INTRODUCTION

Diabetes mellitus (DM) is a persistent hyperglycemia caused by defects in insulin secretion, insulin action, or both [1]. Vitamin D, known primarily as a hormone of bone metabolism, can affect the transcription of a number of genes. In addition, Vitamin D may inhibit the renin-angiotensin system, reduce parathyroid hormone levels, decrease coagulation, reduce inflammation thereby reducing atherosclerosis, and increase insulin production [2]. Other studies found an association with asthma [3]. This persistent hyperglycemia may lead to long-term damage and loss of functions of many organs [4]. Symptoms of hyperglycemia include polyuria, polydipsia, polyphagia, blurred vision, and decrease in weight and to less common are blurred vision, and decrease in weight and to less common are blurred vision, and decrease in weight.

Stressful environmental, and immunological factors can cause the destruction of the pancreatic β-cells and insulin deficiency in T1DM, and mostly, this process results from autoimmune β-cell destruction, although not all cases have the evidence of islet-directed autoimmunity [7].

Individuals with a genetic susceptibility have normal β-cell mass at birth but start to lose their β-cells secondary to the autoimmune destruction that may occur over months to years, and this autoimmune process is thought to be triggered effect and continuously progress through a β-cell-specific molecule. The disease becomes clinically clear after the development of immunologic markers in the presence of the stimulating event. After that, β-cell mass begins to decline or about 80% of β-cells are destroyed, and insulin secretion becomes gradually impaired, but the glucose tolerance remained normal [8]. In vitro study suggested that interleukin-1 (IL-1) and tumor necrosis factor, two cytokines mostly produced by macrophages, induce structural changes of β-cells and suppression of their insulin releasing ability [9]. The children were given 2000 IU of Vitamin D daily in early childhood associated with reduced risk of T1DM. However, the mechanism still unclear [10,11]. Studies on a systemic defect in immune regulation suggested that natural killer T-cells (NKTC) activity and antigen presenting cells (APC) function are all decreased in diabetic patients [12]. On the other hand, lack of NKTC activity also results in the fewer amount of IL-4 production and subsequently lower amount of APC activation than required to maintain the normal immunity functions [13]. Vitamin D influences the pathogenesis, risks, and complications of DM. Studies have shown that Vitamin D supplementation in infancy reduces the risk of developing T1DM later in early adulthood [14]. Vitamin D receptors have immune-modulating effects, and the development of T1DM may be associated with polymorphisms in the Vitamin D receptor gene [15,16].
25 patients receive 2000 IU Vitamin D, once daily only in addition to the normal insulin regimen and Group 2 with 25 patients receive insulin ( soluble and lente) twice daily.

From all eligible subjects, demographic (age and gender) and laboratory markers (Vitamin D, ionized calcium, alkaline phosphatase [ALP], IL-1β, and IL-4) were recorded at baseline and after 45 and 90 days for both the groups.

About 5 ml of fasting venous blood collected and serum stored at −20°C after separation, and this sample was used to assess the serum levels of fetal bovine serum, hemoglobinA1C, ionized calcium, ALP, Vitamin D, IL-1β, and IL-4, as illustrated in Table 1.

Statistical analysis
Data were presented in simple measures of frequency, mean, and standard error. The significance of the difference of different means (quantitative data) was tested using students’ t-test for the difference between two independent means or paired t-test for difference of paired observations (or two dependent means). The significance of the difference of different percentages (qualitative data) was tested using Pearson Chi-square test with application of Yate’s correction or Fisher’s exact test whenever applicable. SPSS version 20 (USA, Chicago, IL) software package was used for the statistical analysis. Statistical significance was considered whenever the P value was ≤0.05.

RESULTS

There was no significant difference in age and gender between Groups 1 and 2, as illustrated in Table 2.

Serum Vitamin D3 was significantly higher in Group 1 compared to Group 2 after 45 and 90 days, and in Group 1, serum Vitamin D increased from 45 to 90 days, but it was not statistically significant, while for Group 2, there was a significant reduction in serum Vitamin D, from 45 days to 90 days. In Group 1, serum IL-1β significantly reduced after 90 days, while no significant change in Group 2 was observed. In Group 1, serum IL-1β was significantly increased after 90 days, while in Group 2, there was a significant reduction in IL-1β after 90 days, as illustrated in Table 3.

There was no significant association between Vitamin D with inflammatory markers after supplementation of Vitamin D, as illustrated in Table 4.

Table 1: List of various laboratory markers used in the study with its supplier

<table>
<thead>
<tr>
<th>Suppliers</th>
<th>Diagnostic kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANDOX, UK</td>
<td>Fasting blood glucose kit (manual), ELISA</td>
</tr>
<tr>
<td>Cobas 411 (ROCH), Germany</td>
<td>Insulin kit (auto-analyzer)</td>
</tr>
<tr>
<td>RANDOX, UK</td>
<td>Ca kit, ELISA</td>
</tr>
<tr>
<td>bioMerieux, France</td>
<td>ALP kit, ELISA</td>
</tr>
<tr>
<td>bioMerieux, France</td>
<td>Vitamin D3 kit, auto-analyzer</td>
</tr>
<tr>
<td>Bio-Rad, USA</td>
<td>HbA1c kit auto-analyzer</td>
</tr>
<tr>
<td>Elabscience, China</td>
<td>IL-1β kit, ELISA</td>
</tr>
<tr>
<td>Elabscience, China</td>
<td>IL-4 kit, ELISA</td>
</tr>
</tbody>
</table>

ALP: Alkaline phosphatase, IL: Interleukin

Table 2: Demographic data of the patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>T1DM and Vitamin D</th>
<th>T1DM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8.2±2.5</td>
<td>8.8±2.6</td>
<td>0.410</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17 (68%)</td>
<td>13 (52%)</td>
<td>0.248</td>
</tr>
<tr>
<td>Male</td>
<td>8 (32%)</td>
<td>12 (48%)</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean±SD, T1DM: Type 1 diabetes mellitus

DISCUSSION

In the current study serum, IL-1β was significantly decreased in patients receiving Vitamin D, in addition to insulin while those who did not receive Vitamin D, had a non-significant increase in IL-1β, and also, serum IL-1β was significantly increased after 90 days for those receiving Vitamin D, while those receiving insulin only had a significant decrease in IL-1β serum levels.

It is understandable now that Vitamin D displays an anti-inflammatory effect as one of its major roles, and its deficiency is often accompanied with increased risk of any inflammatory, immunological, and autoimmune disorders. The active biological metabolite of Vitamin D [Vitamin D3] is a potent regulator of the immune response and functions by binding to the Vitamin D receptor with immunomodulatory properties [17].

Contact et al. study shows an inhibitory effect for Vitamin D3 on Th1 cell activation where these cells play an essential part in immune and inflammatory diseases by producing inflammatory cytokines, such as IL-1, and thus, Vitamin D3 plays an important key role in the inhibition, differentiation, and proliferation of both T and B cells, reduces polarity of Th1 cells to Th2 cells, and inhibits the generation and production of cytokines which leads to destruction of pancreatic islet cells and then induces endoplasmic reticulum stress and apoptosis by pro-inflammatory cytokine [18-20]. Furthermore, these cells produce Vitamin D3 which then regulates their proliferation and function [21].

Our findings were in agreement with Cantorna et al. study that production of the Th2-associated cytokine IL-4 could be upregulated by...
Vitamin D3 treatment [22], also Vitamin D3 inhibited Th1 cell expansion and cytokine production and resulted in Th2 cell expansion and increased IL-4 production [22,23] with promoting the Th2 cytokine production of IL-4, for that reason orientated T cell response towards Th2 dominance thus it increase the production of more anti-inflammatory Th2 cytokines (IL3, IL4, IL5, IL10) and inhibits Th1 response activity seems to play a major role in the treatment of autoimmune diseases [24]. The present study compatible with Virtanen et al. studies showed that Vitamin D3 administration had been shown to increase IL-4 and improve Vitamin D3 that may make the clear deviation in the immune system, specifically in THC [25]. The role of regulation IL-4 production is controversial as Gregori et al. showed that the inhibition of both Th1 and Th2 cell cytokine production, including the inhibition of IL-4 [which then may trigger humoral immunity leads to antibody production] [26].

CONCLUSION

Daily vitamin D₃ in addition to insulin offers favourable immunological effect in paediatric patients with Type 1 DM.

CONFLICTS OF INTEREST

No financial, personal, or any other type of interest will present a conflict concerning this work.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally in this research.

REFERENCES

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