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], and other study 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 insufficient growth and increase exposure to infections [1,5]. Type 1 DM (T1DM) accounts for 5–10% of all cases of diabetes [6]. Genetic, environmental, and immunologic 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 receptor have immune-modulating effects, and the development of T1DM may be associated with polymorphisms in the Vitamin D receptor gene [15,16].

METHODS

This is a prospective randomized controlled open-label study carried out in Child Central Teaching Hospital in the outpatient clinic of the endocrinology department during the period from April 2015 to May 2016. The study was approved by the Ethics Committee by College of Pharmacy/University of Al-Mustansiriyah, and written informed consent was taken from all participants (their legal guardian since all of them are minors). The study was conducted on 50 newly diagnosed T1DM patients [with duration of disease ≤1 year], and ages were between 4 and 12 years. The patients' records were kept for the next 3 months after their initial visit to the hospital; they were observed for Vitamin D₃ that measured before and after 45 days and after 90 days of treatment. All the patients kept on similar management and dose of Vitamin D₃ as well as their regular medications for DM. The patients were randomized into two groups: Group 1 with
25 patients receive 2000 IU Vitamin D3 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, hemoglobinAlc, 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 D3 increased from 45 to 90 days, but it was not statistically significant, while for Group 2, there was a significant reduction in serum Vitamin D3 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.

### DISCUSSION
In the current study serum, IL-1β was significantly decreased in patients receiving Vitamin D3 in addition to insulin while those who did not receive Vitamin D3 had a non-significant increase in IL-1β, and also, serum IL-4 was significantly increased after 90 days for those receiving Vitamin D3, 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 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 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

### Table 3: Assessment of various markers in pediatric patients with T1DM

| Variables | T1DM and vitamin D | T1DM | p-value |
|-----------|-------------------|------|---------|
| Vitamin D (ng/ml) Baseline 11.0±0.99± 15.0±1.565± 0.038 After 45 days 18.5±1.081± 14.1±1.051± 0.009 After 90 days 26.4±1.796± 10.5±0.808± <0.001 Calcium ionized (mmol/L) Baseline 1.39±0.089± 1.76±0.093± 0.127 After 45 days 1.54±0.079± 1.65±0.078± 0.327 After 90 days 1.74±0.089± 1.50±0.076± 0.046 ALP (U/L) Baseline 291.2±26.613± 333.81±18.591± 0.196 After 45 days 293.12±21.905± 327.82±20.429± 0.252 After 90 days 306.80±20.027± 351.66±17.280± 0.096 IL-1β (pg/ml) Baseline 9.43±5.25± 6.587±2.205± 0.401 After 45 days 3.01±4.052± 11.73±4.316± 0.049 After 90 days 3.65±4.2064± 5.589±19.728± 0.537 IL-4 (pg/ml) Baseline 47.297±27.430± 13.832±21.993± 0.231 IL-1β | IL-1β | IL-4 | p-value |

| Markers | Serum vitamin D | p-value |
|---------|-----------------|---------|
| IL-1β | Baseline | After 90 days |
| r | p-value | r | p-value |
| IL-1β | −0.198 | 0.343 | −0.165 | 0.431 |
| IL-4 | −0.017 | 0.936 | 0.022 | 0.919 |

r: Correlation coefficient, IL-1β: Interleukin-1β, IL-4: Interleukin-4, ALP: Alkaline phosphatase, T1DM: Type 1 diabetes mellitus, SEM: Standard error of the mean.
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.

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