Role of Vitamin D in Premature Atherosclerosis in Adolescents Type 1 Diabetes through Transforming Growth Factor-β1, Interferon-γ, Interleukin-10, and Interleukin-17

Harjoedi Adji Tjahjono1,2,3, Wisnu Barlianto2,3, Dian Handayani4, Handono Kalim5

1Doctoral Program of Medical Science, Faculty of Medicine Universitas Brawijaya, Malang, Indonesia; 2Saiful Anwar Hospital, Malang, Indonesia; 3Department of Pediatric, Faculty of Medicine Universitas, Brawijaya, Malang, Indonesia; 4Nutrition Science Program, Medical Faculty, Universitas Brawijaya, Malang, Indonesia; 5Department of Internal Medicine, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

Abstract

BACKGROUND: Cardiovascular disease (CVD) is one of the causes of mortality in patients with type 1 diabetes (T1D). The development of CVD is mainly triggered by atherosclerosis, which is associated with the inflammatory process.

AIM: The current study was aimed to investigate the association of Vitamin D level and premature atherosclerosis in adolescents with T1D, mainly through the regulation of various cytokines (interferon-γ [IFN-γ], IL-17, interleukin-10 [IL-10], and transforming growth factor-β1 [TGF-β1]).

METHODS: This study was designed as a cross-sectional study involving 40 T1D and 40 healthy control who came to the outpatient clinic, Saiful Anwar Hospital, Malang, Indonesia, within the study period (January 2019-July 2019).

RESULTS: Our data demonstrated that the IFN-γ and IL-17 levels were significantly higher (p < 0.001), whereas the TGF-β1 and IL-10 levels were significantly lower (p < 0.001) in T1D group compared with control. Furthermore, T1D also has higher carotid intima-media thickness (cIMT) value and lower flow-mediated dilatation (FMD) value compared to the control group (p < 0.001). Level of 25(OH)D was strongly associated with reduced cIMT and elevated FMD (p < 0.005). The direct effect of 25(OH)D on cIMT and FMD was higher than the indirect effect of Vitamin D through TGF-β1, IL-10, IL-17, and IFN-γ. The cutoff value of 25(OH)D levels for the risk of atherosclerosis was 12.8 ng/dL (sensitivity 85.7% and specificity 86.7%).

CONCLUSION: The level of Vitamin D in the T1D group was significantly lower than those in healthy children and Vitamin D deficiency substantially influences the formation of premature atherosclerosis.

Introduction

Type 1 diabetes (T1D) is caused by insulin deficiency and predominantly occurred in the pediatric population. International Diabetes Federation estimates as many as 1.1 million children and adolescents will be affected by T1D [1]. In Indonesia, the incidence of T1D was approximately 0.2–0.42 per 100,000 children in 2012 [2]. The primary cause of mortality in T1D is cardiovascular disease (CVD) [3]. Evidence from the prospective study showed that T1D patients have a higher risk for the development of myocardial infarction, stroke, and heart failure compared to non-diabetic patients [4].

The increased risk of T1D-associated CVD cannot be described adequately by traditional risk factors (dyslipidemia, hypertension, smoking, and age) and microvascular complications, including nephropathy and neuropathy [2], [3], [4], [5], [6]. The possible explanation for CVD, particularly atherosclerosis, in patients with T1D might be linked to endothelial dysfunction, which is strongly associated with oscillating hyperglycemia and oxidative stress [3], [7], [8], [9]. Moreover, in T1D, absolute insulin deficiency also leads to elevated lipolytic activity, causes a release of pro-atherogenic lipoprotein (very low-density lipoprotein [LDL], intermediate-density lipoproteins, and LDL), and contributes to the development of atherosclerotic plaque [3], [10]. Subclinical atherosclerosis is defined as endothelial dysfunction, coronary calcification, and increased carotid intima-media thickness (cIMT) [11]. Increased cIMT and plaque formation are biologically and genetically distinct entities [12], [13]. A cIMT is a risk factor for cardiovascular events in addition to the other conventional vascular risk factors [13], [14]. Both calcified and non-calcified plaques are independent predictors of vascular disease for all age groups.

Increasing evidence supports a role for Vitamin D that extends beyond that of bone health [15]. Vitamin D deficiency is a known risk factor for CVD [16]. Low Vitamin D level in a patient with T1D is associated with future development of microalbuminuria, retinopathy, and coronary artery calcification as well as increased risk of all-cause mortality [17]. In addition,
Vitamin D deficiency has also been associated with the activation of the renin-angiotensin-aldosterone system and increased levels of inflammatory mediators resulting in target organ injury [18].

The balance between pro-inflammatory and anti-inflammatory cytokines is crucial in terms of both beta-pancreatic cell destruction and atherosclerotic plaque formation. Interferon-γ (IFN-γ) and interleukin-17 (IL-17) are secreted by Th1 and Th17 subset, which associated with beta-cell destruction and pro-atherogenic, while IL-10 and transforming growth factor-β1 (TGF-β1) are anti-inflammatory cytokines which conversely inhibit beta-cell damage and plaque formation [19], [20]. Although some data are in favor of the role of Vitamin D in subclinical atherosclerosis, the association has not been settled yet. We aimed to evaluate the relationship between Vitamin D level and cIMT in patients with T1D through assessing the level of IFN-γ, IL-17, IL-10, and TGF-β1.

Subjects and Methods

Study design

This study was designed as a cross-sectional, which aimed to investigate the association of Vitamin D status with the level of various cytokines (IFN-γ, IL-17, IL-10, and TGF-β1) and the marker for premature atherosclerosis (cIMT and flow-mediated dilatation [FMD]). The procedures conducted in this study have been approved by the Research Ethical Committee, Faculty of Medicine, Universitas Brawijaya (No. 2018/14/03/302/2018). Research subjects were recruited using the consecutive sampling method, where each patient who met the inclusion criteria was included in the study from the period of January to July 2019 until the minimum sample size was achieved (40 subjects) based on the calculation of sample size as described in the literature [21]. All the children included in this study were subjected to general history taking, clinical, and laboratory assessment continued with specific examinations (cytokines and vitamin D level, cIMT, and FMD). This study was conducted at Policlinic of Pediatric Endocrinology, Saiful Anwar Hospital, Malang, Indonesia.

Inclusion criteria for the T1D group were defined as follows: has been diagnosed with T1D, aged 10–18 years old, and the parents/guardian allowed him/her to be involved in this study after being given an explanation (informed consent). Exclusion criteria were defined as follows: Systemic infections, liver disorders, impaired kidney function, malignancy, and anemia (hemoglobin levels <11 g/dL, history of taking Vitamin D supplementation within the previous 3 weeks, amlodipine, valsartan, and statin treatment). For the control group, to rule out the diagnosis of T1D, we conducted a GAD-65 assay. GAD-65 assay was conducted by using an indirect enzyme-linked immunoabsorbent assay (ELISA) method, as previously described by literature [22]. Nutritional status was defined by plotting body mass index (BMI) into a standardized WHO chart for children aged 10–19 years old, then categorized as obese, overweight, normal, and underweight.

Measurement of cytokines and 25(OH)D$_3$ level

The level of cytokines was measured by ELISA methods as instructed by the manufacturer (IFN-γ, IL-17, IL-10, and TGF-β1; R&D systems). The level of 25(OH)D$_3$ was quantitatively measured by the ELISA kit produced by Cusabio (cat. CSB-E07900h). Briefly, after collecting 5 mL of whole blood within the EDTA-coated tube, the samples were then centrifuged for 2500 rpm for 15 minutes at room temperature. The supernatant then removed by using Pasteur pipettes into a new tube. The plasma samples then stored at -80°C to be analyzed together with the other samples.

ELISA method was performed after all the samples obtained. The reagents, standard dilutions, and samples were prepared at room temperature (RT). After adding 200 µL of assay diluent to each well, 100 µL of standard, control, or sample were added into each well, covered with adhesive strip, then incubated at room temperature for two hours. Sequentially, 400 µL of washing buffer was added into each well and aspirated. This washing process was repeated four times. A 100 µL of antibodies were added into each well, then incubated at RT for 30 minutes. The washing process then repeated for four times, continued with the addition of 100 µL of substrate solution and incubation period at RT for 30 minutes. Finally, 100 µL of stop solution was added into each well. Optical density (OD) of each plate was measured by using ELISA reader at the wavelength of 450 nm. The level of cytokines in each sample was derived from the standard curve. The procedure for 25(OH)D$_3$ measurement is quite similar to the measurement of cytokines except the antibody were replaced by enzyme conjugate, and the OD was analyzed at the wavelength of 650 nm.

Measurement of cIMT

Analysis of cIMT was performed as previously described in the literature [23]. Briefly, the diameter of the left and right common carotid arteries were obtained from the M-mode picture of echocardiographic analysis (Philip Type 50G). This procedure was performed by a cardiologist. The measurement of carotid intima-media thickness (cIMT) was taken only from common carotid arteries (without internal carotid and carotid bulb) since it was technically feasible to be done in pediatric subjects. The premature atherosclerotic plaque was defined as cIMT of more than 0.5 mm.
**Measurement of FMD**

FMD analysis was conducted to evaluate the function of the artery and has been done as previously described by the literature [24]. Briefly, the diameter of the brachial artery was measured while the position of the right arm was relaxed and supine so that the ultrasound examination is performed on the brachial artery 5–10 cm above the antecubital fossa. The cuff was inflated at supra systolic pressure (40–50 mmHg above systolic pressure) for 5 min; then sequentially, the cuff was quickly deflated and cause rapid blood flow. Arterial diameter is measured up to 5 min after the cuff was deflated, and the highest width is determined. Baseline diameter was defined as the diameter of the artery before the stimulation using an inflated sphygmomanometer cuff. The percentage of FMD is determined using a standardized formula as follows: FMD% = (peak diameter – baseline diameter)/baseline diameter. While the peak diameter is the largest diameter after reactive hyperemia or after the cuff is suddenly deflated before returning to the base diameter, measured up to the 5th min after reactive hyperemia.

**Statistical analysis**

Statistical analysis was performed using SPSS for Windows software version 24.0. Patient demographic data, which includes age, sex, BMI, and laboratory examination results, are displayed in descriptive data. IL-10 and cIMT data were tested for normality (to determine the distribution of normal data or not) with Kolmogorov–Smirnov and variance homogeneity tests (to find out the same data variant or not). For the comparison study, if the variables were normally distributed and homogeneous, then the independent t-test was used. However, if the variables were not normally distributed and were not homogeneous, the Mann–Whitney test was used. Meanwhile, to see the correlation between IFN-γ, IL-17, IL-10, TGF-β1 with cIMT, and FMD performed Pearson correlation test. A value of p < 0.05 indicates a statistically significant difference.

**Results**

**Characteristics of research subjects**

During the study period, we enrolled 80 subjects consisting of 40 T1D patients and 40 healthy control. Briefly, there were no significant differences between diabetic and control groups based on age, gender, complete blood count parameters, renal function test, and C-reactive protein (CRP) level (p > 0.05). Moreover, the HbA1c level was significantly higher in the diabetic group compared with the control group (p < 0.001). Furthermore, lipid assay panels showed that the T1D group had a significantly higher LDL and total cholesterol level as compared to healthy subjects, but no difference was found in the TG and HDL levels. The characteristics of the subjects of this study are presented in Table 1.

**Table 1: Characteristics of research subjects**

| Characteristic | T1 diabetes (n=40) | Control (n=40) | p-value |
|----------------|-------------------|----------------|---------|
| Age (years)    | 14.42 ± 2.85      | 14.6 ± 0.98    | 0.236   |
| Duration of diabetes (year) | 4.75 ± 1.33      | 4.75 ± 1.33    |         |
| Gender         |                   |                |         |
| Male           | 17 (43%)          | 14 (35%)       | 0.453   |
| Female         | 23 (57%)          | 26 (65%)       | 0.542   |
| Hemoglobin (g/dL) | 14.15 ± 1.11    | 14.57 ± 1.83   | 0.128   |
| Leucocyte (10³/cells/L) | 8.484 ± 2.789  | 7.528 ± 1.337  | 0.149   |
| Thrombocyte (10³/cells/L) | 362.92 ± 62.49  | 346.12 ± 86.05 | 0.341   |
| Urea (mg/dL)   | 23 ± 12           | 19.88 ± 5.2    | 0.315   |
| Creatinine (mg/dL) | 0.6 ± 0.4       | 0.64 ± 0.15    | 0.007   |
| C-reactive protein (mg/dL) | 0.14 ± 0.14     | 0.11 ± 0.22    | 0.056   |
| Nutritional status |                |                |         |
| Normal         | 29 (72%)          | 34 (85%)       |         |
| Underweight    | 7 (18%)           | 2 (5%)         |         |
| Overweight     | 14 (35%)          | 4 (10%)        |         |
| HbA1c (%)      | 9.5 ± 2.02        | 4.7 ± 0.24     | <0.001* |
| <6.5%          | 3 (8%)            | 4 (10%)        |         |
| ≥6.5%          | 37 (92%)          | 0              |         |
| Triglycerides (mg/dl) | 107.5 ± 42.7   | 95.2 ± 45.01   | 0.087   |
| Normal ≥100 mg/dl | 38 (90%)        | 36 (90%)      |         |
| High >150 mg/dl | 2 (5%)           | 4 (10%)        |         |
| Total cholesterol (mg/dl) | 173.9 ± 36.8   | 132.5 ± 29.4   | <0.001* |
| Normal ≥200 mg/dl | 39 (98%)        | 39 (98%)      |         |
| High >240 mg/dl | 1 (2%)           | 1 (2%)         |         |
| LDL (mg/dl)    | 127 ± 32          | 90.18 ± 25.34  | <0.001* |
| Normal ≥100 mg/dl | 33 (85%)        | 40 (100%)     |         |
| High >160 mg/dl | 6 (15%)          | 0             |         |
| HDL (mg/dl)    | 56.3 ± 14.5       | 51.48 ± 10.78  | 0.100   |
| Low <40 mg/dl  | 27 (68%)          | 37 (93%)       |         |
| High >50 mg/dl | 13 (32%)         | 3 (7%)         |         |

Data were presented as mean±SD, *p<0.05 indicates significant.
Correlation between 25(OH)D$_3$ level and the cytokines level

To examine whether the Vitamin D status is associated with the balance between pro-inflammatory and anti-inflammatory markers, we performed a correlation analysis. Our data revealed that the 25(OH)D$_3$ level was positively associated with anti-inflammatory cytokines (IL-10 and TGF-β1). On the other hand, the 25(OH)D$_3$ level was negatively correlated with pro-inflammatory cytokine (IL-17 and IFN-γ). This result indicates that Vitamin D modulates the inflammatory response through the attenuation of pro-inflammatory processes. The results of correlation analysis between vitamin D and cytokine levels is presented in Table 3.

| Cytokine | r     | p      |
|----------|-------|--------|
| IFN-γ    | −0.795| < 0.001|
| IL-17    | −0.797| < 0.001|
| IL-10    | 0.822 | < 0.001|
| TGF-β1   | 0.746 | < 0.001|

*IFN-γ: Interferon-γ, IL: Interleukin.*

Correlation between cytokines level and cIMT

To determine the association between atherosclerotic plaque formation and inflammatory processes, we conducted a correlation analysis. Our data demonstrated that cIMT is negatively correlated with IL-10 level (p < 0.001, r = −0.629) and TGF-β1 level (p < 0.001, r = −0.547). On the other hand, cIMT was positively correlated with IL-17 level (p < 0.001, r = 0.569) and IFN-γ level (p < 0.001, r = 0.650). These data suggest that the development of atherosclerosis plaque, particularly in the carotid arteries, is strongly associated with the inflammatory response. The graph presented the correlation between cytokine levels and cIMT value is shown in Figure 1.

Correlation between cytokines level and FMD

To investigate the association between inflammatory response and the function of the carotid artery, we performed a correlation study. Our data demonstrated that FMD was positively correlated with IL-10 level (p < 0.001, r = 0.635) and TGF-β1 level (p < 0.001, r = 0.592). On the other hand, FMD was negatively correlated with IL-17 level (p < 0.001, r = −0.640) and IFN-γ level (p < 0.001, r = −0.655). Taken together with the cIMT data, these results indicate that pro-inflammatory cytokines have a positive impact on the stiffness of the artery, thus decreasing the FMD. The graph presented the correlation between cytokine levels and FMD value is shown in Figure 2.

The relationship between T1D and premature atherosclerosis

Based on the Chi-square test, there was a significant relationship between the incidence of T1D with the incidence of atherosclerosis based on cIMT value (OR = 37,000; 95% CI = 9.3–146.6), indicating that patient with T1D has 37-fold higher risk to develop premature atherosclerosis compared to healthy control. The association analysis of type 1 diabetes and premature atherosclerosis is shown in Table 4.

| Group       | Carotid intima-media thickness | Total | p-value |
|-------------|--------------------------------|-------|---------|
| Control     | −0.5 mm (negative)              | 37    | <0.001  |
| Type 1 diabetes | −0.5 mm (positive)          | 30    | 0.044   |
| Total       |                                | 67    | 0.003   |
| OR(95% CI)  |                                |       | 9.3–146.6 |

Predictor variables of premature atherosclerosis

To investigate the predictor variables for the development of premature atherosclerosis, we performed logistic regression analysis on gender, age, duration of diabetes mellitus, lipid profile, HbA1c level, BMI, cytokines level, and 25(OH)D$_3$ level. Interestingly, a preliminary correlation study showed that gender, age, duration of diabetes mellitus, lipid profile, HbA1c level, and BMI did not significantly correlate with premature atherosclerosis (p > 0.05) (Table 5). However, the level of 25(OH)D$_3$ is significantly correlated with cIMT value (p < 0.001).

| Predictor | OR 95% CI |
|-----------|-----------|
| Gender    | 2.9–11.4  |
| Age       | 1.01–1.03 |
| Duration of DM | 1.01–1.05 |
| HbA1c     | 0.99–1.01 |
| BMI       | 0.99–1.01 |
| IL-10     | 0.99–1.01 |
| IL-17     | 0.99–1.01 |
| TGF-β1    | 0.99–1.01 |
| 25(OH)D$_3$ | <0.001   |

Other variables, such as cytokines level (IL-17, IFN-γ, IL-10, and TGF-β1) and 25(OH)D$_3$ were significantly correlated with premature atherosclerosis (p < 0.001). Surprisingly, logistic regression analysis by including the cytokines and 25(OH)D$_3$ levels showed that these variables did not significantly influence premature atherosclerosis (Table 6).

| Variables | B     | SE    | Ward  | p    | OR   | 95% CI     |
|-----------|-------|-------|-------|------|------|-----------|
| 25(OH)D$_3$ | 0.863 | 1.377 | 0.393 | 0.531| 2.170| 0.2–35.2  |
| IFN-γ     | 1.533 | 0.962 | 2.537 | 0.111| 4.631| 0.7–30.5  |
| IL-10     | −0.218| 1.307 | 0.028 | 0.868| 0.804| 0.1–10.4  |
| IL-17     | 0.959 | 0.122 | 0.546 | 0.596| 2.610| 0.1–63.8  |
| TGF-β1    | 0.120 | 0.122 | 0.957 | 0.328| 1.127| 0.9–1.4   |
| Constant  | −56.1 | 26.5  | 4.5   | 0.034| 0.000|           |

*IFN-γ: Interferon-γ, IL: Interleukin, TGF-β1: Transforming growth factor-β1.*
Receiver operating characteristic (ROC) analysis on the 25(OH)D$_3$ level and premature atherosclerosis

ROC curve shows that the cutoff value for 25(OH)D$_3$ levels for the risk factor of premature atherosclerosis is 12.80 ng/dL. Based on the cut-off value, 25(OH)D$_3$ ≤ 12.80 ng/dL is categorized as a higher risk for the development or progression of premature atherosclerosis (sensitivity 85.7%, specificity 86.7%, accuracy 86.3%, p < 0.001). The result of ROC analysis is shown in Figure 3.

Discussion

Our data demonstrated that the T1D group had a significantly higher level of LDL and total cholesterol, but no differences were found in the TG and HDL values. Quantitative lipoprotein abnormalities could be found in T1D patients with poor glycemic control (elevated plasma triglycerides and LDL cholesterol) or nephropathy (elevated triglycerides and LDL cholesterol, high-density lipoprotein levels). T1D with optimal glycemic control, plasma triglycerides, and LDL cholesterol are normal or slightly decreased, whereas HDL cholesterol is normal or slightly increased. Several qualitative abnormalities of lipoprotein, which are potentially atherogenic, were observed in patients with T1D, even with good metabolic control [25], [26].

The current study suggests that patients with T1D mellitus have significantly reduced Vitamin D levels, higher pro-inflammatory activity, and lower anti-inflammatory activity. These factors are possibly contributing to carotid stiffness and plaque deposition, as represented by elevated cIMT and reduced FMD. Correlation study also supports the notion that Vitamin D deficiency is strongly associated with the imbalance between pro-inflammatory and anti-inflammatory activity. Consequently, the profound activation of pro-inflammatory cells contributes to the development of atherosclerotic plaque.

In the diabetic state, a chronic inflammatory process is well-known as an important risk factor for β cell damage, insulin resistance, and diabetic...
vascular complications, endothelial dysfunction, and procoagulant imbalance [27], [28], [29]. An observational study demonstrated that T1D is an independent risk factor for increased carotid IMT in children [30], [31]. Inflammatory processes occur in atherosclerotic conditions characterized by increased CRP, pro-inflammatory cytokines, and decreased anti-inflammatory cytokines. The levels of hs-CRP, IL-6, IL-10 are independently related to the level of atherosclerosis [8], [32]. On the other hand, higher serum IL-10 levels are associated with a long-term risk of cardiovascular events [33].

Atherosclerosis is a chronic inflammatory disease of the arterial wall driven by innate and adaptive immune responses. Inflammation controls the development and the destabilization of arterial plaque. Cells involved in the atherosclerotic process secrete and are activated by soluble factors, known as cytokines. Important recent advances in the comprehension of the mechanisms of atherosclerosis have provided evidence for a dual role of cytokines: Pro-inflammatory and T helper-1-related cytokines promote the development and progression of the disease, whereas anti-inflammatory and regulatory T cell-related cytokines exert clear antiatherogenic activities [34], [35], [36].
Recent studies showed that several pro-atherogenic factors, including cholesterol, oxidized LDL, and fatty acids, regulate the production of IL-17 and IL-17-promoting cytokines from innate and adaptive immune cells. Given that IL-17 is associated with a number of autoimmune diseases in humans, dissecting the mechanisms beyond the mutual regulation of pro-atherogenic factors and IL-17 might provide a novel pathophysiology between atherosclerosis and autoimmune diseases [37], [38]. The mechanism of protection appeared to be related to overt decreases in inflammation as levels of serum cytokines such as IL-6, IFN-γ, and IL-12 were reduced into the inflammatory process in atherosclerosis [39].

Vitamin D deficiency is related to enhance pro-inflammatory or autoimmune activity and atherosclerosis formation in T1D patients [40]. Low 25-hydroxyvitamin D levels were associated with increased intima-media thickness and maximal carotid plaque thickness in those with plaque, and 25-hydroxyvitamin D contributed in a robust manner to the variance in both. These results confirm and extend data on the association of low Vitamin D levels with subclinical carotid atherosclerosis [41], [42], [43], [44]. In addition, in advanced disease, there was a significant negative correlation between 25(OH)D and IL-17 in the diabetic neuropathy groups, suggesting that Vitamin D is a potentially modifiable risk factor for diabetes and may regulate inflammatory mediators, for example, IL-17 and IL-13 [45]. Clinically, Vitamin D levels were associated with HbA1c levels and IL-10 levels in T1DM [46]. An animal study also showed that 1,25-(OH)_2 D_3 had a partially protective effect on diabetic rats, which might be through the inhibition of growth factors, VEGF, and TGF-β [47].

IL-10 plays a role in cardiac remodeling and has a protective effect on autoimmune diseases, one of which is T1D. In the process of atherosclerosis, IL-10 can be both an agent that causes atherosclerosis and an atheroprotective agent [28], [29]. The functions of IL-10 are to control cell proliferation, cell migration, matrix synthesis, wound contraction, calcification, and immune response and all are major components of the atherosclerotic process. However, many of the effects of IL-10 are important for tissue repair; hence, IL-10 is also considered atheroprotective. The role of IL-10 in blood vessels is to inhibit the proliferation of endothelial and smooth muscle of blood vessels and play a role in tissue repair. Clinically, lower levels of IL-10 are correlated with atherosclerosis [48]. As the cIMT represents atherosclerosis, whereas IL-10 acts as an anti-atherosclerosis agent, the IL-10 level would be negatively correlated with cIMT [34], [49], [50]. In agreement with the previous studies, our data showed the same finding. Furthermore, our study showed that the TGF-β1 level was also negatively associated with cIMT. Conversely, a previous study demonstrated that elevated TGF-β levels in diabetes play a pathogenic role in the development of accelerated atherosclerosis in diabetes. Consequently, blocking growth factor actions on proteoglycan synthesis with many known cardiovascular and diabetes drugs attenuates lipid binding and potentially provides pleiotropic activities that assist in the prevention of atherosclerosis [51].

Our study showed important data regarding the relation of anti-inflammatory and pro-inflammatory cytokines and the development of premature atherosclerosis in children with type 1 diabetes. Nonetheless, because the current study was designed as a cross-sectional, it has less capability to explain the causative role of cytokine levels and plaque deposition. A prospective cohort might be required to address this highlighted issue. Furthermore, although the sample size has been fulfilled the minimum requirement for the statistical analysis, obtaining more subjects, or recruiting the subjects from different age groups might be beneficial to improve its clinical applicability.

Conclusions

We concluded that low Vitamin D levels are associated with premature atherosclerosis as determined by the elevation of intima-media thickness and the reduction of FMD of the common carotid artery. The pathogenesis of how Vitamin D affects the development of atherosclerosis might be related to the imbalance between pro-inflammatory (IL-17 and IFN-γ) and anti-inflammatory (TGF-β1 and IL-10) cytokines. Our data also suggests that T1D patient with Vitamin D level ≤12.80 ng/dL has a higher risk for the development of premature atherosclerosis.

References

1. International Diabetes Foundation. Diabetes atlas. 6th ed. Vol. 6. International Diabetes Foundation; 2017. p. 142-5.

2. Kemenkes Republik Indonesia. Hasil Utama Riset Kesehatan Dasar Tahun. Indonesia: Kementrian Kesehatan Republik Indonesia; 2018. https://doi.org/10.6066/jtip.2013.24.2.121

3. Hoffman RP. Vascular endothelial dysfunction and nutritional compounds in early Type 1 diabetes. Curr Diabetes Rev 2014;10(3):201-7. https://doi.org/10.2174/157339981066614061324326

4. Lee SI, Patel M, Jones CM, Narendran P. Cardiovascular disease and Type 1 diabetes: Prevalence, prediction and management in an ageing population. Ther Adv Chronic Dis 2015;6(6):347-74. PMID:24925525

5. Krantz JS, Mack WJ, Hodis HN, Liu CR, Kaufman FR. Early onset of subclinical atherosclerosis in young persons with Type 1 diabetes. J Pediatr 2004;145(4):452-7. https://doi.org/10.1016/j.
31. Atwa HA, Shora HA, Elsayed A. Hormonal, metabolic and radiological markers of subclinical atherosclerosis in Egyptian children with Type 1 diabetes. Rep Endocr Disord 2018;2(1):3.

32. Trigona B, Aaggoun Y, Maggio A, Martin XE, Marchand LM, Begghti M, et al. Preclinical noninvasive markers of atherosclerosis in children and adolescents with Type 1 diabetes are influenced by physical activity. J Pediatr 2010;157(4):533-9. https://doi.org/10.1016/j.jpeds.2010.04.023

33. Kamaly N, Fredman G, Fojas JJ, Subramanian M, Choi WI, Zepeda K, et al. Targeted interleukin-10 nanotherapeutics developed with a microfluidic chip enhance resolution of inflammation in advanced atherosclerosis. ACS Nano 2016;10(5):5280-92. https://doi.org/10.1021/acsnano.6b01114

34. Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. Recent advances on the role of cytokines in atherosclerosis. Arterioscler Thromb Vasc Biol 2011;31(5):969-79. https://doi.org/10.1161/ata.vbaha.110.207415

35. Ross R. Atherosclerosis an inflammatory disease. N Eng J Med 1999;340(2):115-26.

36. Ryu H, Chung Y. Regulation of IL-17 in atherosclerosis and regulatory pathways. Physiol Rev 2006;86(2):515-81.

37. Tedgui A, Mallat Z. Cytokines in atherosclerosis: Pathogenic and regulatory pathways. Physiol Rev 2006;86(2):515-81.

38. Allam G, Abdel-Moneim A, Gaber AM. The pleiotropic role of interleukin-17 in atherosclerosis. Biomed Pharmacother 2018;108(5):18-18. https://doi.org/10.1152/physrev.00024.2005

39. Major AS, Harrison DG. What fans the fire: Insights into mechanisms of inflammation in atherosclerosis and diabetes mellitus. Circulation 2011;124(25):280911. https://doi.org/10.1161/circulationaha.111.070565

40. Tiwari S, Pratyush DD, Gupta SK, Singh SK. Vitamin D deficiency is associated with inflammatory cytokine concentrations in patients with diabetic foot infection. Br J Nutr 2014;112(12):1938-43. https://doi.org/10.1017/s0007114514003018

41. Carrelli AL, Walker MD, Lowe H, McMahon DJ, Rundek T, Sacco RL, et al. Vitamin D deficiency is associated with subclinical carotid atherosclerosis: The Northern Manhattan study. Stroke 2011;42(8):2240-5. https://doi.org/10.1161/strokeaha.110.608539

42. Devaraj S, Yun JM, Duncan-Staley CR, Jialal I. Low Vitamin D levels correlate with the proinflammatory state in Type 1 diabetic subjects with and without microvascular complications. Am J Clin Pathol 2011;135(3):429-33. https://doi.org/10.1309/ajcpgzq4z2xiali

43. Young KA, Snell-Bergeon JK, Naik RG, Hokanson JE, Tarullo D, Gottlieb PA, et al. Vitamin D deficiency and coronary artery calcification in subjects with Type 1 diabetes. Diabetes Care 2011;34(2):454-8. https://doi.org/10.2327/dc10-0757

44. Sachs MC, Brunzell JD, Cleary PA, Hoofnagle AN, Lachin JM, Molitch ME, et al. Circulating Vitamin D metabolites and subclinical atherosclerosis in Type 1 diabetes. Diabetes Care 2013;36(8):2423-9. https://doi.org/10.2327/dc12-2020

45. Bilir B, Tulubas F, Bilir BE, Atlle NS, Kara SP, Yildirim T, et al. The association of Vitamin D with inflammatory cytokines in diabetic peripheral neuropathy. J Phys Ther Sci 2016;28(7):2159-63. https://doi.org/10.1589/jpts.28.2159

46. Wulandari D, Cahyono HA, Widjajanto E, Puryatni A. Low levels of Vitamin D correlate with hemoglobin A1c and interleukin-10 levels in pediatric Type 1 diabetes mellitus patients. J Trop Life Sci 2014;4(3):182-6. https://doi.org/10.11594/jtls.04.03.04

47. Ren Z, Li W, Zhao Q, Ma L, Zhu J. The impact of 1,25-dihydroxy vitamin D3 on the expressions of vascular endothelial growth factor and transforming growth factor-β1 in the retinas of rats with diabetes. Diabetes Res Clin Pract 2012;98(3):474-80. https://doi.org/10.1016/j.diabres.2012.09.028

48. Geovanini GR, Libby P. Atherosclerosis and inflammation: Overview and updates. Clin Sci (London). 2018;132(12):1243-52. https://doi.org/10.1042/cs20180306

49. Pillay AK, Naidoo DP. Atherosclerotic disease is the predominant aetiology of acute coronary syndrome in young adults. Cardiovasc J Afr 2018;29(1):36-42. https://doi.org/10.5830/cvja-2017-035

50. Dalla Pozza R, Beyerlein A, Thilmany C, Weissenbacher C, Netz H, Schmidt H, et al. The effect of cardiovascular risk factors on the longitudinal evolution of the carotid intima media thickness in children with Type 1 diabetes mellitus. Cardiovasc Diabetol 2011;10:53. https://doi.org/10.1186/1475-2840-10-53

51. Yang SN, Burch ML, Tannock LR, Evanko S, Osman N, Little PJ. Transforming growth factor-β regulation of proteoglycan synthesis in vascular smooth muscle. Contribution to lipid binding and accelerated atherosclerosis in diabetes. J Diabetes. 2010;2(4):233-42. https://doi.org/10.1111/j.1753-0407.2010.00089.x

PMid:21799770

PMid:21799770

PMid:20923499

https://www.id-press.eu/mjms/index