Vitamin D level in patients with type 1 diabetes and its relation to tissue transglutaminase immunoglobulin A antibodies

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Introduction
Type 1 diabetes mellitus (T1DM) is a lifelong metabolic disorder, which accounts for ~10% of all cases of diabetes. Vitamin D deficiency is extensively studied in the pathogenesis of T1DM. Vitamin D regulates both innate and adaptive immunity, and this indicates its potential role in preventing and treating T1DM. T1DM is also associated with celiac disease which is an autoimmune-mediated, chronic inflammatory disorder of the small intestine. Vitamin D deficiency has been described in patients with celiac disease and T1DM.

Aim
The study’s primary aim was to investigate the 25-hydroxyvitamin D [25(OH)D] level in patients with T1DM and relation between vitamin D level and the presence or absence of tissue transglutaminase immunoglobulin A [tTG (IgA)] antibodies.

Patients and methods
The study estimated 25(OH)D level in 75 patients with T1DM and 15 healthy participants. It also studied the relation between vitamin D level and the presence or absence of tTG (IgA) antibodies.

Results
Patients with T1DM had significantly lower level of 25(OH)D (82.7%) compared with control participants (46.7%) \( (P=0.003) \). In all, 5.3% of patients were positive for tTG (IgA) antibody, and antibody titer was significant higher in patients with T1DM, with mean of 1.57±3.38, compared with control participants, with mean of 0.31±0.07 \( (P=0.001) \). Vitamin D level was low in diabetic patients with positive tTG (IgA) antibody than diabetic patients with negative tTG (IgA) antibody, but this change did not achieve significant value.

Conclusion
Vitamin D deficiency is high in children and adolescents with T1DM. Its level was decreased in diabetic patients with positive tTG (IgA) antibody than diabetic patients with negative tTG (IgA) antibody.

Keywords:
celiac disease, tissue transglutaminase immunoglobulin A antibody, type 1 diabetes mellitus, vitamin D level

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β-cells. Insulin secretion is a calcium-dependent process, so alterations in calcium flux can have adverse effects on insulin secretion by B cell. Vitamin D insufficiency through changing the balance between the extracellular and intracellular B-cell calcium pools may interfere with normal insulin release in response to glucose [4].

Celiac disease (CD) is an immune-mediated, chronic inflammatory disease of the small intestine. It occurs in genetically susceptible individuals by the ingestion of proline-rich and glutamine-rich proteins present in wheat, rye, and barley, although other factors seem to play a secondary role. There is a clear association of T1DM with CD [3]. The prevalence of CD among children and adults with T1DM is observed to range between 0.6 and 16.4% compared with 0.01–0.03% in the general population [5]. Vitamin D deficiency has been described in patients with CD [6] and in patient with T1DM [7].

The study aims to investigate the 25-hydroxyvitamin D [25(OH)D] level in patients with T1DM and its relation to tissue transglutaminase immunoglobulin A [tTG (IgA)] antibodies.

Patients and methods
This study was conducted at Minia University Hospital in the outpatient clinics of endocrine and metabolism from March 2014 to November 2014. It included 75 patients with T1DM and 15 control participants matched for age and sex after approval of hospital ethics committee and informed patient consents. The age of diabetic patients ranged from 9 to 30 years old. The study enrolled 15 control participants matched for age and sex. Patients were included in the study if they had no history suggestive of vitamin deficiency (rickets or osteoporosis) or undiagnosed or untreated CD. Patients were excluded if they have any of the following: taking multivitamin supplements including vitamin D; having past history of irritable bowel syndrome (for adults) or lactose intolerance (for children); having treated with systemic immunomodulatory agents in the prior 30 days, having complications of T1DM or CD; having treated with systemic immune-modifying biological agents, for example, infliximab in the prior 6 months; and known patients with CD. The 25(OH)D level was assessed by enzyme-linked immunosorbent assay. Vitamin D deficiency is defined as a 25(OH)D level less than 20 ng/ml, insufficiency is defined as a 25(OH)D concentration of 20–30 ng/ml, and normal level of vitamin D is defined as a 25(OH)D concentration greater than 30 ng/ml [8]. tGA (IgA) antibody was estimated by Immunometric Enzyme Immunoassay (Chemux Bio Science, Inc company, USA) with cutoff value of 10 U/ml. Fasting and postprandial blood glucose, glycated hemoglobin (HbA1c), lipid profile, renal function tests, and liver enzymes all were determined by automated chemistry analyzer. HbA1c was also assessed, and the following levels were taken as a reference range: children <6 years old: <8.5%, children 6–12 years old: <8%, and teens 13–19 years old: <7.5%.

Results
Table 1 shows the baseline characteristics of the 75 enrolled diabetic patients with low BMI in diabetic patients in comparison with control group (P=0.03). The study revealed a significantly low level of vitamin D in diabetics patients, with mean of 17.6±17.2, than the control group, with mean of 44.4±31.8 (P=0.001) (Table 2 and Figs 1 and 2). Of the 75 diabetic patients studied, 5.3% (four patients) were positive for tTG (IgA) antibody and 94.7% patients (71 patients) were

| Demographics | Cases (N=75) [n (%)] | Controls (N=15) [n (%)] | P |
|--------------|----------------------|-------------------------|----|
| Age (mean±SD)| 16.9±5.5             | 17.9±5.94               | 0.5|
| Sex          |                      |                         | 0.7|
| Male         | 34 (45.3)            | 6 (40)                  |    |
| Female       | 41 (54.7)            | 9 (60)                  |    |
| Weight       | 47.9±12.4            | 50.1±14.4               | 0.5|
| Height       | 146.2±14.8           | 152.8±10.3              | 0.1|
| BMI          | 22.1±3.01            | 24±3.07                 | 0.03*|
| Underweight  | 7 (9.3)              | 0                       |    |
| Normal       | 60 (80)              | 10 (66.7)               | 0.09|
| Overweight   | 7 (9.3)              | 4 (26.7)                |    |
| Obese        | 1 (1.3)              | 1 (6.7)                 |    |
| Duration of diabetes mellitus (years) | | | |
| <5           | 53 (70.7)            |                         |    |
| ≥5           | 22 (29.3)            |                         |    |

*means statistically significant values.
negative. These results showed significant difference regarding tTG (IgA) antibodies between patients with T1DM (5.3%) and control participants (0%) \((P=0.001)\) and also revealed that tTG (IgA) antibody titer was significant higher in patients with T1DM compared with control participants \((P=0.001)\) (Table 3). The study showed that vitamin D level was decreased in diabetic patients with positive tTG (IgA) antibodies, with mean of 6.7±10.9, than diabetic patients with negative tTG (IgA) antibody, with mean of 18.02±17.3, but this change did not achieve statistically significant value (Table 4).

### Discussion

T1DM is a chronic autoimmune disease that usually manifests during early childhood and puberty [9]. In Egypt, T1DM incidence and prevalence are increasing, being higher in females, and more cases are found to originate from rural areas [10]. Vitamin D deficiency is one environmental factor that has long been discussed to influence the risk of T1DM [11]. Suggested role of vitamin D in the pathogenesis of T1DM include the important functions of this hormone in immunoregulation and immunoactivation [12], the genetic association of vitamin D receptor with T1DM [13], and the results of epidemiological studies showing that lower serum vitamin D levels and inadequate supplementation during infancy increase the risk of T1DM [14]. In this study, vitamin D level was significantly low in patients with T1DM, with mean of 17.6±17.2, compared with control participants, with mean of 44.4±31.8 \((P=0.001)\), and 37.3% of the patients with T1DM were deficient compared with 6.7% in control group. These results are similar to the results of Borkar et al. [15] in North India, as 58% of patients with T1DM were deficient in 25(OH)D compared with 32% of controls, but lower than the study of Bener et al. [16] in Qatar, as of 170 children with T1DM and 170 controls, 90.6% of children with T1DM versus (85.3%) of nondiabetic children had vitamin D deficiency. All these studies showed significantly lower mean 25(OH)D level in T1DM compared with controls [17]. These results are in disagreement with a study conducted in Florida, a solar-rich region in the USA, which demonstrated that there were no differences in 25(OH)D levels in diabetics (recently or more than 5 months diagnosed) compared with their first-degree relatives and controls [18].

The range of prevalence difference might be explained by differences in geographical location, the age of patients, duration since diagnosis of diabetes, and glycemic control [19]. This can also be explained by the fact that variance in levels of 25(OH)D is related to the occurrence of malabsorptive state among patients with T1DM. It is evidently described that vitamin D

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**Table 2 Vitamin D level in diabetic patients and controls**

| Vitamin D level | Cases \((N=75)\) [n (%)] | Control \((N=15)\) [n (%)] | \(P\) |
|-----------------|---------------------------|----------------------------|------|
| Mean±SD         | 0.005–68                  | 4–106                      | 0.001* |
| Median          | 17.6±17.2                 | 44.4±31.8                  |      |
| Deficient       | 28 (37.3)                 | 1 (6.7)                    | 0.009* |
| Insufficient    | 34 (45.4)                 | 6 (40)                     | 0.3   |
| Sufficient      | 13 (17.3)                 | 8 (53.3)                   | 0.02  |

*means statistically significant values.

**Table 3 Tissue transglutaminase immunoglobulin A antibodies between diabetics and controls**

| tTG (IgA) antibody level | Cases \((N=75)\) [n (%)] | Controls \((N=15)\) [n (%)] | \(P\) |
|--------------------------|---------------------------|----------------------------|------|
| Mean±SD                  | 1.4±3.07                  | 0.3±0.06                   | 0.1  |
| Median                   | 0.6                       | 0.3                        | 0.001* |
| Positive                 | 4 (5.3)                   | 0 (0.0)                    | 0.001*|
| Negative                 | 71 (94.7)                 | 15 (100)                   |      |

IgA, immunoglobulin A; tTG, tissue transglutaminase. *means statistically significant values.

**Table 4 Vitamin D level in diabetic patients with positive tissue transglutaminase (immunoglobulin A) antibody and negative one**

| Vitamin D level | Diabetic with negative tTG (IgA) \((N=71)\) [n (%)] | Diabetic with positive tTG (IgA) \((N=4)\) [n (%)] | \(P\) |
|-----------------|---------------------------------------------------|-----------------------------------------------|------|
| Range           | 0.005–68                                          | 0.005–23                                      | 0.2  |
| Mean±SD         | 18.02±17.3                                        | 6.7±10.9                                      |      |
| Deficient       | 25 (35.2)                                         | 3 (75)                                        | 0.05 |
| Insufficient    | 33 (46.5)                                         | 1 (25)                                        | 0.2  |
| Sufficient      | 13 (18.3)                                         | 0 (0.0)                                       | 0.1  |

IgA, immunoglobulin A; tTG, tissue transglutaminase.
can prevent the death of islet cells [20]. Thus, there is a
direct association of β-cell dysfunction, hypovitaminosis D, and insulin resistance [21].

The study revealed that vitamin D deficiency was
predominant in female (64.3 vs. 35.7%). This result
was in agreement with Al-Agha et al. [22] who
demonstrated that 61.5% of the girls had inadequate
amounts of vitamin D versus 38.5% of the boys. This
result was inconsistent with the results by Littorin et al. [23] which showed lower levels of plasma 25(OH)D in
men than in women with T1DM. Higher level of 25
(OH)D in men could be explained by the difference in
sun exposure between the two sexes, as a higher sun
index score was shown in boys. In addition, faster growth spurt during puberty in girls and more vitamin D requirement for bones might be a further reason for the higher prevalence of vitamin D deficiency among girls than boys [24].

Our study showed that patients with low vitamin D level (deficient and insufficient) were overweight than vitamin D sufficient patients but without statistically significant changes, and this agrees with a study by Çizmecioglu et al. [25], which revealed that vitamin D deficiency and insufficiency were common in obese and overweight schoolchildren, especially in girls. Obesity could be a risk factor in terms of hypovitaminosis D in adolescent [26]. This result was in conflict with the results of Bin-Abbas et al. [27], which demonstrated no significant changes between vitamin D level and BMI.

The excess body fat may disrupt hormonal pathways important for skeletal health. For example, leptin, an adipocyte-derived hormone that binds to osteoblasts, appears to activate a pathway that inhibits renal synthesis of the active form of vitamin D [28].

Of 75 diabetic patients studied, 5.3% patients were positive for tTG (IgA) antibody and 94.7% were negative. A higher percentage of tTG (IgA) antibody-positive patients (17%) was reported by Abdulrahman et al. [29], as among the 106 children with T1DM who had been screened for CD over a 2-year period (2008–2010), tTG (IgA) ab assessment revealed 17% (19 children) of the children were positive for tTG (IgA) antibody.

The study revealed that 25(OH)D level was lower in diabetic patients with positive tTG (IgA) antibody than diabetic patients with negative tTG (IgA) antibody, but this change did not achieve significant value. This matched with the study by Setty-Shah et al. [30] (62 patients and 49 controls) which demonstrated a higher occurrence of vitamin D deficiency in children with T1DM+CD (27.3%) compared with the controls (18.4%), CD (22.2%), and T1DM (13.6%).

Patients with CD often present with nutritional deficiencies as a result of villous atrophy and subsequent malabsorption. Vitamin D is one such nutritional deficiency that has been noted in patients with CD [31].

Our study showed that patients with low vitamin D level (deficiency and insufficiency) had higher HbA1C (25.8%). This result was in agreement with a study by Littorin et al. [24] and Janner et al. [32]; both demonstrated that there was no correlation between the concentrations of 25(OH)D and HbA1C. This may indicate that the diabetic state per se is a reason for low 25(OH)D levels and is not secondary to any hyperglycemic or insulin-resistant state. This result was in disagreement with a study of Nahla Abdelkarim et al. [33] which showed that HbAlc is higher in T1DM patients with low vitamin D level. Moreover, Tunc et al. [34] showed that children with T1DM had lower serum 25(OH)D concentrations and that these vitamin D-deficient children often require increased amounts of insulin to maintain normal glycemic control. A less likely explanation is the presence of altered dietary habits, or nutritional status in patients with worse glycemic control. Moreover, the relation between vitamin D status and HbA1C may be explained by the effects of vitamin D on β-cell secretory function, on insulin action, and on systemic inflammation [35].

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Conflicts of interest
There are no conflicts of interest.

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