Detection of vitamin D in patients with gestational diabetes mellitus and its effects on insulin resistance, adipokines and TNF-α

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Objective: To detect vitamin D levels in patients with gestational diabetes mellitus and the influence and clinical effect of Vitamin D supplement on insulin resistance, fatty factors and TNF-α.

Methods: A total of 100 patients with GDM from September 2014 to May 2015 in our hospital were selected as object of observation (GDM Group). 52 cases patients with Vitamin D deficiency were randomly divided into two groups. At the same time, 50 cases of healthy pregnant women were selected as normal group. Biochemical indexes of observation group and normal group were detected. Biosynthetic Human Insulin Injection were given to the patients in the control group. The patients in the observation group were supplemented with vitamin D drops on the basis of the treatment of control group. The level of insulin resistance, adipokines and TNF-α were detected in the 2 groups.

Results: FBG, PBG, FINS, TG, Visfatin, TNF-α and HOMA-IR in GDM group were higher compared with that in normal group. 25(OH)D3 and APN in GDM group decreased significantly compared with that in normal group. The comparison of TC, HDL-C and LDL-C in the two groups were not statistically significant. PBG, FINS, HOMA-IR, Visfatin and TNF-α in both groups after treatment significantly decreased compared with that before treatment. PBG, Visfatin and TNF-α in treatment group after treatment decreased more significantly than that in control group. FINS, HOMA-IR in treatment group after treatment increased more significantly than that in control group. The decrease of FBG was not obvious and there was no significant difference between the two groups after treatment. APN and 25(OH)D3 in both groups after treatment significantly increased compared with that before treatment. And they in treatment group after treatment increased more significantly than that in control group. In the correlation analysis, 25(OH)D3 in serum was positively correlated to the level of APN. Also, it was negatively correlated to HOMA-IR, Visfatin and TNF-α.

Conclusion: Vitamin D levels in patients with gestational diabetes mellitus decreased more significantly compared with that in healthy pregnant women. And the patients with vitamin D deficiency have higher risk to get GDM. Vitamin D can treat GDM by regulating the degree of insulin resistance and the level of adipokines. And it has clinical value in the treatment of GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is a common complication in pregnant women, which is easy to cause symptoms such as high blood pressure, macrosomia, neonatal complications and so on. It is a serious threat to maternal and child health. Its pathogenesis is still not clear. Insulin resistance (IR) and pancreatic Islet β Cells dysfunction play the important role in GDM. In addition to genetic, placenta and other factors, adipokines and inflammatory factors are also involved in the occurrence of GDM. Some studies found that vitamin D has the function of controlling blood sugar, affecting the synthesis of adipokines and involving in inflammatory reaction. In this study, the Vitamin D levels of the patients with GDM were detected. And its effects on insulin resistance, adipokines and TNF-α were discussed. We hope to provide theoretical basis for the
mechanism of vitamin D.

2. Materials and methods

2.1 General information

A total of 100 patients with GDM from September 2014 to May 2015 in our hospital were selected as object of observation (GDM Group). Selection criteria: (1) all pregnant women were consistent with the diagnosis standard of gestational diabetes mellitus in the “Guidelines for the diagnosis and treatment of pregnancy complicated with diabetes” (2) All pregnant women had no diabetes, hypertension, polycystic ovary syndrome, liver and kidney dysfunction, autoimmune and other chronic diseases. (3) The patients took vitamin D and calcium preparations in the last 6 months and other drugs such as insulin and analogues. Then, we selected the other 50 healthy pregnant women in our hospital as the normal group. (The results of their blood glucose test do not meet the criteria of “Guidelines for the diagnosis and treatment of pregnancy complicated with diabetes” issued by Chinese Medical Association in 2014). In the GDM group, there were 100 pregnant women, aged from 23 to 35 years old, with an average 30 years. The body weight was 56 to 73.5 kg, with an average of (60.5±6.3) kg. They were at 24 to 26 weeks of pregnancy, with an average of (25.4±1.3) weeks. In the normal group, there were 50 pregnant women, aged from 24 to 32 years old, with an average 29 years. The body weight was 56 to 75.5 kg, with an average of (65.5±8.3) kg. They were at 24 to 28 weeks of pregnancy, with an average of (26.4±2.2) weeks. There had no differences in the age, weight and gestational weeks, and there was no statistical significance ($P>$0.05).

2.2 Method

2.2.1 Diagnostic criteria of GDM

75 g OGTT (oral glucose tolerance test) was performed during pregnancy. The diagnostic criteria are: Venous blood glucose values of the women were lower than 5.1 mmol/L, 10.0 mmol/L and 8.5 mmol/L after fasting, drinking glucose water, 1 h and 2 h later. If any of the detection indexes exceeds the upper limit standard, they can be determined as gestational diabetes mellitus. This study was approved by the ethics committee and the subjects have signed the informed consent.

2.2.2 Diagnostic criteria of vitamin D deficiency

Serum vitamin D levels were detected in the 100 cases of patients with GDM. The Vitamin D grading standard: vitamin D deficiency ($25(OH)D_3$ $\leq 25$ nmol/L), vitamin D insufficient ($25(OH)D_3$ $< 25$ $(OH)D_3$ $\leq 75$ nmol/L), vitamin D adequate ($25(OH)D_3$ $> 75$ nmol/L). The result showed that patients with vitamin D deficiency accounts for 52%, vitamin D insufficient accounts for 24%, vitamin D suitable accounts for 8% and vitamin D adequate accounts for 16%. The 52 cases of vitamin D deficiency were divided into treatment group (n=26) and control group (n=26).

2.2.3 Intervention methods

Control group: Biosynthesis Short acting Neutrul Insulin Injection were given to the patients on the basic of planning diet and proper exercise (Chinese medicine standard word: J20100036 Novonordisk Pharmaceutical Co. Ltd), subcutaneous injection half an hour before 3 meals. The initial dose were 0.3-0.4 U/kg.d. After that, we adjusted the injection dose appropriately according to the blood glucose, continuous treatment for 1 months.

Observation group: The patients in the observation group were supplemented with vitamin D drops on the basis of the treatment of control group. (Chinese medicine standard word: H20113033 QingDao city ShuangQi Pharmaceutical Co. Ltd), 400 U/time, 2 times/d, continuous treatment for 1 months.

2.3 Detection method

4 mL and 3 mL of fasting peripheral venous blood of 150 cases (100 cases in GDM group and 50 cases in normal group) were collected for biochemical index detection and centrifugation. Centrifugal extraction of serum placed in the upper -80℃ refrigerator prepared test. In addition, venous blood of 150 pregnant women were collected 2 h after meals. Set aside.

(1)Detection of blood fat and blood glucose: Fasting blood glucose (FBG), postprandial 2 h blood glucose (PBG), total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) were detected by Olmpus and the 5 400 type of full automatic biochemical analyzer. Fasting insulin (FINS) and postprandial 2 h insulin (PINS) were detected by chemiluminescent immunoassay. The level of $25(OH)D_3$ was detected by HPLC. Insulin resistance index (HOMA-IR)=FINS×FBG/22.5

(2)Detection of adipokines and TNF-α: Adipokines include adiponectin (APN) and adiponectin (Visfatin). Centrifugal extraction of serum placed in the upper -80℃ refrigerator prepared for testing APN, Visfatin and TNF-α. ELISA was used. The operation process was performed according to the instruction strictly. The kits were purchased from Shenzhen JingMei Biological Technology Co., Ltd.

2.4 Statistical Methods
SSPS 17.0 statistical package was conducted for statistical analysis. Relevant data indexes were described as mean ± standard deviation. The means comparison between the two samples was done by the t-test. And counting material used a Chi-square test. Linear correlation regression analysis were used to do the correlation analysis. Values of $P<0.05$ were considered to be statistically significant.

3. Results

3.1 Comparison of biochemical indicator between the GDM group and the normal group

Comparison of biochemical indexes in two groups can be seen in table 1. FBG, PBG, and FINS in the GDM group were higher than that in the normal group obviously ($P<0.01$). TC, LDL-C in the GDM group were also higher than that in the normal group. HDL-C in the GDM group was lower than that in the normal group. But the difference between 2 groups was not obvious ($P>0.05$). TG, Visfatin, TNF-α and HOMA-IR in the GDM group increased more significantly compared with that in normal group ($P<0.05$). 25(OH)D3, and APN in the GDM group decreased more significantly compared with that in normal group ($P<0.05$).

Table 1.

Comparison of biochemical indicator between the GDM group and the normal group.

<table>
<thead>
<tr>
<th>Detection indexes</th>
<th>GDM (n=100)</th>
<th>Normal (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mmol/L)</td>
<td>6.90±1.49*</td>
<td>4.93±0.40</td>
</tr>
<tr>
<td>PBG (mmol/L)</td>
<td>11.06±1.87*</td>
<td>6.27±0.89</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>3.82±0.38</td>
<td>3.81±0.32</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.15±0.36</td>
<td>1.93±0.31</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.28±0.35</td>
<td>1.31±0.23</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.49±0.27</td>
<td>2.40±0.23</td>
</tr>
<tr>
<td>FINS (mU/L)</td>
<td>17.56±2.45*</td>
<td>13.76±1.52</td>
</tr>
<tr>
<td>25(OH)D3 (nmol/L)</td>
<td>27.31±5.79</td>
<td>38.64±8.18</td>
</tr>
<tr>
<td>APN (pg/mL)</td>
<td>140.62±26.39</td>
<td>208.40±41.66</td>
</tr>
<tr>
<td>Visfatin (ng/mL)</td>
<td>6.60±1.01</td>
<td>4.15±0.86</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>43.71±8.65</td>
<td>28.57±6.37</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.22±0.68</td>
<td>1.59±0.35</td>
</tr>
</tbody>
</table>

Note: *means that compared with the normal group, the difference was statistically significant $P<0.05$; #means the difference was statistically significant, $P<0.01$.

3.2 Comparison of blood glucose, insulin and the level of insulin resistance between the treatment group and control group before and after treatment

Blood glucose, insulin and the level of insulin resistance can be seen in table 2. The comparison of FBG, PBG, FINS and HOMA-IR in the treatment group and control group before treatment was not statistically significant ($P>0.05$). FBG, PBG, FINS and HOMA-IR in both groups after treatment decreased compared with that before treatment and the difference between 2 groups after treatment was not statistically significant ($P>0.05$). Also, the PBG in treatment group decreased more significantly compared with that in control group after treatment ($P<0.05$). FINS and HOMA-IR in treatment group increased more significantly compared with that in control group ($P<0.05$).

3.3 Comparison of 25(OH)D3, adipokines and TNF-α between the treatment group and control group before and after treatment

The comparison of 25(OH)D3, APN, Visfatin and TNF-α in the treatment group and control group before treatment was not statistically significant ($P>0.05$). 25(OH)D3 in both groups after treatment increased in different degrees compared with that before treatment. But the increase in the treatment group was not obvious ($P>0.05$). 25(OH)D3 in the treatment group after treatment increased significantly compared with that before treatment. Also, 25(OH)D3 in the treatment group increased more significantly compared with that in control group ($P<0.05$). Visfatin and TNF-α in both groups decreased significantly compared with that before treatment. And the two in the treatment group decreased more significantly compared with that in control group. See table 3.

3.4 Correlation analysis of 25(OH)D3 and HOMA-IR, adipokines and TNF-α in GDM group after treatment

25(OH)D3 in GDM group was positively correlated to the level of APN ($r=0.318$, $P<0.05$). Also, it was negatively correlated to

Table 2.

Comparison of FBG, PBG, FINS and HOMA-IR in the two groups before and after treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>FBG (mmol/L)</th>
<th>PBG (mmol/L)</th>
<th>FINS (mU/L)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Before treatment</td>
<td>8.04±0.67</td>
<td>11.23±1.47</td>
<td>13.56±1.25</td>
<td>4.23±0.56</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>7.31±0.62</td>
<td>7.79±0.98</td>
<td>11.52±0.61</td>
<td>3.34±0.76</td>
</tr>
<tr>
<td>Control</td>
<td>Before treatment</td>
<td>7.99±1.01</td>
<td>11.35±1.57</td>
<td>13.87±1.24</td>
<td>4.42±1.63</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>7.59±0.53</td>
<td>9.16±1.33</td>
<td>9.84±1.03</td>
<td>2.83±1.20</td>
</tr>
</tbody>
</table>

Note: compared with control group before treatment, $P<0.05$; compared with control group after treatment, $P<0.05$. 
The research shows that the 25(OH)D3 in the serum is related to vitamin D, which is commonly used as a measure of vitamin D. Symptoms of offspring. In addition, 25(OH)D3 plays an important role in the preterm birth and the rickets, bone density, asthma and other symptoms of offspring. The abnormality of adipokines and inflammatory factors can cause IR, which aggravates the occurrence of GDM. The research shows that vitamin D can regulate adipokines to inhibit the formation of fat cells, which slows down the IR of peripheral tissues. By promoting the synthesis of APN, inhibiting the expression of TNF-α (which can inhibit the phosphorylation of insulin receptor substrate-1, causing IR) and other inflammatory factors, it can improve the sensitivity of insulin. Visfatin, a new type of adipokine, has the function of insulin and can active insulin receptor. The research showed that the APN in the treatment group increased significantly, Visfatin and TNF-α decreased significantly compared with that in the control group. In the correlation analysis, 25(OH)D3 in GDM group was positively correlated to the level of APN. Also, it was negatively correlated to HOMA-IR, Visfatin and TNF-α.

In conclusion, Vitamin D levels in patients with gestational diabetes mellitus decreased more significantly compared with that in healthy pregnant women. And the patients with vitamin D deficiency have higher risk to get GDM. Vitamin D can treat GDM by regulating the degree of insulin resistance and the level of adipokines. And it has clinical value in the treatment of GDM.

### Table 3.
Comparison of 25(OH)D3, APN, Visfatin and TNF-α in the two groups before and after treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>APN (pg/mL)</th>
<th>Visfatin (ng/mL)</th>
<th>TNF-α (pg/mL)</th>
<th>25(OH)D3 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Before treatment</td>
<td>308.19±17.81</td>
<td>8.91±0.35</td>
<td>117.47±6.98</td>
<td>15.36±2.29</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>321.09±17.25</td>
<td>6.18±0.41</td>
<td>64.61±10.16</td>
<td>24.39±2.78</td>
</tr>
<tr>
<td>Control</td>
<td>Before treatment</td>
<td>305.29±17.22</td>
<td>9.01±0.38</td>
<td>119.68±9.18</td>
<td>15.25±2.01</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>311.12±18.41</td>
<td>7.98±3.11</td>
<td>103.1±8.36</td>
<td>17.41±1.56</td>
</tr>
</tbody>
</table>

Note: compared with control group before treatment, *P*<0.05; compared with control group after treatment, *P*<0.05.

HOMA-IR, Visfatin and TNF-α (r = -0.569, -0.325 and -0.282 respectively, *p*<0.05 all).

### 4. Discussion

The incidence rate of GDM in pregnant women is increasing, which is affected by genetic, environmental and other factors. From the beginning of early pregnancy, insulin sensitivity of pregnant women can be decreased by 50%–60% compared with that before pregnancy, resulting in the utilization ratio of insulin to glucose decreased significantly. So, to maintain the body’s normal glucose level, the secretion of insulin tripled, which is extremely easy to cause physiological IR. In the study of the correlation of GDM, research shows that abnormalities of vitamin D are closely related to the dysfunction of pancreatic β-cell and IR. After Vitamin D binding to its receptor, it will regulate and promote the secretion of ancreatic islet β-cell, which improves the utilization of the glucose and the sensitivity of insulin. Also, the study confirmed that in addition to abnormal glucose metabolism, the patients with GDM also has a mild inflammatory reaction. By detecting the biochemical indexes of GDM group and the normal group, the research found that the blood glucose, serum insulin level and insulin resistance of the pregnant women in the GDM group significantly increased compared with that in normal group. The vitamin D decreased significantly, the adipokine RBP4, Visfatin and TNF-α increased significantly and the APN decreased significantly. The results are consistent with the research content above.

Vitamin D deficiency is common in pregnant women. The vitamin D in pregnant women decreased obviously compared with the non pregnant women. In the United States, there are 2/3 pregnant women with vitamin D deficiency. The pregnant women with Vitamin D deficiency have high risk of pregnancy complications (such as preeclampsia). 25(OH)D3 is the main active ingredient of vitamin D, which is commonly used as a measure of vitamin D. The research shows that the 25(OH)D3 in the serum is related to the preterm birth and the rickets, bone density, asthma and other symptoms of offspring. In addition, 25(OH)D3 plays an important role in the metabolism of glucose and insulin, and the proper supplementation of vitamin D can increase the sensitivity of insulin. In the research, the patients with vitamin D deficiency were given vitamin D supplement on the basis of the treatment of insulin aspart. After treatment, blood glucose, insulin levels and insulin resistance decreased, compared with the control group, the level of blood glucose decreased, insulin levels and insulin resistance increased. Vitamin D supplementation can repair the function of islet β-cells, stimulate the expression of insulin receptor and improve the utilization rate of glucose, which can alleviate insulin resistance.

The abnormality of adipokines and inflammatory factors can cause IR, which aggravates the occurrence of GDM. The research shows that vitamin D can regulate adipokines to inhibit the formation of fat cells, which slows down the IR of peripheral tissues. By promoting the synthesis of APN, inhibiting the expression of TNF-α which can inhibit the phosphorylation of insulin receptor substrate-1, causing IR) and other inflammatory factors, it can improve the sensitivity of insulin. Visfatin, a new type of adipokine, has the function of insulin and can active insulin receptor. The research showed that the APN in the treatment group increased significantly, Visfatin and TNF-α decreased significantly compared with that in the control group. In the correlation analysis, 25(OH)D3 in GDM group was positively correlated to the level of APN. Also, it was negatively correlated to HOMA-IR, Visfatin and TNF-α.

In conclusion, Vitamin D levels in patients with gestational diabetes mellitus decreased more significantly compared with that in healthy pregnant women. And the patients with vitamin D deficiency have higher risk to get GDM. Vitamin D can treat GDM by regulating the degree of insulin resistance and the level of adipokines. And it has clinical value in the treatment of GDM.

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[4] Lacroix M, Battista MC, Doyon M. Lower vitamin D levels at first
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