THE EFFECT OF VITAMIN D AND CALCIUM SUPPLEMENT ON HBA1C AND BLOOD PRESSURE IN PATIENTS WITH TYPE 2 DIABETES: A QUASI-EXPERIMENTAL STUDY

INTRODUCTION

Diabetes is one of the stressful diseases which has been globally on the rise. According to the investigations carried out by Casagrande in 2010, 285 million people were affected by diabetes\(^1\). It is predicted that this number reaches 439 million patients by 2030\(^2\). Furthermore, 90-95% of them are affected by type 2 diabetes\(^3\). Diabetes can cause metabolic bone disorders and bone defects due to the unusual imbalance between bone formation and absorption. Bone marrow mesenchymal stem cells form the base of cytology for bone regeneration, especially bone formation and multiplication\(^4\). They can also change in pathological diabetic microenvironments due to different mechanisms. Type 2 diabetes sugar is associated with the inhibition of ossification of bone marrow mesenchymal stem cells\(^5\).

The results of a study indicate that the serum level of vitamin D is lower in diabetic patients than in healthy people\(^6\). Moreover, Hossein Nejad et al. showed that the loss of vitamin D was more prevalent, especially in gestational diabetes\(^7\). Other studies point out that vitamin D can also have a role in insulin secretion and dysfunction\(^8\).
In fact, vitamin D is known to have a role in maintaining glucose tolerance and normal insulin secretion. The presence of vitamin D receptors and vitamin-D-dependent calcium-binding proteins in pancreatic beta cells indicates the fact that beta cells are the target tissues of vitamin D\(^9\). On one hand, it has been reported that diabetes increases the risk of low bone minerals and bone loss in patients\(^8\). Because of having a major role in calcium homeostasis, vitamin D is one of factors influencing the turnover and formation of bone mass. The loss of vitamin D decreases the bone mass and finally results in osteomalacia\(^10\). The studies conducted on the calcium serum levels of these patients indicated significant reductions in ionized serums in comparison with healthy people. The prospective cohort studies show that the reduced risk of diabetes is negatively related to calcium consumption and vitamin D intake. Such observations lead to the idea that improving the levels of vitamin D and calcium in patients with type 2 diabetes can improve their glycemic statuses\(^10\).

In this regard, previous studies show the direct relationship between HbA1c and blood sugar\(^11\). Glycosylated hemoglobin (HbA1c) is a marker of glucose in the diagnosis of type 2 diabetes. Measuring HbA1c is essential for diagnosis and displaying glycemic control in both types 1 and 2 diabetes. The HbA1c test results reflects the average glycemic index in 100-120 days. It is the value predicted for the development of macro and micro vascular complications of diabetes. In addition to the efficiency of HbA1c in the diagnosis of diabetes, this marker is an important index used for measuring microvascular complications and plasma glucose\(^12\).

In addition, hypertension is one of the risk factors which accelerates the development of diabetes if it is not controlled. Therefore, preventing and treating hypertension would decrease mortality and morbidity. Another study indicated that every 10-mmHg decrease in systolic blood pressure reduces the risk of each of the diabetic complications by 12% and the risk of dying from diabetes by 15% (13). In this regard, the 25-hydroxy level of cholecalciferol (the precursor of vitamin D in the body) is low in the majority of patients with hypertension. Therefore, many researchers believe that the vitamin D and calcium supplementation can be useful for correcting the symptoms such as hypertension which causes some health problems\(^14\).

Considering vitamin D reduction in diabetic patients and the effects which the loss of vitamin D may have on blood calcium level, it appears essential to investigate various treatment methods to resolve different problems of these patients. Moreover, one of the complications of diabetes is hypertension which can cause diabetes itself, and the most important index of diabetes control is HbA1c. Therefore, this study was conducted to investigate the effect of vitamin D and calcium supplement on HbA1c and blood pressure in patients with type 2 diabetes.

**Materials and methods**

**Participants**

This quasi-experimental study was conducted on 48 patients with type 2 diabetes visiting the Diabetes Clinic of Imam Khomeini Hospital from March to August 2016 in Zabol city southeast of Iran. The following formula was used to calculate the sample size with 80% of power and 5% of test error for each group. Sampling method was Purposive sampling.

\[
\hat{n} = \left(\frac{z_{1-\alpha/2} + z_{1-\beta}}{e}\right)^2 \left(\frac{p(1-p)}{2}\right) - 0.086 \times 0.84\left(0.087^2 + 0.06^2\right) - 13.25 - 14
\]

\[14 + 1.4 = 16\]

**Inclusion criteria**

Inclusion criteria were being aged between 30 and 70 years old, having type 2 diabetes, having an FBS lower than 200\(^15\).

**Exclusion criteria**

Exclusion criteria were having liver, renal or bone diseases, taking any vitamin D and calcium supplements and diuretics in the past three months\(^16\).

**Sampling and Procedure**

Considering the decline risk of subjects, 16 individuals were selected for each group (total: 48). There were three groups including Group A (receiving vitamin D supplements and routine treatments), Group B (receiving calcium and vitamin D supplements and routine treatments), and Group C (receiving placebos and routine treatments). For a month, they were orally treated with 50000 units of vitamin D supplement and 150-250 milligrams of calcium on a daily basis. The purposive random sampling method employed to select the first, second and third individuals for Group A, Group B, and Group C, respectively.
First, the subjects were referred to a laboratory for measuring the serum levels of vitamin D and calcium, FBS, blood pressure, and HbA1c. The enzyme-linked immunosorbent assay (ELISA) was conducted using a Monobind kit (made in the USA) to measure the serum levels of vitamin D in ML/N within the normal range (30-100). Furthermore, the Selectra method was employed using a Hayan kit (made in Iran) to measure the calcium serum level within the normal range (8.6-10.3). Then a Pishtaz kit (made in Iran) was utilized in the Selectra method to conduct the HbA1c test within the normal range (4-6). A Hayan (made in Iran) kit was also used in the Selectra method to measure FBS within the normal range (70-115). Blood pressure was measured by one individual using the cuffed sphygmomanometer ALPK2 (made in Japan). Vitamin D and calcium below 30 and 8.5 nanograms were regarded as deficiency, respectively. Systolic blood pressure higher than 130 mmHg or diastolic blood pressure higher than 85 mmHg were regarded as hypertension. After conducting the primary tests and evaluations, Group A received vitamin D and routine treatments, and Group B received calcium and vitamin D supplements as well as routine treatments. Group C (control group) received only placebos and routine treatments. Then the HbA1c test and blood pressure were checked again after a specific period of time. The study has been approved by the Research deputy of Zabol University of medical science. Written informed consent was obtained from all patients.

Statistical analysis

Descriptive tests of the frequency, mean and standard deviation (SD) were used to describe sample demographics. ANOVA and paired t-tests tests were applied to interpret results. SPSS Version 18.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Confidence interval of 95% and a significance level of P-value less than 0.05 was considered significant.

Results

The average age of patients was 46 years old in all three groups. Women and men accounted for 58.3% and 41.7% of the population, respectively. The Shapiro Wilk test was carried out to ascertain whether the blood pressure and HbA1c were normal. According to Table 1, the results indicate that this test is significant for blood pressure (p=0.001).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>K-S Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure</td>
<td>13.6</td>
<td>1.58</td>
<td>0.182</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>7.37</td>
<td>0.8</td>
<td>0.098</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 1: The Kolmogorov-Smirnov Test.

Therefore, blood pressure does not have a normal distribution, and parametric techniques cannot be used to investigate it. However, it is not significant for HbA1c (p=0.2) which has a normal distribution, and parametric analyses can be used to investigate it. Blood pressure decreased on average in the first and second groups after the intervention, although no significant decrease was observed in Group C (Figure 1). HbA1c decreased averagely in all three groups; however, no significant decrease was observed in Group C (Figure 2).

Figure 1: Blood Pressure Before and After the Intervention in the Three Groups.

Figure 2: HbA1c Before and After Intervention in the Three Groups.

The intergroup and intragroup comparisons drawn by conducting the nonparametric Wilcoxon test indicated that the three groups were significantly different from each other in blood pressure (p<0.01 and p=0.05). Given the paired or dependent t-test as shown in table 2, HbA1c decreased significantly by 1.76 in Group A. It decreased by 1.85 and 1.4 in Groups B and C, respectively. These data show that the interventions decreased HbA1c levels in the three groups. According to ANOVA in the intragroup comparison of HbA1c after the intervention, the three groups were significantly different.
In other words, HbA1c decreased among all of the subjects (28.08 ± 2 to 20.50 ± 2, p = 0.122). Considering the Tukey’s test in the intergroup comparison of HbA1c after the intervention, Groups A and B were not significantly different (p=0.08). However, there was a significant decrease in HbA1c (1.88) in Group A compared with Group C. The intergroup comparison did not show any significant differences between A and B (p=0.08). However, there was a significant decrease in HbA1c (2.41) in Group C compared with Group B, something which shows that Group B had the greatest effect on the decrease in HbA1c (Table 3).i

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD before the Intervention</th>
<th>Mean ± SD after the Intervention</th>
<th>Mean Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.47 ± 0.8</td>
<td>0.5 ± 30.7</td>
<td>-1.76</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>0.617 ± 0.04</td>
<td>0.519 ± 0.57</td>
<td>-1.85</td>
<td>0.000</td>
</tr>
<tr>
<td>C</td>
<td>8.99 ± 2.36</td>
<td>7.59 ± 0.36</td>
<td>-1.4</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 2: The comparison of HbA1c changes among the three groups before and after the intervention.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Mean Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>-0.52</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-1.88</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>-0.52</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>-2.41</td>
<td>0.000</td>
</tr>
<tr>
<td>C</td>
<td>A</td>
<td>1.88</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.41</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3: The intergroup comparison of HbA1c after the intervention.

Discussion

In this study, blood pressure decreased on average in Groups A and B after the intervention. However, it did not change much in Group C. HbA1c decreased in the three groups, although Group C (control group) did not show a significant decrease, something which points out that vitamin D and calcium supplementations had an effective role in decreasing blood pressure in patients with type 2 diabetes. According to the research results, calcium and vitamin D supplement had a greater effect on reduction in blood pressure and HbA1c than vitamin D and placebos did. Freud et al. investigated the effect of vitamins C and E supplement on reduction in blood pressure(19). Like our study, vitamin supplements could reduce blood pressure in patients with type 2 diabetes. Anyanwu et al. conducted a study to investigate the effect of vitamin D on blood sugar control in people with type 2 diabetes. They concluded that vitamin D had a very effective role in the reduction of HbA1c in people with type 2 diabetes(18).

Talay et al. prove the positive effect of vitamin D the reduction of FBS, insulin serum, and HOMA-IR. However, our study investigated the effect of vitamin D on HbA1c, which is an index of diabetes. In both studies, vitamin D had favorable effects on the reduction of diabetes indices, something which shows the higher the initial level of vitamin D is, the greater the effect it has on the reduction of diabetes indices(19). Despite observational studies, there are some quantitative studies on the effect of treatment with calcium and vitamin D supplements on the glycemic control of patients with type 2 diabetes. It is noteworthy that the effects of calcium and vitamin D supplementations were separately investigated in previous studies, although the metabolisms of calcium and vitamin D are related to each other very much(20). Pittas et al. indicated that the supplementations of vitamin D and calcium improved the glycemic profile of people with normal glucose tolerance for three years(18).

In another study, the daily supplementations of vitamin D improved insulin secretion when it was accompanied by the supplementation of calcium twice a day(21). Nevertheless, our study is the first work of research investigating the effects of calcium and vitamin D supplementations on HbA1c and blood pressure exclusively in patients with type 2 diabetes. The two previous studies were conducted on relatively healthy people or those having blood sugar disorders and not taking hypoglycemic agents. Therefore, our findings show that the supplementation of calcium and vitamin D improved the better control of HbA1c and blood pressure in patients with type 2 diabetes. Foroughi et al. investigated the effect of vitamin D on the control of blood sugar indices. They found out that vitamin D supplement had useful effects on the control of blood sugar and other indices; however, they did not emphasize the effects on HbA1c. In their study, other blood sugar indices decreased, something which inspired our study.

According to the results, this index decreased, too, after using vitamin D supplement(22). Vitamin D has a greater effect on the cellular genome or the genome elements responding to vitamin D. Therefore, the effects of vitamin D at these levels.
can influence the cellular genome to create responses at the levels or receptors or intercellular messengers to convey the insulin message to the cell. As a result, diabetic indices decrease. It appears that increasing calcium through vitamin D in muscular tissues can be responsible for increasing glucose transfer to muscles. Thus, glucose is consumed on another path, and it decreases blood sugar finally. Moreover, vitamin D regulates the environmental core receptors which play an important role in insulin sensitivity\(^{(22, 23)}\).

**Limitation**

The limitation of present study was hard access to samples.

**Conclusion**

The research results indicate that treatment with vitamin D and calcium supplement has greater effects on the reduction of blood pressure and HbA1c in patients with type 2 diabetes. In fact, findings help understand the roles of vitamin D and calcium in the control of blood sugar in patients with type 2 diabetes. Therefore, it is recommended to investigate the levels of vitamin D and calcium in patients with type 2 diabetes. Vitamin D and calcium supplements should be prescribed to improve the control of blood pressure and HbA1c in case of deficiency. It also appears necessary to conduct more studies to ascertain the appropriate doses of vitamin D and calcium and increase the treatment periods in which patients with type 2 diabetes are treated with such supplements.

**References**


7) Holick MF. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. Current Opinion in Endocrinology, Diabetes and Obesity. 2002; 9(1): 87-98.


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