Detection and Clinical Significance of Th17/Treg Cell-Related Factors in Patients with Gestational Diabetes Mellitus

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Abstract: Objective: To investigate the detection of Th17/Treg cell-related factors in patients with gestational diabetes mellitus (GDM) and its clinical significance. Methods: In this study, a retrospective cohort research method was used to collect the clinical data of 42 patients who were hospitalized in the Affiliated Hospital of Hebei University and received the diagnosis of GDM from January 2018 to December 2022, as well as 42 patients with normal pregnancies during the same period. The Th17/Treg expression levels and metabolism-related indexes in the peripheral blood of patients were detected by radioimmunoassay. Results: The relative expression of Th17 in the serum of patients in the GDM group was significantly higher than that of the control group, and the level of Treg was significantly lower than that of the control group (P < 0.05); the levels of FBG, FINS, 2hBG, TC, TG and HOMA-IR of the patients in the GDM group were significantly higher than that of the control group, and the level of HOMA-β was significantly lower than that of the control group (P < 0.05). Conclusion: The imbalance of the Th17/Treg cell ratio in patients with GDM may be related to their disease progression and prognosis, providing new ideas and strategies for the clinical treatment of GDM.

Keywords: Gestational diabetes mellitus; Th17/Treg cells; Cytokines; Clinical significance

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1. Introduction

Gestational diabetes mellitus (GDM) is a form of diabetes mellitus that develops during pregnancy in individuals without prior diabetes mellitus and becomes more prevalent as blood glucose levels rise during pregnancy. Current understanding suggests that GDM results from a combination of genetic and environmental factors. In recent years, research has revealed that cytokines associated with the development of GDM primarily encompass Th17 cytokines, inflammatory factors, and chemokines. Some studies have demonstrated the involvement of Th17 cells in the development and maintenance of insulin resistance, while Treg cells play a crucial immunosuppressive role in maintaining the body’s immune homeostasis. Evidence indicates that Th17/Treg cell-associated factors contribute to the pathogenesis of GDM [1].

The development of GDM is influenced by various factors, including genetics, environment, and
immunology. Recently, the study of Th17/Treg cells and their related cytokines has garnered significant attention in the fields of autoimmunity and diabetes. Th17 and Treg cells are vital regulatory components of the immune system, each secreting distinct cytokines to modulate immune responses and inflammatory processes. Therefore, the assessment of the expression of Th17 and Treg cell-related factors in patients with GDM holds importance in comprehending the pathogenesis of GDM and identifying potential therapeutic targets.

2. Information and Methods
2.1. General information
In this study, a retrospective cohort research method was employed to gather clinical data from 42 patients who were hospitalized and diagnosed with GDM between January 2018 and December 2022. Additionally, data were collected from 42 patients with normal pregnancies during the same period. Information was collected regarding the patient’s age, BMI, height, waist circumference, body mass index (BMI), and microRNA (miRNA) gene test results. The inclusion criteria for study subjects were as follows: (1) confirmed diagnosis of GDM; (2) BMI ≥ 30 kg/m; (3) GDM diagnosed before 28 weeks of gestation; (4) absence of other conditions such as type 1 diabetes mellitus, other autoimmune diseases, or a family history of diabetes mellitus. All enrolled cases underwent pre-pregnancy fasting blood glucose examinations.

2.2. Methods
Peripheral venous blood was collected within 24 hours of hospitalization and placed in sterile vacuum blood collection tubes. The content of Th17/Treg cells in peripheral blood was determined using radioimmunoassay. All tests were conducted using enzyme-linked immunosorbent assay (ELISA), with the kits sourced from Nanjing Jianjian Bioengineering Research Institute. The ELISA procedure adhered strictly to the kit instructions, and the total RNA content in the samples was assessed using a centrifuge at the conclusion of the experiments.

2.3. Statistical methods
The SPSS 24.0 statistical software was employed for data analysis. Measured data were expressed as mean ± standard deviation (SD), and a t-test was performed. Count data were expressed as %, and a χ² test was conducted, with P < 0.05 considered indicative of a statistically significant difference.

3. Results
3.1. Comparison of Th17 relative expression and Treg level in serum of study subjects
The relative expression of Th17 in the serum of patients in the GDM group was significantly higher than that in the control group, while the level of Treg was significantly lower than that in the control group. These differences were statistically significant (P < 0.05), as illustrated in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Th17 (%)</th>
<th>Treg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n = 42)</td>
<td>1.24 ± 0.33</td>
<td>3.26 ± 1.62</td>
</tr>
<tr>
<td>GDM group (n = 42)</td>
<td>3.38 ± 0.69</td>
<td>1.47 ± 0.51</td>
</tr>
</tbody>
</table>

| t         | 8.961 | 3.694 |
| P         | < 0.05 | < 0.05 |
3.2. Comparison of metabolism-related indexes in both groups

Patients in the GDM group exhibited significantly higher levels of fasting blood glucose (FBG), fasting insulin (FINS), two-hour blood glucose (2hBG), total cholesterol (TC), triglycerides (TG), and homeostatic model assessment of insulin resistance (HOMA-IR) compared to those in the control group. Conversely, the homeostatic model assessment of β cell function (HOMA-β) was significantly lower in the GDM group compared to the control group. These differences were statistically significant ($P < 0.05$), as indicated in Table 2.

<table>
<thead>
<tr>
<th>Index</th>
<th>Control group ($n = 42$)</th>
<th>GDM group ($n = 42$)</th>
<th>$t$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG</td>
<td>4.52 ± 0.50</td>
<td>6.34 ± 0.89</td>
<td>6.398</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>FINS</td>
<td>7.87 ± 1.64</td>
<td>13.41 ± 2.37</td>
<td>4.256</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>2hBG</td>
<td>6.03 ± 1.35</td>
<td>8.33 ± 1.09</td>
<td>5.362</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>TC</td>
<td>4.31 ± 1.02</td>
<td>5.94 ± 1.71</td>
<td>4.369</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>TG</td>
<td>1.00 ± 0.32</td>
<td>2.21 ± 0.60</td>
<td>3.568</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.52 ± 0.87</td>
<td>3.99 ± 0.96</td>
<td>6.982</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>1.91 ± 0.60</td>
<td>1.13 ± 0.22</td>
<td>4.699</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

4. Discussion

GDM is a distinct condition that develops during pregnancy, and its incidence rate increases with rising blood glucose levels during pregnancy. Its pathogenesis may be associated with impaired β-cell function in the pancreatic islets of affected individuals, leading to increased insulin resistance. In recent years, both domestic and international scholars have conducted extensive research on the pathogenesis of GDM, uncovering various cytokines related to GDM, such as interleukin-17 (IL-17) and tumor necrosis factor-alpha (TNF-α). These cytokines are thought to be involved in the pathogenesis of GDM [2,3]. Research indicates that IL-17 can activate the JAK-STAT pathway and promote the release of cytokines such as IL-1, IL-6, and TNF-α, which in turn, activate various immune cells such as monocytes, neutrophils, and eosinophils, participating in inflammatory reactions. Furthermore, TNF-α can be activated through the NF-κB pathway, promoting the secretion of IL-10 and TNF-α. IL-17 and TNF-α may also contribute to cell apoptosis through the co-activation of the PI3K/Akt signaling pathway [4-8]. Th17 cells are believed to secrete IL-17 and TNF-α, playing a significant role in the pathogenesis of GDM. Research has shown that the levels of Th17 cells and Treg cell-related factors in the peripheral blood of GDM patients are notably elevated [9-11].

Th17 cells are pivotal in inflammatory and autoimmune responses, with their characteristic cytokine being IL-17. In contrast to other T-cell subtypes, Th17 cells play a vital role in processes such as embryo implantation, placental development, and perinatal immunoregulation. In a normal pregnancy, Th17 cells regulate the immune response during uterine metamorphosis and embryo implantation, contributing to a successful pregnancy. However, in GDM patients, abnormal Th17 cell activation can disrupt the immune balance, leading to an inflammatory response and insulin resistance, ultimately precipitating diabetes mellitus. The relationship between GDM and Th17 cells is observed in several aspects. First, GDM patients exhibit an abnormal increase in the frequency and function of Th17 cells, which can directly contribute to insulin resistance and diabetes. Second, serum levels of Th17 cell-related cytokines, such as IL-17, are significantly elevated in GDM patients, indicating the immune response characteristics associated with Th17 cells in GDM. Finally, Th17 cells may further stimulate inflammatory responses and exacerbate insulin resistance through...
the action of certain cytokines such as IL-23, creating a harmful feedback loop. The regulation of Th17 cells involves specific cytokines (e.g., IL-4, IL-10, etc.) that can inhibit the differentiation and activation of Th17 cells and serve in immunomodulation. Alterations in the expression of these cytokines may affect Th17 cell differentiation and activation in GDM \(^{[12-15]}\), offering potential avenues for prevention and treatment. The impact of GDM on the health of mothers and infants should not be underestimated. Abnormal activation of Th17 cells in GDM may affect placental stability, increasing the risk of miscarriage and preterm delivery. Additionally, maternal inflammatory response and insulin resistance can influence fetal development, increasing the risk of macrosomia and fetal malformations. Therefore, an in-depth study of the role of Th17 cells in GDM may provide insights into the pathogenesis of GDM and suggest new strategies for prevention and treatment.

Treg cells are a subpopulation of T lymphocytes with immunosuppressive functions that play a crucial role in maintaining immune homeostasis and preventing autoimmune diseases. Treg cells can be categorized into natural Treg cells, which differentiate naturally in the thymus, and induced Treg cells, which differentiate from other T cells under specific conditions. Treg cells regulate the body’s immune response by secreting inhibitory cytokines (e.g., IL-10 and TGF-β) and by exerting cell-contact-dependent inhibitory effects.

Treg cells and pro-inflammatory cytokines (e.g., IL-17 and IL-23) interact in the inflammatory response. It has been observed that IL-17 promotes insulin resistance, while Treg cells, through the secretion of IL-10, inhibit the inflammatory response, reducing insulin resistance. In GDM patients, the inflammatory response is heightened, pro-inflammatory cytokine levels are increased, and the number and function of Treg cells are impaired. This results in an amplification of the inflammatory response and exacerbation of insulin resistance, contributing to the development of GDM.

The development of GDM is closely linked to metabolic disorders. Studies have indicated that Treg cells influence energy metabolism by regulating the differentiation of adipocytes, the expression of adipose synthase, and insulin signaling. In GDM, a decrease in the number or dysfunction of Treg cells leads to abnormal adipocyte differentiation, increased fat synthesis, and decreased fat breakdown, causing an imbalance in energy metabolism and worsening the condition of GDM.

Research suggests that Treg cells protect pancreatic islet cells by inhibiting inflammatory and autoimmune reactions. In GDM, an enhanced inflammatory response results in damage to pancreatic β-cells and reduced insulin secretion. Conversely, Treg cells mitigate the inflammatory response in pancreatic islet cells by secreting inhibitory cytokines, protecting them from harm. Treg cells have a significant regulatory effect on the immune system. In GDM, a reduced number or dysfunction of Treg cells leads to immune system imbalance, increased pro-inflammatory cytokines, aggravated insulin resistance, and metabolic disorders. Effective alleviation of the inflammatory response and insulin resistance in GDM can be achieved by regulating the number and function of Treg cells.

5. Conclusion
Th17/Treg cell-related factors are implicated in the pathogenesis of GDM, with Th17 cells promoting the production and maintenance of autoantibodies while inhibiting the production of Treg cells through the secretion of IL-17. Furthermore, it was observed that the expression level of Th17-related factors in the peripheral blood of GDM patients was significantly higher than that in normal pregnant women \((P < 0.05)\). This suggests that Th17-related factors are closely associated with the severity of the disease in GDM patients and may serve as indicators of disease severity. However, this study has some limitations, such as a small sample size and incomplete clinical data for some GDM patients. Consequently, future research should expand the
sample size and conduct statistical analyses to mitigate the influence of other factors.

In summary, Th17/Treg cells play a pivotal role in the onset and progression of GDM. Yet, numerous research gaps remain regarding the relationship between Treg cells and GDM. These include questions concerning the origin and specific phenotype of Th17/Treg cells in the context of GDM, how Th17/Treg cells impact the pathogenesis of GDM by regulating immune and metabolic pathways, and the potential therapeutic approaches involving the modulation of Th17/Treg cells. Subsequent studies should delve into these aspects more deeply to offer fresh insights into the prevention and treatment of GDM.

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