosis\(^{(17)}\). Clinical studies have confirmed that continuously high blood glucose levels can lead to the non-enzymatic glycosylation of the extracellular matrix of the glomerular cell nucleus, which leads to the production of end-glycosylation products, for which galectin-3 is the receptor. Thus, the expression level of galectin-3 increases as the end-products of glycosylation increase, accelerating the production of many cytokines, leading to glomerular lesions and renal interstitial fibrosis, and ultimately leading to kidney damage and diabetic nephropathy\(^{(18)}\).

Nesfatin-1 can induce insulin β cells to release insulin\(^{(19)}\). Clinical studies have shown that nesfatin-1 co-localizes with insulin. They have also found serum nesfatin-1 production in diabetic patients with insulin resistance\(^{(20)}\). Increased levels of IL-18, IL-6, hs-CRP, and other inflammatory mediators can independently affect the progress of patients with diabetic nephropathy, leading to poor prognoses.

In this study, we measured the levels of TEF3, SFRP5, Klotho, galectin-3, nesfatin-1, and related inflammatory factors in the serum of the two groups of experimental subjects, and analyzed their correlation. The results showed that the levels of TEF3, IL-18, IL-6, and hs-CRP in the diabetic nephropathy group were significantly increased compared with the control group, whereas the levels of SFRP5, Klotho, galectin-3, and nesfatin-1 were significantly decreased (P<.05). Serum TEF3 was negatively correlated with SFRP5, Klotho, galectin-3, and nesfatin-1 (P<.05) and positively correlated with IL-18, IL-6, and hs-CRP (P<.05). This suggests that TEF3, SFRP5, Klotho, galectin-3, and nesfatin-1 are important reference indicators in the evaluation and clinical monitoring of patients with diabetic nephropathy.

References

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