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Original Research Article

Vitamin D administration in type 1 diabetes and its impact on T lymphocyte subsets

Mamdouh Allahyani[1]*, Abdulelah Aljuaid[1], Ahmad A Alghamdi[1], Abdullah F Aldairi[2], Mazen Almehmadi[1], Ayman S Alhazmi[1]

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P.O. Box 11099, Taif 21944, [2]Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Al Abdeyah, PO Box 7607, Makkah 21961, Saudi Arabia

For correspondence:* ***Email:* *m.allahyani@tu.edu.sa;* ***Tel:*** *00966-500881380*

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Abstract

Purpose: *To investigate the effect of vitamin D supplementation on glycated hemoglobin A1c (HbA1c); CD3+, CD4+, CD8+, CD4+/CD25+, and CD8+/CD25+ ratios.*
Methods: *Thirty mice were equally assigned to three cohorts: control, untreated T1 diabetic (T1), and T1 diabetic treated with vitamin D for 4 months. Body weight, blood glucose levels, HbA1c percentages, and lipid profiles were measured in mice in each of the three groups. Additionally, total lymphocytes and CD4+, CD3+ cells, CD8+ cells, CD4+/CD25+, and CD8+/CD25+ ratios were assayed using flow cytometry in all mice.*
Results: *Data showed significantly lower blood glucose levels, HbA1c levels, and lipid profiles ($p < 0.0001$); total lymphocyte counts ($p < 0.05$), and counts of CD3+ ($p < 0.05$), CD4+, and CD8+ cells ($p < 0.0001$) in vitamin-D-treated diabetic mice than in the untreated diabetic mice. In contrast, there were significantly higher CD4+/CD25+ and CD8+/CD25+ ratios in treated mice with diabetes ($p < 0.01$).*
Conclusion: *Vitamin D may have promising immunomodulatory properties that could help mitigate the harmful effects of T1DM.*

Keywords: *CD4+ cells, CD8+ cells, CD25+ cells, Type 1 diabetes mellitus, T lymphocyte, Vitamin D*

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INTRODUCTION

The incidence of type 1 diabetes mellitus (T1DM) has consistently increased over the past several decades in most countries that regularly report data [1]. In a 2021 estimate, the global population of people with T1DM was approximately 8.4 million, with 500,000 new cases reported that year [2]. It is anticipated that by 2040, the population of T1DM patients may range from 13.5 to 17.4 million people [2]. Significant complications such as cardiac diastolic dysfunction and dyslipidemia are associated with T1DM [2]. The destruction of pancreatic β-cells occurs through a complex autoimmune process involving the activation of macrophages, dendritic cells, B lymphocytes, and $CD4^+$ and $CD8^+$ T lymphocytes. Additionally, T1DM disrupts the balance between anti-inflammatory $CD25^+$ Tregs (reduced functionality) and inflammatory Th17 cells (heightened functionality) [3].

Vitamin D influences both innate and acquired immunity: it both plays a role in

shaping the immune system during early life, and it is crucial for the proper development and maintenance of self-tolerance [4]. Reduced vitamin D signaling, particularly in early life, increases the likelihood of developing autoimmunity [5]. Thus, vitamin D deficiency may be a contributing factor to the increasing prevalence of T1DM. Moreover, vitamin D may be useful for preventing and treating immunological dysregulation in patients with T1DM. A human study found that maintenance of sufficient vitamin D levels, especially during early life, offers protection against the onset of T1DM [6].

Studies have also highlighted the critical role of CD4+ T lymphocytes in T1DM [7], with one study confirming that vitamin D regulates biological processes in CD4+ T cells [8]. Other studies suggest that vitamin D supplementation enhances insulin production, enhances insulin activation in β-cells of rat pancreas, and corrects impaired insulin production in vitamin D-deprived rabbits and rats [9].

Type 1 diabetes mellitus (T1DM) is a considerable burden for healthcare systems with continuous demand for the development of more efficient, cost-effective therapies. Vitamin D may facilitate immunological tolerance and T cell anergy while diminishing the inflammatory response, thereby yielding positive outcomes in the management of T1DM [10]. This study investigated the influence of vitamin D supplementation on CD4+ and CD8+ cell populations, as well as its effect on glycated hemoglobin (HbA1c), and CD4+/CD25+ and CD8+/CD25+ ratios.

EXPERIMENTAL

Design

Thirty male BALB/c mice aged two months and weighing 20 – 25 g were purchased from the animal house at Umm Al-Qura University. The mice were housed in standard rodent enclosures with woodchip bedding in a spacious, well-ventilated room maintained at 25 °C in a 12-hour light and 12 – h dark environment. Throughout the study, all mice were provided with standard rodent chow and tap water. After adjusting to their new environment, the mice were randomly assigned to three equal-sized groups designated A - C, with 10 mice in each group. Mice in group *A* (negative control group) were not subjected to any interventions. An untreated diabetic group (group B) served as positive control, intraperitoneally administered 0.1 mg/mL streptozotocin in deionized water at a dose of 55 mg/kg body weight for five consecutive days for induction of T1DM. The mice were considered diabetic when fasting blood glucose levels rose above 200 mg/dL. Group C mice were diabetic vitamin D-treated group: T1DM was induced in this group as was done for group B, but one week after the final dose of streptozotocin, the mice were given 300 international units (IU) of vitamin D3 daily via intragastric gavage for two months [11].

Body weight measurement

The body weight of each mouse was measured over 4 months at four-week intervals using a digital balance (OHAUS, Model: Scout Pro SPU601, Shanghai, China).

Blood collection

Blood samples were collected from the retro-orbital venous plexus of each mouse using non-heparinized tubes, before and six days after administering streptozotocin. Blood glucose levels were estimated at three-day intervals following vitamin D3 administration. At the end of the trial (four months), blood samples were collected from each mouse in all groups for determination of HbA1c and lipid profiles. The blood samples were promptly centrifuged at 2500 rpm for 15 min, and the sera obtained were kept frozen at –80 °C prior to analysis.

For flow cytometric analysis, blood sample was obtained from each mouse under anesthesia by puncturing the heart with a needle (A 23 – 25-gauge needle). Each mouse was anesthetized intraperitoneally with a 2 % solution of sodium pentobarbital at a dosage of 50 mg/kg. Following anesthesia, the mice were sacrificed *via* spinal dislocation. Blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes and kept on ice until processed.

Evaluation of blood glucose and HbA1c

Blood glucose levels were estimated using a colorimetric assay. Additionally, HbA1c levels were measured after the four-month experiment using a glycohemoglobin kit (Lot

no. 111001; POINTE Scientific Inc., Canton, MI, USA).

Determination of lipid profile

The concentrations of triacylglycerols, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in each mouse were measured using colorimetric assays.

Sample preparation for flow cytometry

The blood samples collected in EDTA tubes were carefully layered onto a Ficoll-Paque density gradient medium in a 15 mL conical tube and the samples were centrifuged continuously at 400×g for 30 min at 4 °C. The mononuclear cell layer in each tube was carefully removed and transferred to a new tube. The cells were rinsed twice with phosphate-buffered saline (PBS) and subsequently incubated in a staining solution composed of 2 % fetal bovine serum and 0.1 % NaN_3.

Assessment of CD3+, CD4+, CD8+, and CD25+ cells, and total lymphocytes

The cell suspension was aliquoted into four tubes, with each containing 100 µL of the sample. The samples were incubated in the dark with fluorochrome-conjugated monoclonal antibodies against CD4, CD8, CD25, and CD3 for 30 min at 4 °C, with strict precautions taken to avoid exposure to light. After staining, the cells were washed with staining buffer and fixed with 1 % paraformaldehyde for 30 min at 4 °C, in the dark. Thereafter, the samples were washed and reconstituted in fluorescence-activated cell sorting (FACS) buffer before being analyzed using a flow cytometer (FACSCanto II system; BD Bioscience, San Jose, CA, USA).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical analysis was done with SPSS 17 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used, followed by Tukey's post hoc test for multiple comparisons. For data determined at multiple time points, repeated measures ANOVA was applied. Flow cytometry data were analyzed using FlowJo software, version 7.10 (Tree Star, Ashland, OR, USA). Differences were considered significant at $p < 0.05$.

RESULTS

Body weight changes

Figure 1 shows the changes in weight for all groups over the 16 weeks of study. The diabetic mice treated with vitamin D had greater body weights than the untreated diabetic mice after 12 weeks ($p < 0.001$) and 16 weeks ($p < 0.0001$).

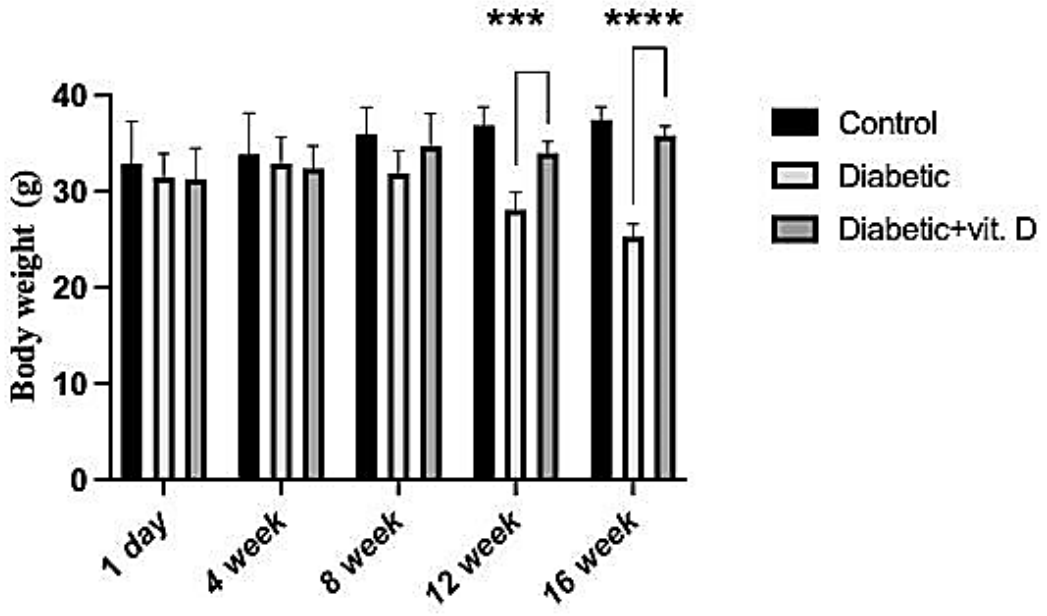

Figure 1: Estimation of body weight in mice. ***$p \leq 0.001$ and ****$p \leq 0.0001$

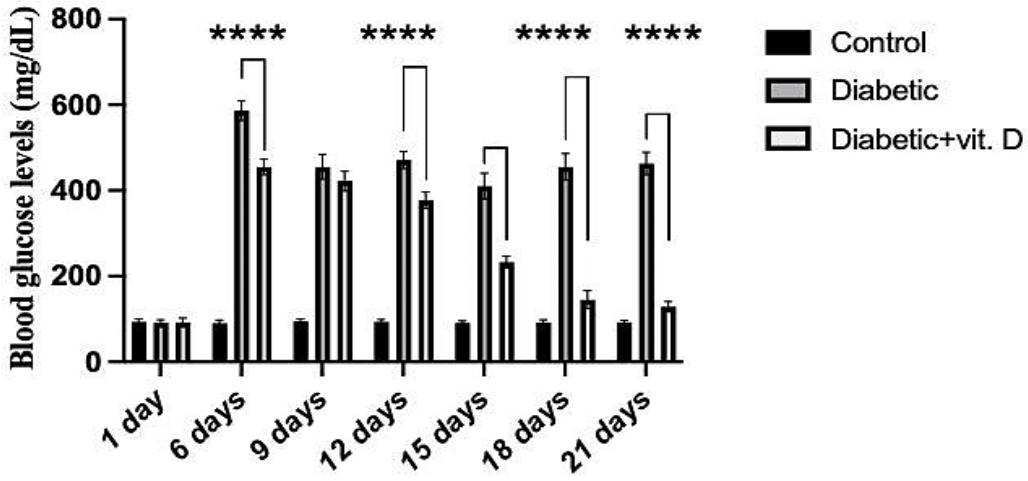

Figure 2: Blood glucose levels in all groups over 21 days. ****$p \leq 0.0001$

Fasting blood glucose levels

Figure 2 shows the fasting blood glucose values for all groups at 18 days after streptozotocin treatment. The diabetic mice treated with vitamin D exhibited lower levels of fasting blood glucose when compared to the untreated diabetic mice on days 6, 12, 18, and 21 ($p < 0.0001$).

Levels of HbA1c

Figure 3 shows the HbA1c percentages for all groups. Data showed that the HbA1c percentages were lower in the vitamin D-treated groups (6.20 ± 7.40 %) compared to mice in the untreated diabetic group (11.88 ± 2.20 %, $p < 0.0001$).

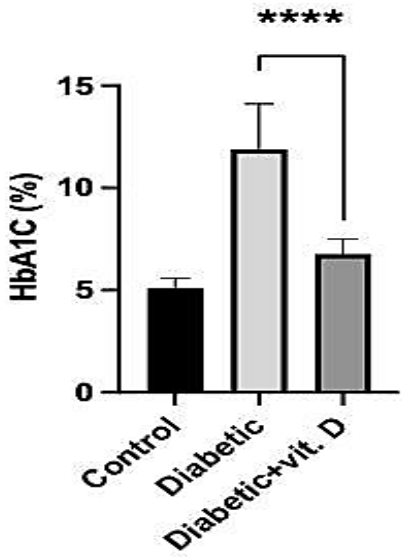

Figure 3: The levels of glycated hemoglobin (HbA1c) were estimated in all groups. ****$p \leq 0.0001$

Lipid profile

Figure 4 presents the lipid profiles for all groups. The vitamin D-treated diabetic mice had significantly lower triglycerides (123.40 ± 5.99) when compared to mice in the untreated diabetic group (251.50 ± 8.40, $p < 0.0001$). The vitamin D-treated diabetic mice also had significantly lower total cholesterol levels (118.28 ± 7.05) than the untreated diabetic mice (247.40 ± 5.30, $p < 0.0001$). The diabetic mice treated with vitamin D had lower levels of LDL (88.03 ± 9.06), when compared to the untreated diabetic mice (201.30 ± 6.99, $p < 0.0001$). In contrast, HDL levels were significantly higher in the vitamin D-treated diabetic mice (68.30 ± 2.64) than in mice in the untreated diabetic group (22.05 ± 2.50, $p < 0.0001$).

Peripheral CD3+ cells, CD4+ cells, CD8+ cells, and CD25+ Subsets

Results showed that the total lymphocyte count was significantly lower in diabetic mice treated with vitamin D (14.57 ± 2.08) than in mice in the untreated diabetic group (18.90 ± 1.01, $p < 0.05$; Figure 5). The data also indicated a significant reduction in CD3+ cell levels in diabetic mice administered vitamin D (39.13 ± 1.20), when compared to the untreated diabetic mice (45.43 ± 0.98, $p < 0.05$; Figure 5).

Additionally, diabetic mice treated with vitamin D had a marked reduction in CD4+ T cell counts (25.83 ± 3.30) relative to the untreated diabetic group (52.93 ± 1.07, $p < 0.0001$; Figure 6). Similarly, there was a lower population of CD8+ T lymphocytes in the vitamin D-treated diabetic

mice (24.27 ± 1.75) than the untreated diabetic mice (46.50 ± 1.32, $p < 0.0001$; Figure 6).

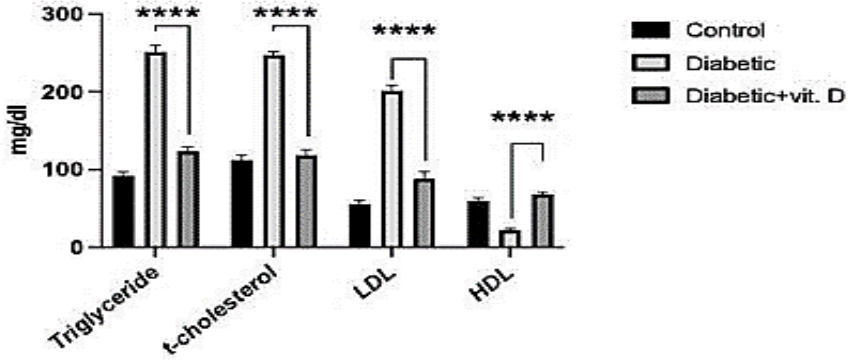

Figure 4: Serum triglyceride, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) of mice in all groups after four months. ****$p \leq 0.0001$

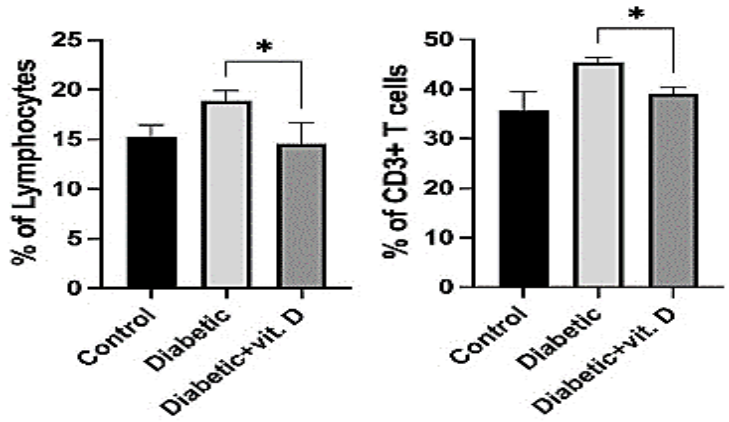

Figure 5: Total lymphocytes and T lymphocytes in all groups. The percentage of these cells in the blood of mice was measured using flow cytometry. Data are presented as mean ± standard deviation. *$p \leq 0.05$

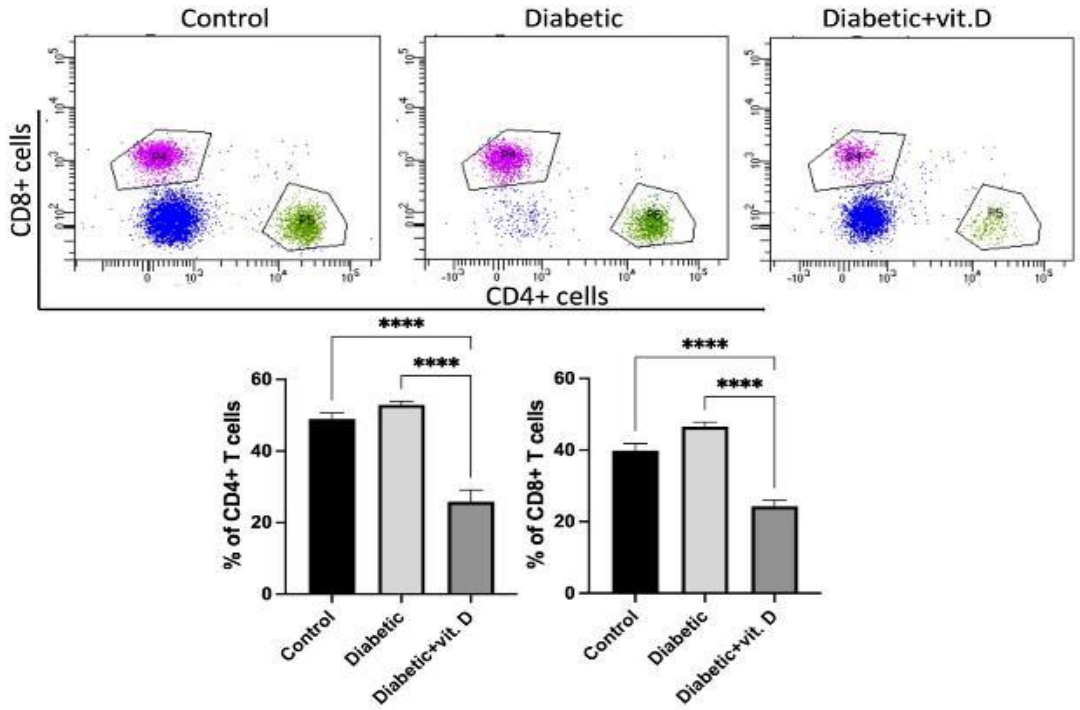

Figure 6: The CD4+ and CD8+ cells in all groups. The percentage of CD4+ and CD8+ cells was measured in the blood of mice using flow cytometry. Data are presented as mean ± standard deviation. ****$p \leq 0.0001$

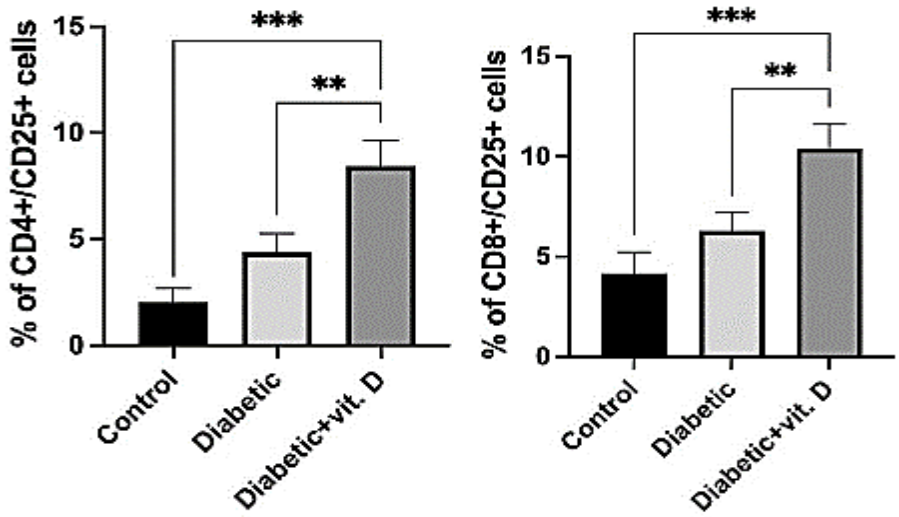

Figure 7: The CD4+/CD25+ and CD8+/CD25+ ratios in all groups. The ratios of CD4+/CD25+ and CD8+/CD25+ were measured in the blood of mice using flow cytometry. Data are presented as mean ± standard deviation. ***P* ≤ 0.01, ****p* ≤ 0.001

The CD4+/CD25+ ratio was significantly higher in diabetic mice treated with vitamin D (8.43 ± 1.19) than in the untreated diabetic group (4.40 ± 0.87, $p < 0.01$; Figure 7). Moreover, CD8+/CD25+ ratio was significantly higher in diabetic mice treated with vitamin D (10.40 ± 1.21) than in the untreated diabetic mice (6.30 ± 0.90, $p < 0.01$; Figure 7).

DISCUSSION

Type 1 diabetes mellitus (T1DM), a chronic autoimmune disorder marked by elevated fasting blood glucose levels and infiltration of pancreatic islets, is triggered by a combination of genetic and environmental factors [2]. The impact of vitamin D supplementation on immune function regulation is inconclusive., The regulatory effects of vitamin D supplementation in the context of T1DM development were investigated in this study.

The results obtained revealed that vitamin D supplementation reduced fasting blood glucose levels and normalized HbA1c levels in mice with T1DM. Data from previous studies suggests that a deficiency in vitamin D may qualify as an environmental predisposing factor for the development of T1DM [12]. Consistent with our findings, it was shown in a previous study that vitamin D supplementation normalized blood glucose levels [4]. A comprehensive analysis of four case-control studies and one cohort study also indicated that vitamin D supplementation significantly decreases the likelihood of developing T1DM [13]. Furthermore, Lai *et al* [14] found that vitamin D supplementation improved pancreatic β-cell function, suggesting that it may reduce β-cell apoptosis, while another study suggested that vitamin D supplementation may induce β-cell autophagy, inhibit apoptosis, and enhance insulin secretion [15]. However, in contrast to the findings in the present investigation, a study among children in Sudan found no significant changes in HbA1c levels following vitamin D treatment, while there were significant reductions in fasting blood glucose levels [12]. This may be ascribed to the dosage and period of vitamin D supplementation. Nonetheless, vitamin D shows a promising immunomodulatory effect in preventing and treating T1DM [10]. However, the impact of vitamin D treatment on human insulin production remains uncertain, and clinical intervention trials of vitamin D supplementation have produced inconsistent data.

Vitamin D affects the biological functions of CD4+ cells, which are key immune-mediated cells involved in the development of T1DM [7,8]. A study demonstrated that vitamin D reduced the activation and proliferation of CD4+ cells [16]. Moreover, vitamin D has been found to inhibit the excessive activation of CD8+ cells, thereby preventing them from releasing proinflammatory cytokines such as IFN-γ and TNF-α [17]. In the present study, it was found that vitamin D supplementation decreased the populations of CD4+ and CD8+ cells. Thus, the anti-inflammatory activity of vitamin D appears to help protect pancreatic β-cells and maintain insulin secretion through a calcium-dependent pathway. Moreover, in the current study, diabetic mice treated with vitamin D exhibited a higher number of CD4+/CD25+ ratios than the untreated

diabetic mice. A previous report indicated that vitamin D enhanced the total count and characteristics of T regulatory cells in diabetic patients [18]. This supports the therapeutic potential of vitamin D supplementation in patients with newly diagnosed T1DM. The CD8+/CD25+ subset consists of CD8+ T regulatory cells, which release immunosuppressive factors and anti-inflammatory cytokines, e.g., IL-10. The present study found a higher frequency of this subset in the vitamin D-treated group than in the untreated diabetic group. It has been suggested that the suppression of experimentally induced diabetes occurs through the inhibition of IFN-γ by vitamin D, along with the activation of IL-10 and T regulatory cells [14].

In summary, vitamin D has the potential to promote immunological tolerance, downregulate processes associated with adaptive immunity, and reduce inflammation caused by auto-aggression in T1DM [12]. Mechanistically, vitamin D decreases the invasion of beta cells by inflammatory lymphocytes and aids in the regeneration of beta cells. Studies have also found that vitamin D supplementation effectively reverses insulin impairment in vitamin D-deprived rabbits and mice [9].

Study limitations

This study has limitations. Diabetes was induced in this study using streptozotocin treatment. However, the destruction of beta cells was not confirmed through histological examination. In addition, there are inherent limits to the generalizability of findings from an animal T1DM model study to humans and the extrapolation of vitamin D doses. Different doses of vitamin D and varied durations of administration should be used in future studies to obtain more comprehensive results.

CONCLUSION

This study has demonstrated that vitamin D supplementation improves glycemic status in T1DM by modulating the levels of CD4+, CD8+, and T regulatory cells. Thus, vitamin D may be an immunomodulatory agent with promising potential for the reduction of T1DM-induced harmful effects. However, further studies in human models are needed to validate these findings.

DECLARATIONS

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Ethical considerations

The animal study protocol was accredited by the National Committee for Bioethics at Taif University, and the Committee determined that the study meets its requirements (approval no. 45-238).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Mamdouh Allahyani, Abdulelah Aljuaid, and Ayman S Alhazmi contributed to the concept and designed the study and wrote the original draft. Mamdouh Allahyani, Abdulelah Aljuaid, and Mazen Almehamdi performed the research. Ahmad A Alghamdi and Abdullah F Aldairi contributed to the analysis and interpretation of the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript for publication.

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