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Levels of 25(OH)D_3, IL-2, and C-peptide in Children with Type 1 Diabetes Mellitus (T1DM) Receiving Vitamin D_3 Supplementation

Tjahyo Suryanto *, Harjoedi Adji Tjahjono, Edi Widjajanto

Faculty of Medicine, Brawijaya University, Malang, Indonesia

ABSTRACT

Type 1 Diabetes Mellitus (T1DM) has become a health problem in many countries. T1DM is the consequence of autoimmune destruction process of β cells. There was relationship between vitamin D deficiency with T1DM. The destruction process was caused by an imbalance of pro-inflammatory and anti-inflammatory cytokines. One of the pro-inflammatory cytokines is IL-2. C-peptide examination to see the function of beta cells due to destruction of pancreatic beta cell. Administration of vitamin D_3 supplementation still cause controversy and give varying results. This randomized clinical trial was conducted to determine the levels of 25(OH)D_3, IL-2, and C-peptide in people with T1DM who received vitamin D_3 supplementation. The subjects were 26 children with T1DM, divided into K1 group (received vitamin D_3 supplementation) and K2 group (received placebo). The results showed higher levels of 25(OH)D_3 in the K1 group and statistically found a significant difference ($p = 0.00$). Higher levels of IL-2 and lower C-peptide were obtained in the K1 group and no statistically significant differences were found ($p = 0.76$ and $p= 0.26$). The insignificant relationship and the negative correlation were found between 25(OH)D_3 and IL-2 ($p = 0.71$; $r = - 0.12$), 25(OH)D_3 and C-peptide ($p = 0.59$; $r = -0.16$), also levels of IL-2 and C-peptide ($p = 0.13$; $r = -0.44$) in children with type 1 diabetes who received vitamin D3 supplementation. From this study can be concluded that administration vitamin D_3 supplementation in patients with T1DM can increase levels 25(OH)D_3 significantly. This increase has not significantly lowered levels of IL-2 and increased levels of C-peptide. However, there was an absolute decrease in the rate of slower C-peptide in the supplemented group than in the placebo group.

Keywords: *Type 1 DM, 25(OH)D_3, C-peptide, IL-2*

INTRODUCTION

Type 1 Diabetes Mellitus (T1DM) has become a health problem in many countries, with an overall increase estimation is 3% per year. In 2010, children who suffer from T1DM has reach 480,000 patients worldwide and the number of new cases which diagnosed each year reach 75,800 children [1]. The prevalence of T1DM in Indonesia was 3.1 per 100,000 population in 2011 [2]. While the number of children with T1DM at Saiful Anwar Hospital (RSSA) Malang between the years 2005 to 2012 was 1.8 per 100,000 population [3].

Type 1 Diabetes Mellitus is the consequence of autoimmune destruction process of β cells, which produce insulin hormone, and the result of interactions between multiple genes and environmental exposures [4, 5]. Recently, researcher has discovered relationship between vitamin D deficiency with the risk of chronic diseases, including autoimmune diseases such as T1DM. Vitamin D3 turns out to has immunosuppressive effect, thus it can prevent insulitis and further destruction of pancreatic cells [6, 7]. The process of autoimmune destruction of β cells occurs due to an imbalance of pro-inflammatory and anti-inflammatory cytokines. One of the pro-inflammatory cytokines that play a role in T1DM is interleukin-2 (IL-2). IL-2 and IFN-γ will activate cytotoxic T cells to destroy target cells presented by the Major Histocompatibility Complex (MHC). In addition, this cytokine combination will activate the natural killer (NK) cell [8]. Autoimmune destruction of pancreatic beta cell in T1DM causes the need for an examination

*Corresponding author:
Tjahyo Suryanto
Faculty of Medicine, Brawijaya University
Jalan Veteran, Malang, Indonesia 65145
E mail: sunotnay_qt@yahoo.co.id

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that is sensitive to see the function of β cells. Examination of C-peptide can be used to assess secretion of endogenous insulin released by the β cells of the pancreas [9, 10].

Research in various countries, such as in the United States, Switzerland, Australia, and Indonesia mentioned that people with T1DM suffered from vitamin D deficiency and insufficiency [11, 12, 13, 14]. Administration vitamin D3 supplementation can prevent or reduce autoimmune damage and destruction of pancreatic β cells [7]. Many studies were conducted in various countries to determine the effect of vitamin D_3 supplementation on levels of 25(OH)D_3, IL-2, and C-peptide in children with T1DM. The results obtained are still varied and still cause controversy [15, 16]. Current research on the effect of vitamin D3 supplementation on levels of 25(OH)D_3, IL-2, and C-peptide in children with T1DM is not present in Indonesia. Therefore, the purpose of this study was to determine the effect of vitamin D_3 supplementation on levels of 25(OH)D_3, IL-2, and C-peptide in children with T1DM.

MATERIALS AND METHODS

Study design

This experimental study was designed as randomized clinical trial (RCT) double blind, pre- and post-test control group. Vitamin D_3 supplementation was administered for 6 months. Before treatment, subjects with T1DM was measured for 25(OH)D_3, IL-2, and C-peptide level. Then, all subjects were randomly divided into 2 groups (K1 and K2) as follow: K1: subjects were treated with insulin 0.5-2 IU/day + vitamin D_3 2000 IU/day (D-Vit, PT. Gracia Pharmindo) for 6 months, K2: subjects were treated with insulin 0.5-2 IU/day + placebo (flour containing capsules, D_3 Farmasi. Ltd) for 6 months. All procedures in this study had been approved by Ethical Committee of Research, RSSA, Malang, Indonesia.

Subjects

As many as 26 subjects were included in this study (13 subjects K1 and 13 subjects K2). Subjects were taken from Pediatric Endocrinology Outpatient Care, Saiful Anwar General Hospital, Malang, Indonesia during April-October 2016. Inclusion criteria for subjects are as follows: diagnosed as T1DM, age 1-18 years old, not on vitamin D_3 supplementation, and allowed by his/her parents (informed consent). Exclusion criteria for subjects is T1DM patients with other autoimmune disease, severe infection, hepatic dysfunction, renal dysfunction, anemia and on vitamin D_3 supplementation.

Measurement of 25(OH)D_3 level

Based on the manufacturer's instruction (Orgentec, Alegria, Germany), this research used Enzyme Linked Immunosorbent Assay to assess the level of Vitamin D (25(OH)D3). The samples used were plasma stored at -20°C. The method of inspection is to prepare a polypropylene tube, one for calibrator, control and sample. Conjugate enzymes, substrate enzymes, sample buffers and specific test controls was included in the kit with 8-well-microstrips. One patient sample used each strip for a single determination. We pipetted sample into well No 1 and mixed with tracer reagent and the 25-OH vitamin D3/D2 obtained from vitamin D binding protein. 25-OH vitamin D and tracer reagent was coated with buffer suspension at well 2. A well-reacted reaction in which 25-OH vitamin D3/D2 and 25-OH vitamin D tracer reagents compete to bind 25-OH vitamin D3/D2 antibodies to form complex 25-OH vitamin D3/D2 and antibodies or 25 -OH vitamin D reagent tracer and antibody happened when samples and controls was transferred to wells No. 3 and No. 4. Covered the plate with plastic and incubated at 18 – 25°C for 2 hours. Washed the plate to remove unbound and unspecifically bound molecules. Subsequently enzyme conjugate binds to the immobilized tracer-antibody complexes was added. Plate is closed and wrapped with plastic, incubated at 18 – 25°C for 30 minutes. We washed the plate to remove unbound enzyme conjugate. Added TMB substrate enzymes to well than closed and wrapped the plate in a plastic bag. The plate was incubated 18 – 25°C for 30 minutes in order to get hydrolyzation and color development during incubation. We added Stop Solution to the well to stop the reaction. Read at 650 nm using the ELISA reader for the intensity of the blue color. Vitamin D level > 30 ng/mL was defined as a normal level [17].

Measurement of C-peptide level

Based on the manufacturer's instruction (Roche, Cobas e, USA), this research used Enzyme Linked Immunosorbent Assay to assess the level of C-peptide. This assay based on the the quantitative sandwich enzyme principle. The examination time is 18 minutes at 37°C. Examination of C-peptide levels was performed by taking 20 µL of plasma samples, controls and calibrators at well. During the first stage of incubation of the C-peptide examination, the antigen from the sample formed a sandwich complex with biotinylated monoclonal C-peptide-specific antibody and a monoclonal antibody C-

peptide-specific antibody labeled with a ruthenium compound. The 100 µL conjugate is mixed in each well for 5 minutes. Incubation for 30 minutes at room temperature. In the second stage incubation, streptavidin-coated microparticles is added and the complex becomes attached to a solid phase through the interaction of biotin and streptavidin. The incubated mixture was removed and well washed 3 times using a buffer solution. TMB solution is mixed into each well including the empty one. Incubation for 15 minutes at room temperature. We added 50 µL of the Stop Solution at each well to stop the reaction and read at 450 nm using the ELISA reader for the absorbance.

Measurement of IL-2 level

Based on the manufacturer's instruction (Biolegend, Legend Max, USA), this research used Enzyme Linked Immunosorbent Assay to assess the level of IL-2. All reagents are prepared at room temperature. Prepare 500 µL standard solution of 1,000 pg/mL by diluting 25 µL standard stock solution in 475 µL Assay Buffer B. Dilute 6 times from standard solution 1,000 pg/mL in separate tube using Assay Buffer B as diluent. We could see the Human IL-2 standard concentrations in the tubes from 1,000 pg/mL to 15.6 pg/mL, respectively, and Assay Buffer B was functioning as a standard zero (0 pg/mL). Plate is washed 4 times using 1X Wash Buffer solution at least 300 µL at each well and remove remaining 1X Wash Buffer solution with filter paper every time wash. Assay Buffer B solution was added as much as 50 µL in each well which will each contain 50 µL the standard solution and samples in the appropriate well. Closed the plate and incubated for 2 hours while vibrating. Washed the plate with 1× Wash Buffer solution 4 times as above. The second incubation phase is carried out after being added Human IL-2 antibody solution in each well. This process is done at room temperature for 1 hour while vibrating. The plate was washed with 1× Wash Buffer solution 4 times as above. The third incubation phase is carried out after being added Avidin-HRP solution to each well. This process is done at room temperature for 30 minutes while vibrating. The plate was washed with 1× Wash Buffer solution 5 times as above, for 30 seconds to 1 minute for each wash in order to minimize the background. The next incubation phase is carried out after being added Substrate Solution F to each well, incubated for 20 min in dark condition and the plate need not be closed. The wells should turn into a blue color with intensity that is proportional to its concentration. Stopped the process by adding a Stop Solution at each well. We could see that the color of the solution changed from blue to yellow. We read at 450 nm using the ELISA reader for the absorbance.

Statistical analysis

Statistical test was based on data distribution and homogeneity by using Shapiro-Wilk test. Statistical differences of $25(OH)D_3$ level after treatment between group was analyzed by independent t-test. For differences of IL-2 and C-peptide level after treatment between group were analyzed by Mann-Whitney test. Correlation of $25(OH)D_3$, IL-2, and C-peptide level were analyzed by Spearman correlation test. Data was analyzed at 95% confidence interval ($\alpha = 0.05$) using *SPSS* for *Windows version 17.0*.

RESULTS AND DISCUSSION

Subject characteristics

Subject characteristics such as sex, age, duration of disease (T1DM), insulin dose, and nutritional status was shown in Table 1. Data obtained from Table 1 shows the most distribution of sex in the two research groups are female. For the duration of illness, both groups were found to be approximately equal. Nutritional status in both groups found more children with well-nourished status. Vitamin D status in both groups found more children with abnormal vitamin D status (16/26) than children with normal vitamin D status (10/26). Age and insulin dose was listed as mean ± standard deviation dan between two groups showed no statistically significant difference, which obtained $p > 0.05$, that is 0.80 and 0.57, respectively.

As many as 26 children with T1DM were subjected to study and divided into 2 groups, group 1 consisting of T1DM patients receiving vitamin D supplementation and group 2 consisting of T1DM patients receiving placebo. The most distribution of sex in the two research groups are female. This is in accordance with previous research in which the incidence of T1DM in Indonesia with male sex ratio compared to female 45 : 55, besides that female has proportion 3 times more than male [18]. But contrary to data from the IDF in 2011 that says that male more than female as much as 1.5 times. However, based on ISPAD data in the same year mentioned that gender differences to the incidence of T1DM did not occur as a whole worldwide [1]. These differences are due to differences in population, race, and number of research subjects [19].

Nutritional status in both groups found more children with well nourished. This is consistent with a re-

Table 1. Subject Characteristics

Characteristics	*K1 (n = 13)*	*K2 (n = 13)*
Sex		
Male	6 (6/13)	5 (5/13)
Female	7 (7/13)	8 (8/13)
Duration of disease		
≤ 5 years	6 (6/13)	7 (7/13)
> 5 years	7 (7/13)	6 6/13)
Nutrional status		
Well nourished	8 (8/13)	11 (11/13)
Undernourished	5 (5/13)	2 (2/13)
$25(OH)D_3$ status		
Deficiency	5 (5/13)	5 (5/13)
Insufficiency	2 (2/13)	4 (4/13)
Sufficiency	6 (6/13)	4 (4/13)
Age (years)	12.46 ± 2.99	12.15 ± 3.26
Insulin dose (IU/kg BW)	1.14 ± 0.16	1.19 ± 0.29

Table 2. Results of comparison of levels of $25(OH)D_3$. IL-2, and C-peptide in the group receiving vitamin D supplementation compared with the placebo group

Variable	*K1*	*K2*	*p-value*
$25(OH)D_3$	104.75 ± 39.51	18.56 ± 23.31	0.00
C-peptide	-0.08 ± 0.08	-0.04 ± 0.03	0.26
IL-2	110.12 ± 378.96	31.58 ± 316.46	0.76

search in Brazil where 59% of T1DM people with well nourished, 1% with malnutrition, and 40% with obesity [20]. In theory, nutritional status has no direct effect on T1DM because decreased insulin secretion that occurs due to autoimmune process is not due to decreased insulin sensitivity as occurs in other types of DM [21].

Puberty is a transitional period between childhood and adulthood that is influenced by complex factors. At this time there are physical and psychological changes that arise due to changes in endocrine activity sequentially and regularly. In the United States most girls will experience puberty at the age of 8 – 13 years, while boys are aged 9 – 14 years [22]. Onset, peak work, and duration of insulin work are the decisive factors in the management of DM patients. Insulin dose adjustment aims to achieve optimal metabolic control, without increasing the risk of hypoglycemia and without neglecting the quality of life of patients both short and long term. During the 'honeymoon' period the total daily dose of insulin is < 0.5 IU/kgBW/day, the child before puberty (outside the 'honeymoon' period) in the dose range of 0.7-1.0 IU/kgBW/day, while during puberty the need increases above 1 IU to 2 IU/kgBW/day [23, 24]. This may be the case in this study, with mean age of 12.46 ± 2.99 years for the K1 group and 12.15 ± 3.26 years for the K2 group which is the age of puberty, so that an average insulin dose of 11.14 ± 0.16 and 1.19 ± 0.29 IU/kgBW/day for each group in order.

Level of 25(OH)D3. IL-2, dan C-peptide in the group receiving vitamin D supplementation compared with the placebo group

Based on data normality test, it was found that levels of $25(OH)D_3$ in both groups were normally distributed ($p > 0,05$) while the levels of IL-2 and C-peptide were not normally distributed ($p < 0.05$). From different test, there was a significant difference in the levels of $25(OH)D_3$ among the group receiving vitamin D supplementation and the group receiving placebo ($p = 0.00$). But non-significant differences were found in the levels of IL-2 ($p = 0.76$) and C-peptide ($p = 0.26$) between two groups (Table 2).

Vitamin D status in the study sample was dominated by abnormal levels of $25(OH)D_3$, either deficiency or insufficiency, with a total of 16 people. This indicates that 61.5% of patients with T1DM who are the subjects of our study have low levels of $25(OH)D_3$. Similarly, when the subjects were divided into two groups: those receiving vitamin D supplementation and placebo, both were dominated by low levels of $25(OH)D_3$, i.e. 7 people (54%) and 9 people (69%), respectively. Our research is in line with previous research conducted in RSSA Malang, 90% of children with T1DM had low levels of $25(OH)D_3$ [14]. The same result also obtained in various countries, such as in the United States, Switzerland, and Australia, where in patients with T1DM suffered from vitamin D deficiency and insufficiency [11, 12, 13].

In this study, higher levels of $25(OH)D_3$ were obtained in the group with vitamin D supplementation. Statistically between the groups receiving vitamin D supplementation and the placebo group there was a significant difference ($p = 0.00$). These results were consistent with a randomized study in which 15 patients with T1DM got 1 year of daily oral vitamin D therapy (2000 IU), DHA (38 mg/kg) and intensive diabetes management found vitamin D levels remained stable in patients receiving supplementation but decreased in the control group [25]. Another study, 8 patients with $25(OH)D_3$ deficiency of 15 patients with T1DM consecutively received vitamin D_3 supplementation for up to one year. Target levels of $25(OH)D_3$ in the blood are

achieved and persist after one year of treatment [26]. Study of T1DM patients in Boston was randomized and divided into 4 groups with each group given placebo, vitamin D_3 1,000, 2,000, or 4,000 IU for 3 months. Higher levels of vitamin D increased after 3 months with the highest levels in the group receiving vitamin D_3 4000 IU [27]. A cross-sectional study of 141 T1DM patients and given 1000 IU/day cholecalciferol as supplementation. There was a significant difference in levels of $25(OH)D_3$ between subjects with control and found to be a significant increase in levels of $25(OH)D_3$ [28].

Correlations among levels of 25(OH)D_3, IL-2, and C-peptide in the group receiving vitamin D supplementation

Based on Spearman correlation test, it was found that levels of $25(OH)D_3$ with C-peptide levels, $25(OH)D_3$ levels with IL-2 levels, and C-peptide levels with IL-2 levels were all not significantly correlated ($p = 0.59$; $p = 0.71$; $p = 0.13$). It can be concluded that there is no significant relationship between these three variables (Table 3).

In this study we found lower levels of C-peptide in the group with vitamin D supplementation, although the absolute level of decreased C-peptide was slower in the group receiving supplementation than those receiving placebo. Cumulative incidence of progression to undetectable levels of C-peptide reached 38.5% in the group receiving vitamin D supplementation and 69.2% in the placebo group. Statistically no significant differences in C-peptide levels were obtained between groups receiving vitamin D supplementation with the group receiving placebo ($p = 0.26$). This is consistent with studies conducting a case-control intervention study in 30 children with T1DM. After administering oral doses of cholecalciferol 2000 IU/day for 6 months, the decreased tendency was lower than the β-cell function seen. The percentage reduction of C-peptide was also lower in the intervention group compared with the control group. Three (20%) patients in the control group progressed to an undetectable C-peptide during the study period compared to 1 (6%) in the intervention group [29]. In another study 38 patients with T1DM were randomly assigned either daily oral therapy of cholecalciferol 2000 IU, or placebo. The cumulative incidence of progression to undetectable fasting C-peptide level reached 18.7% in the cholecalciferol group and 62.5% in the placebo group. Therefore, cholecalciferol is safely used as an adjunctive therapy with insulin and is associated with protective immunological effects and a slow decline in the function of residual β -cells in patients with T1DM [30]. A study of patients aged 18 – 39 years in Germany who was newly diagnosed with T1DM who received 0.25 µg $1.25(OH)_2D_3$ or placebo daily for 9 months and followed for a total of 18 months. The results obtained no differences in the area under curve (AUC) C-peptide, peak C-peptide, and fast C-peptide between the treated group and placebo [31]. In another study, 34 patients were newly diagnosed with T1DM in a double-blind trial with calcitriol 0.25 µg/day or placebo and followed for 2 years. The levels of C-peptide fell significantly, but in both groups had similar levels, with no significant difference [32].

Different results were obtained in studies in China, where plasma C-peptide, fasting C-peptide (FCP) and postprandial C-peptide (PCP) levels, remained stable in the insulin-assisted group plus 1-alpha$(OH)D_3$, while FCP levels decreased in the insulin-only group during the 12-month intervention. Seventy percent of patients treated with 1-alpha$(OH)D_3$ of their FCP concentrations persisted or increased after 1 year of treatment, while only 22% of the patients treated with insulin alone had stable FCP levels. Further analysis with different time periods of diabetes showed that better beta-cell beta functions were maintained (reflected significantly higher FCP and PCP levels) in the group receiving 1-alpha$(OH)D_3$ therapy plus Insulin [33]. Research in Iran, where the intervention group received alfacalcidol 0.25 µg twice daily, while the control group received placebo. There was a higher FCP result and lower daily insulin dosage per body weight (DID) in the intervention group, with males having a stronger response to alfacalcidol. No side effects were observed [34]. Another study, 8 patients with $25(OH)D_3$ deficiency, of 15 patients with T1DM consecutively received vitamin D_3 supplementation for up to one year. Target levels of $25(OH)D_3$ in blood were achieved and persisted after one year of treat-

Table 3. Results of correlation *between* levels of $25(OH)D_3$. IL-2, and C-peptide in the group receiving vitamin D supplementation

Variable	*p-value*	*r-value*
Level of $25(OH)D_3$ with level of C-peptide	0.59	- 0.164
Level of $25(OH)D_3$ with level of IL-2	0.71	- 0.115
Level of C-peptide with level of IL-2	0.13	- 0.441

ment and the C-peptide value remained stable after one year of treatment [26].

In this study, higher levels of IL-2 were found in the group receiving vitamin D supplementation. Statistically between the groups receiving vitamin D supplementation and the placebo group did not differ significantly (p= 0.76). Some studies reported direct modulation of CD4 + T cells with active vitamin D, and Treg cell induction expressed IL-17, IL-21, IFN-γ CTLA-4 and FoxP3 inhibition. If T cells grow in an environment rich in IL-2 and vitamin D, they express the highest levels of CTLA-4 and FoxP3, and are capable of suppressing CD4 + T cell proliferation [35,36]. But in his latest study, $1.25(OH)_2D_3$ did not significantly affect the overall expression of FoxP3 or IL-2 but that changed the magnitude of the cytokine effect. Nevertheless, the presence or absence of $1.25(OH)_2D_3$ inflammatory cytokines reduced the effects of TGFβ on the increase of FoxP3 and IL-2 [37].

There was no significant association between levels of $25(OH)D_3$ and C-peptide levels in children with T1DM who received vitamin D supplementation, with very weak association and negative correlation (p = 0.59; r = -0.16). Research in Germany and Italy also obtained similar results [31, 32]. This is due to the duration of disease in this study subjects averaged still a decade so obtained low levels of C-peptide despite the increase of $25(OH)D_3$ and this indicates that the progressive destruction of pancreatic β cells occur mainly in the first years of the emergence T1DM disease [38]. In addition, the onset that occurs at a young age also affects the levels of C-peptide [39]. This result is different from the studies conducted in China where there was a significant positive correlation between vitamin D levels and C-peptide levels [33]. Similarly, the results of a study conducted in Boston showed a significant positive correlation between vitamin D and C-peptide levels [27].

There was no significant association between levels of $25(OH)D_3$ and IL-2 levels in children with T1DM who received vitamin D supplementation, with very weak association and negative correlation (p = 0.71; r= -0.12). Overbergh *et al*, showed that in NOD rats the shift between Th1 / Th2 cells also occurs in the periphery and is not limited to the pancreas. Furthermore, this change only occurs in immune responses with specific autoantigen (GAD65 exposure, B-chain insulin, heat shock protein 65), and not observed in the immune response associated with other antigens (ovalbumin, tetanus toxin, and others). Vitamin $1.25(OH)_2D_3$ clearly causes a decrease in IL-12 and 2 tested Th1 cytokines (IFN-γ and IL-2) after administration of GAD65. Decreased expression of IL-12 is not surprising since $1.25(OH)_2D_3$ can directly inhibit IL-12 transcription. Because of the central role of IL-12 as the main stimulant for Th1 cytokine, decreased expression of IL-12, as expected, is accompanied by similar reductions in IFN- and IL-2 [40]. In another study of IL-2 effect mechanisms showed that in the absence of these cytokines, Treg cells could not survive or expand their numbers in the thymus and peripheral parts [41] and were unable to suppress T cell [42]. From these data, it can be concluded that IL-2 has an important and not excessive function in development, homeostasis, and Treg cell function [43]. This is not in line with studies reporting direct modulation of CD4+ T cells with active vitamin D, and Treg cell induction expressed IL-17, IL-21, IFN-γ CTLA-4 and FoxP3 inhibition. If T cells grow in an environment rich in IL-2 and vitamin D, they express the highest levels of CTLA-4 and FoxP3, and are able to suppress CD4+ T cell proliferation [35,36].

There was no significant association between IL-2 and C-peptide levels in children with T1DM who received vitamin D supplementation, with moderate correlation and negative correlation (p = 0.13; r = -0.44). From the research conducted by Dragovic *et al*., obtained a negative correlation after vitamin D supplementation between IL-2 and C-peptide. This is indicated by a decrease in the incidence of chronic complications of T1DM and an improvement in the complications resulting from increased C-peptide and IL-2 reduction [44]. The same thing is also shown by research in California, which is obtained a negative correlation between C-peptide and IL-2 [45].

Limitations in our study are limited samples just in Malang, so it is less able to describe the status of vitamin D in patients with T1DM in Indonesia. Difficult factors that may arise in this study also cannot be controlled as ideal as possible, such as low vitamin D dietary factors, sun exposure, ethnicity/race, body surface area, and drugs that may affect vitamin D status research subjects who may be contributing to the occurrence of vitamin D deficiency are not examined in this study. Other factors that can affect the levels of C-peptide and IL-2. In addition, there are other factors that may affect the results of research but have not been studied, among others, IL-17, IL-10, IL-12, TGF-β, IFN-γ, and VDR. It is desirable to do further research with more homogeneous sampling by further tightening the inclusion criteria in order to obtain better and meaningful results.

CONCLUSION

From this study can be concluded that administration vitamin D_3 supplementation in patients with T1DM can increase levels $25(OH)D_3$ significantly. This increase has not significantly lowered levels of IL-2 and increased levels of C-peptide. However, there was an absolute decrease in the rate of slower C-peptide in the supplemented group than in the placebo group.

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