

Available online at [GSC Online Press Directory](https://www.gsconlinepress.com/)

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsconlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



Relationship between vitamin D deficiency and premature atherosclerosis in an adolescents with type 1 DM through inflammatory IFN- γ

Mohamed Faraj Ali Aldaefi^{1*} and Harjoedi Adji Tjahjono²¹ Master program of Biomedical Sciences Faculty of Medicine, Universitas Brawijaya, Malang 65145 Indonesia.² Pediatric department, Saiful Anwar General Hospital Malang 65112 Indonesia.

Publication history: Received on 06 February 2020; revised on 04 April 2020; accepted on 06 April 2020

Article DOI: <https://doi.org/10.30574/gscbps.2020.11.1.0032>

Abstract

Vitamin D deficiency has been related to increased risk of myocardial infarction, stroke. Type 1 DM patients have a higher risk of atherosclerosis, which is the leading cause of death in most patients, and IFN- γ is a pro inflammatory cytokine, and type 1 DM is a chronic inflammation. In our study, we try to detect the premature atherosclerosis in adolescents with type 1 DM who have vitamin D deficiency through inflammatory IFN- γ .

The study is a case control with 31 diabetic children aged 6 to 18 years as a case group, and control group 35 healthy children aged 10-18 years. We analysis the vitamin D, IFN- and HbA1c, and using Doppler US for cIMT of common carotid artery(CCA), and flow mediated dilation index(FMD%) of the brachial artery. And analysis the data through SPSS version16.0.

Type 1 DM group have higher levels of IFN- γ (p-0.005) and cIMT (p-0.01.) and both were significant, while the correlation effect of vitamin D deficiency and IFN- γ on both cIMT and FMD% were (p-0.294) and (p-0.270) (p-0.794)and (p-0.894) respectively and both insignificant, and correlation effect of HbA1c on cIMT was significant with (p-0.002), and correlation effect of HbA1c on FMD% was insignificant with (p-0.222).

We concluded that there is significantly higher of IFN- γ in type 1 DM, also a significant correlation effect of HbA1c on cIMT, and no relationship between vitamin D deficiency and premature atherosclerosis in an adolescents with type 1 DM through inflammatory IFN- γ .

Keywords: Vitamin D deficiency; Type 1 DM; cIMT; FMD%

1. Introduction

Vitamin D is a hormone (steroid), and the most sources of it is the skin, during exposure of the skin to ultraviolet B radiation. Also, it can be supplied from the diet (like salmon) or by supplements (drugs), some studies conducted that its deficiency has increased the risk of autoimmune diseases like diabetes mellitus (DM). Many studies suggest, its deficiency is associated with the risk for many diseases, like (cardiovascular disease, osteoarthritis, and rejection transplantation). [1]

The cells within the immune system can affected by vitamin D. So It discourages generation and differentiation of B cell, and immunoglobulin secretion. Vitamin D also leads to suppression of T cell generation and that is resulting in conversion from “Th1 to a Th2” moreover, it affects the maturity of T cell with a deviation away from the inflammatory Th17 phenotype and facilitates the T regulatory cell induction. These effects lead to decreased production of

* Corresponding author: Mohamed Faraj Ali aldaefi [Email address : mooa82d@gmail.com](mailto:mooa82d@gmail.com)

inflammatory cytokines (IL-21, IL-17) and increased production of anti-inflammatory cytokines such as IL-10. Vitamin D also has effects on dendritic cells (DCs) and monocytes. It inhibits monocyte production of inflammatory cytokines such as IL-12, IL-8, IL-6, IL-1 and TNF α . Also, it inhibits DC maturation and differentiation.[2].

In case of low vitamin D level has been shown to be related to increased risk of myocardial infarction (MI), stroke, and total cardiovascular events. Vitamin D has a variety of favorable effects on endothelial dysfunction, vascular smooth muscle cells (VSMC) proliferation and migration, and calcification, as well as on the atherosclerosis via immune response, furthermore, it has a beneficial effect against systemic conditions that enhanced atherosclerosis such as insulin resistance, RAAS, β -cell dysfunction, dyslipidemia. [3].

Before the Vitamin D is considered as a regulator of bone and mineral metabolism only, but now we believed that it has a potent immune modulator linked to many diseases in human like, insulin resistance glucose homeostasis. In human and animal there is evidence that Vitamin D deficiency can affect the secretion of the insulin. Now a day many evidences suggests the role of vitamin D in the pathogenesis of insulin resistance including several vitamin-D-related gene polymorphisms and vitamin-D-related metabolic and immune pathways. [4].

Type 1 DM is a multisystem disease and it is also a chronic disease of fat, protein, and carbohydrate metabolism, which is due to absence of insulin, which results from the noteworthy and progressive inability of the beta cells of the pancreas to secrete insulin because of its autoimmune destruction. Internationally, type 1 DM is increasing 2-5% per year In Europe, the Australia, and Middle East. About one-fifth of the total number of people with DM in Scandinavia. Chinese and Japanese make up which less than 1% of diabetic person in the world wide. Some of these differences may relate to the completeness of reporting and definitional issues. [5].

Before the most significant cause of morbidity and mortality in diabetic patients is acute complications like DKA and hypoglycemia which are now due to good management significantly decreased. Diseases like coronary artery, cerebrovascular, and peripheral vascular disease most often occur at an earlier age in diabetic patients than the no diabetic population. Diabetic patients have significantly increased the risk of atherosclerotic vascular disease. heart attacks and strokes which are the most common causes of death in these patients which are due to macro-vascular diseases.[6].

The pleiotropic cytokine IFN- γ is a pro-inflammatory mediator that is detected at high levels in atherosclerotic lesions by various cells, including macrophages/ monocytes, natural killer T-cells (NKT) and T_H1 cells,. Activated NKT can produce significant amounts of IFN- γ and are pro-atherogenic in mice. IFN- γ has been involved in the atherosclerotic process via direct effects and indirect via IL-18 and IL-12.[7].

Subclinical atherosclerosis are defined as endothelial dysfunction, coronary calcification, and increase thickness of intima media of the carotid artery, there are also data indicating the possible relation between low vitamin D and subclinical atherosclerosis.[8, 9].

Increased carotid intima-media thickness (cIMT) is a marker of structural atherosclerosis, which correlates with cardiovascular risk factor, and the Low vitamin D levels in childhood were associated with increased carotid cIMT in adulthood.[10].

Endothelial dysfunction is an important early event in the development of atherosclerosis, which forego total clinical symptoms and morphological signs. About 20 years ago the assessment of flow-mediated dilation (FMD) was introduced as a non-invasive technique to examine vasodilator function in vivo. FMD has become common in clinically oriented studies, because it strongly predicts cardiovascular events in patients with established cardiovascular diseases. FMD appears to be predictive of those with constructing cardiovascular diseases or those with asymptomatic cardiovascular events.[11].

There is associated between the supplementation of vitamin D3 and the improvement of FMD in obese African American people who were healthy with no diseases like diabetes and hypertension.[12].

2. Subjects and methods

2.1. Subjects

The study is case control with two groups, the first group was the case group, which was 31 children and all was type 1 DM aged 6 to 12 years (mean age 15.94 ± 1.00 years), while the second group was 35 healthy children aged 6-18 years (mean age 14.63 ± 1.00 years), all children with type 1 DM which were selected undergoing outpatient polyclinic at department of endocrine in Saiful Anwar general Hospital, Malang during the research period, and all children in both groups selected based on inclusion and exclusion criteria, after signed of the family in the consent of paper.

2.2. Study design and sample size

The study design is case control study with 31 patients with type 1 DM as case group and 35 healthy children as the controls group with total number 66 children. Considering a maximum error of 5% and a 95% confidence interval, P value of ≤ 0.05 provide statistically significant.

Examination of the thickness of the common carotid artery can be used as a screening risk of cardiovascular disease which is a macro vascular complication from type1 DM. measurement of the diameter of the left and right common carotid by M-echocardiographic mode to see carotid stiffness [13].

We measured only common carotid artery cIMT because our patients were mostly children, and we measured both right and left and we take maximum one, because internal carotid and carotid bulb measurement depends on anatomical tomography of the patient and difficult to done in children[14].

Blood pressure measurement done for all children after 10 minutes of rest, using appropriate cuff sized sphygmomanometer, children were placed in supine position with sphygmomanometer in their left forearm, cuff was inflated 50mmHg above the systolic blood pressure for 4 minutes and the images taken in 1,3,5 minutes and take a baseline image before the inflated the cuff, this is according to protocol [15].

We use the FMD% index, which is calculated by dividing the 'response' or change in arterial diameter by the initial baseline diameter of the artery and multiplying by 100, and the average normality of FMD% is 10.7[16].

Examination of vitamin D levels was carried out in the Clinical Pathology Laboratory in Saiful Anwar General Hospital, Malang. The sample used is in the form of plasma stored at a temperature of -20°C . The examination method is to prepare a polypropylene tube, one for the calibrator, control and sample respectively. Biotin solution 25-D as much as 1 ml is added to all tubes, vortex for 10 seconds. Each dilution of the calibrator is added 200 UL, controls or samples in suitable wells from coated plate / micro plate antibodies. The plate is covered with plastic. Incubated at $18-25^{\circ}\text{C}$ for 2 hours. All wells were washed 3 times with a wash solution, 200 UL of the conjugate was added to the well using a multichannel pipette. The plate is closed and wrapped in plastic, incubated at a temperature of $18-25^{\circ}\text{C}$ for 30 minutes. 200 UL TMB substrate was added to all wells, plates were closed and wrapped in plastic bags, incubated $18-25^{\circ}\text{C}$ for 30 minutes, then as many as 100 UL stop solutions were added to all wells. The absorbance of each well is measured at 450 nm using an ELISA reader in 30 minutes.[17]

HbA1c examination is done by taking whole blood samples (whole blood) in a tube containing EDTA. The sample is stable for 4 days at $2-8^{\circ}\text{C}$ or for 1 day at room temperature ($15-30^{\circ}\text{C}$). Samples were left at room temperature ($15-30^{\circ}\text{C}$) before analysis. The Samples do not require special preparation. When the sample was less than 2 ml, it was previously dissolved in 1: 300 in a 1.5 ml sample vial (5 ml sample in 1.5 ml of solvent) before being analysed, then examined with the Bio-Rad D-10TM device using the HPLC method. The principle of this method is ion exchange to reach an isoelectric point where the isoelectric point of HbA1c is lower and faster to migrate than other Hb components (BioRad, 2010).

Measurement of levels of IFN- γ using the ELISA method. All reagents, standards and samples are prepared. 200 μl diluent assays were added to the well, incubated for 1 hour. Added 100 μl standardize, control and sample per well, then covered with adhesive strip, incubated 2 hours at room temperature. Each well is aspirated and washed, the process is repeated up to 4 washing times. Washing is done with wash buffer (400 μl). At the last wash washout was removed by aspiration, 100 μl of antibody was incubated for 1 hour. 100 μl of avidin HRP conjugate IFN- γ were added to each well. The plate is covered with adhesive strip, then incubated 30 minutes at room temperature. Aspiration or washing is repeated. 100 μl TMB solution substrate was added to each well, incubated 30 minutes at room temperature. Stop solution 100 μl is added to each well. The color of the well will change from blue to yellow. Optical density in each

well was determined by a micro plate reader at 450 nm. The absorbance obtained is converted to equations in the standard curve so that the levels of FN γ are obtained, and normal level is 0.4-16pg/ml. (biolegend.com).

3. Results

3.1. Characteristic of study sample

The study consists of two groups, the first group is adolescents with type 1 DM and second one is adolescents non-diabetic control group. The first group consists of 31 children and the second group consist of 35 children who had been selected based on inclusion and exclusion criteria. The characterization data were analysed using Kolmogorov-Smirnov normality test, and if it is transformed normally, then we did the unpaired t test. And in case transformed is not normally distributed, then we did the Mann-Whitney test. The detailed characteristics of the sample are shown in table 1 below.

Table 1 Characteristic of study sample

Characteristic of sample	Control (n=35)	Type 1 DM(n=31)	p-value
Male	16	13	0.758a
Female	19	18	0.758a
Age(years)	14.63±1.00	15.94±1.00	0.087b

*a. The correlation result was not significant with Chi-square test*b. The compare mean result was not significant with Mann Whitney test.

The table 1 shows that based on the gender in the control group there were 16 boys and 19 girls, while in the type 1 DM group were 13 boys and 18 girls. It appears that the distribution of the data by sex was insignificantly different between the two groups (p-0.758).this explains that the selection of research samples from sex is evenly distributed. Likewise, the data based on the age (years) in the control group with the average 14.6053±1.00 years and in the type 1 DM with average 15.94 ±1.00 years.it appears that the distribution of the data based on the age was insignificantly different (p-0.087).this explains that the taking or comparing of research samples based on the control group and type 1 DM obtained the age of children who have been homogeneous, because the distribution of the data on the age of the children is almost the same.

3.2. Vitamin D level

Based on the table 2, the mean level of vitamin D in the type 1 DM group was (11.17ng/ml) higher than the mean level of vitamin D in the control group which was (9.68ng/ml), the p value was (0.278 > 0.050), which means that the vitamin D level in the type 1 DM group was slightly higher than the vitamin D level in the control group but insignificant.

Table 2 The results of the vitamin D normality test

Group	Mean	St dev.	Normality Significance
Type 1DM	11.1753	5.64450	0.200
Control	9.6830	5.42223	0.067
p-value		= 0.278	

3.3. HbA1c level

Based on the table 3 below, the mean level of HbA1c in the type 1 DM group was (9.92%) higher than the mean level of HbA1c in the control group which was (4.72%), the p value was (0.000 < 0.050), which means that HbA1c in the type 1 DM group was higher than the HbA1c in the control group and significant.

Table 3 The results of the HbA1c normality test

Group	Mean	St dev.	Normality Significance
Type 1 DM	9.7838	2.52339	0.147
Control	4.7211	0.24179	0.004
p-value		= 0.000	

3.4. IFN- γ level

Based on the table 4 below, the mean level of IFN- γ in the type 1 DM group was (26.06pg/ ml) higher than the mean level of IFN- γ in the control group which was (16.74pg/ ml), the p value was (0.005 < 0.050), which means that the IFN- γ in the type 1 DM group was higher than the IFN- γ in the control group and significant.

Table 4 The results of IFN- γ normality test

Group	Mean	St dev.	Normality Significance
Type 1 DM	26.0618	16.6583	0.000
Control	16.7462	7.20297	0.000
p-value		= 0.005	

3.5. cIMT (CCA)

Based on the table 5 below, the mean of cIMT of the CCA in the type 1 DM group was (0.49mm) higher than the mean in the control group which was (0.44mm), the p value was (0.010 < 0.050), which means that cIMT of CCA in the type 1 DM group was higher than the cIMT of CCA in the control group and significant.

Table 5 The results of cIMT normality test

Group	Mean	St dev.	Normality Significance
Type 1 DM	0.4932	0.08654	0.200
Control	0.4400	0.07585	0.200
p-value		= 0.010	

3.6. FMD%.

Based on the table 6 below, the mean of the FMD% in the type 1 DM group was (13.75%) lower than the mean FMD% in the control group which was (14.26%), the p-value was (0.362 > 0.050), which means that the FMD% in the type 1 DM group was slightly lower than the FMD% in the control group but insignificant.

Table 6 The results of FMD% normality test

Group	Mean	St dev.	Normality Significance
Type 1 DM	13.7465	12.97728	0.001
Control	14.2563	9.36508	0.200
p-value		= 0.362	

3.7. Effect of HbA1c on cIMT

3.7.1. Type 1 DM group

Based on the table 7 below, the HbA1c has a positive and significant effect on the cIMT, seen from the p-value ($0.002 < 0.050$). The negative coefficient indicates that the HbA1c can significantly increase the cIMT.

3.7.2. Control group

Based on the table 7 below, the HbA1c has a positive and not significant effect on the cIMT, seen from the p-value ($0.250 > 0.050$). The negative coefficient indicates that the HbA1c can insignificantly decrease the cIMT.

Table 7 The effect of HbA1c on cIMT

Group	t count	p value	R square
Type 1 DM	3.330	0.002	0.277
Control	1.170	0.250	0.040

3.8. Effect of HbA1c on FMD%

3.8.1. Type 1 DM group

Based on table8 below, the HbA1c has a negative and insignificant effect on the FMD%, seen from the p-value ($0.222 > 0.050$). The negative coefficient indicates that the higher level of HbA1c can decrease the FMD% but insignificant.

3.8.2. Control group

Based on table 8 below, the HbA1c has a positive and not significant effect on the FMD%, seen from the p value ($0.566 > 0.050$). The negative coefficient indicates that the HbA1c can increase the FMD%, but insignificant.

Table 8 The effect of HbA1c on FMD%

Group	t count	p value	R square
Type 1 DM	-1.423	0.222	0.051
Control	3.782	0.566	0.010

3.9. The effect of vitamin D deficiency on cIMT through IFN- γ

3.9.1. The effect of vitamin D deficiency on cIMT through IFN- γ (type 1 DM group).

Based on the table 9 below, the Vitamin D deficiency has a negative and insignificant effect on the IFN- γ , with a p-value ($0.305 > 0.050$). The negative coefficient indicates that the Vitamin D deficiency can decrease the IFN- γ , but insignificant.

The Vitamin D deficiency has a negative and insignificant effect on the cIMT, with a p-value ($0.294 > 0.050$). The negative coefficient indicates that the Vitamin D deficiency can decrease the cIMT, but insignificant.

The IFN- γ has a positive and insignificant effect on the cIMT, with a p-value ($0.794 > 0.050$). The positive coefficient indicates that the IFN- γ can increase the cIMT, but insignificant.

The indirect effect of the Vitamin D deficiency on the cIMT through IFN- γ shows the insignificant effect, with a coefficient of $(-0.190 \times 0.049) -0.009$, because of the direct influence between the Vitamin D deficiency on the IFN- γ , and the direct effect between the IFN- γ on the cIMT, insignificant.

Table 9 The effect of vitamin D deficiency on cIMT through IFN- γ (Type 1 DM group)

Effect	Coefficients	p value	Conclusion	R square
vitamin D on IFN- γ	-0.190	0.305	Not significant	0.036
vitamin D on cIMT	-0.201	0.294	Not significant	0.047
IFN- γ on cIMT	0.049	0.794	Not significant	

3.9.2. The effect of vitamin D deficiency on cIMT through IFN- γ (control group)

Based on the table 10 below, the Vitamin D deficiency has a negative and insignificant effect on the IFN- γ , with a p-value (0.795 > 0.050). The negative coefficient indicates that the Vitamin D deficiency can decrease the IFN- γ , but insignificant.

The Vitamin D deficiency has a negative and not significant effect on the cIMT, with a p-value (0.885 > 0.050). The negative coefficient indicates that the Vitamin D deficiency can decrease the cIMT, but insignificant.

The IFN- γ has a negative and not significant effect on the cIMT, with a p-value (0.332 > 0.050). The negative coefficient indicates that the IFN- γ can decrease the cIMT, but insignificant.

The indirect effect of the Vitamin D deficiency on the cIMT through IFN- γ shows insignificant effect, with a coefficient of (-0.046 \times -0.172) 0.008, because of the direct influence between the Vitamin D deficiency on the IFN- γ and the direct effect between the IFN- γ on the cIMT, insignificant.

Table 10 Effect of vitamin D deficiency on cIMT through IFN- γ (control group)

Effect	Coefficients	p value	Conclusion	R square
vitamin D on IFN- γ	-0.046	0.795	Not significant	0.002
vitamin D on cIMT	-0.025	0.885	Not significant	0.030
IFN- γ on cIMT	-0.172	0.332	Not significant	

3.10. The effect of vitamin D deficiency on FMD% through IFN- γ

3.10.1. The effect of vitamin D deficiency on FMD% through IFN- γ (type 1 DM group)

Based on the table 11 below, the Vitamin D deficiency has a negative and insignificant effect on the IFN- γ , with a p-value (0.305 > 0.050). The negative coefficient indicates that the Vitamin D deficiency can decrease the IFN- γ , but insignificant.

The Vitamin D deficiency has a positive and insignificant effect on the FMD%, with a p-value (0.270 > 0.050). The positive coefficient indicates that the Vitamin D deficiency can increase the FMD%, but insignificant.

The IFN- γ has a negative and not significant effect on the FMD%, with a p-value (0.894 > 0.050). The negative coefficient indicates that the IFN- γ can decrease the FMD%, but insignificant.

The indirect effect of the Vitamin D deficiency on the FMD% through IFN- γ shows the insignificant effect, with a coefficient of (-0.190 \times -0.025) 0.005, because of the direct influence between the Vitamin D deficiency on the IFN- γ and the direct effect between the IFN- γ on the FMD%, insignificant.

Table 11 The effect of vitamin D deficiency on FMD% through IFN- γ (type 1 DM group)

Effect	Coefficients	p value	Conclusion	R square
Vitamin D on IFN- γ	-0.190	0.305	Not significant	0.036
vitamin D on FMD%	0.211	0.270	Not significant	0.047
IFN- γ on FMD%	-0.025	0.894	Not significant	

3.10.2. The effect of vitamin D deficiency on FMD through IFN- γ (control group)

Based on the table 12 below, the Vitamin D deficiency has a negative and insignificant effect on the IFN- γ variable, with a p-value (0.795 > 0.050). The negative coefficient indicates that the Vitamin D deficiency can decrease the IFN- γ variable but insignificant.

The Vitamin D deficiency has a negative and insignificant effect on the FMD%, with a p-value (0.759 > 0.050). The negative coefficient indicates that the Vitamin D deficiency can decrease the FMD%, but insignificant.

The IFN- γ has a positive and not significant effect on the FMD%, with a p-value (0.718 > 0.050). The positive coefficient indicates that the IFN- γ can increase the FMD%, but insignificant.

The indirect effect of the Vitamin D deficiency on the FMD% through IFN- γ shows the insignificant effect, with a coefficient of $(-0.046 \times 0.064) -0.003$, because of the direct influence between the Vitamin D deficiency on the IFN- γ and the direct effect between the IFN- γ on the FMD%, insignificant.

Table 12 The effect of vitamin D deficiency on FMD through IFN- γ (control group)

Effect	Coefficients	p value	Conclusion	R square
vitamin D on IFN- γ	-0.046	0.795	Not significant	0.002
Vitamin D on FMD%	-0.055	0.759	Not significant	0.007
IFN- γ on FMD%	0.064	0.718	Not significant	

4. Discussion

4.1. General characteristic

In the results of this study, we have 66 children divided into 2 groups, namely adolescent with type 1 DM (the case group) which account of 31 children while adolescent control group (non- diabetic) account 35 children. The average age of type 1 DM group was 15 years, which are near to epidemiological data the peak incidence of type 1 DM is 10-14y. [6].

Based on the gender the distribution of boys compare to girls in type 1 DM group in this study shows the more distribution in girls than boys due to randomized selection of the data with ratio of 1.39 : 1 (18/13), which is opposite that in the reports of the epidemiological data that shows the type 1 DM is high in boys than in girls.[18].But is the same results according to Indonesia pediatric society which shows that the incidence of type 1 DM was in male 0,00388 per 100,000 and 0,00483 per 100,000 in female.(Pulungan, 2013).

4.2. Vitamin D levels

The results of this study found in the type 1 DM group that 30 children have vitamin D deficiency, and only 1 children with vitamin D insufficiency while there is no children with normal level of vitamin D While in control group there is 34 children have vitamin D deficiency and 1 children with vitamin D insufficiency, while also there is no children with normal level of vitamin D.in this study shows that the mean of vitamin D in type 1 DM group was (11.17ng/ml) which higher than in the control group which was (9.68ng/ml) and both groups was deficient, our results is opposite the result of study done before in 2015 by liu *et al*, and studies done in 2005 by Pozilli *et al*, which shows that vitamin D3 is low in type 1 DM in comparison to normal children.

To explain our results, it is because the type 1 DM group have regular follow up at endocrine polyclinic in our hospital (Saiful Anwar General Hospital Malang) which give them advice about the type 1 DM in general and its complications and the benefit of vitamin D in diabetic and give them advice to the appropriate time to sun exposure and the source of the vitamin D in the food, also Applying sunscreen which used as a sun protection factor may reduce vitamin D synthesis in the skin by more than 95%. Many factors can affect the vitamin D status, including skin colour, clothing, latitude, season, time spent outdoors, weight status, , and some medications and some medical conditions which are not included in our research.[19].

4.3. IFN- γ level

The result of this study found that in the type 1 DM group the children have higher level of IFN- γ with a mean (26.06pg/ml), while in the mean in the control group was (16.74pg/ml) that's been because IFN- γ is a Th1 type cytokine that is playing a role to activate the cytotoxic T cells and enhancement of inflammatory reaction, and type 1 DM is a chronic inflammation that's why IFN- γ was very high in group of patient of our study in compare to a normal control group. the IFN- γ is a strong activator of inflammation and cellular immunity and response for activation of macrophages[20].

IFN- γ is produced predominantly by natural killer (NK) cells as a part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocytes (CLT) effector T cells once antigen -specific immunity develops [21].

4.4. HbA1c level

In this study, we found that the HbA1c in the type 1 DM group was(9.91%) which was higher than in the normal level and also higher than in what's seen in the control group which was(4.72%) which is normal, that's because all patients in the type 1 DM group were diabetic while a control group were healthy.

HbA1c is the Glucose levels that are bound to hemoglobin (glycated haemoglobin) to see blood glucose levels averaged over a period of 3 months, in units of%.[22].

The high level of HbA1c means that our patient have poor metabolic control and those related to the age which is the age of puberty and hormonal changes play a role in insulin resistance also change of diet, activity, and poor compliance of insulin treatment. [23].

4.5. cIMT

In this study, we found that the mean of the cIMT level was significantly higher in type 1 DM group (0.4932mm) than in the control group (0.440mm),that's mean that the type 1 DM have increased thickness of the carotid artery cIMT. This is consistent with the hypothesis that the anatomical damage of the arterial wall which is characterized by thickening of the intima media of the arteries is the initial stage of atherosclerosis .The results of this study are the same results with the research done in 150 Caucasian children who have type 1 DM and concluded that the cIMT results in children and adolescents with type 1 DM were increased compared to control subjects. [24].

Measurement of intima-media thickness in the carotid arteries using B-mode duplex ultrasound (DUS) ultrasound, which has the advantage of being non-invasive, safe, fast and easy to repeat, is simple and describes the structure of the arterial wall with better resolution than other radiographic techniques. The weakness of this technique is that it depends on the skill and experience of the examiner and is difficult to do with the location of blood vessels other than the carotid artery, also, no data for the normality value of cIMT because it affected by many factors.

4.6. FMD%

In this study, we found that the mean of the FMD% level was insignificantly lower in type 1 DM group (13.74%) than in the control group (14.25%), which is the same of the results done in Egypt at Ismailia hospital during period 05-2016 to 2-2017 in 68 patients with type 1 DM and shows lower FDM in children with type 1 diabetes compared to control group. [25]

Flow-mediated dilation (FMD) is a non-invasive examination in the brachial artery using the principle of reactive hyperemia after stimulation by making short ischemia in the arm by evaluating the percentage increase in brachial artery diameter (FMD%). Measurement of arterial elasticity using FMD has the advantage like non-invasive, easily repeated and applied. But has disadvantage like cannot be done in small sized diameter which is less than 2.5 mm in diameter and also affected by time of day used and also different from doctor to doctor who do it (experience skills). [26].

4.7. Effect of HbA1c on cIMT

In this study, we found that the effect of HbA1c on the cIMT in type 1 DM group was positive and significant, the exact mechanism leading to an increase of cIMT in diabetic patients remains unclear[27].and because the HbA1c is one of the risk factor of atherosclerosis and as we know the cIMT is considered as subclinical of atherosclerosis, our results also same with the results done in type 1 DM to evaluated the Correlation between the Carotid Artery Intima-Media Thickness and HbA1c Levels in Oslo during 18 years follow up [28].

4.8. Effect of HbA1c on FMD%

In this study, we found that the effect of HbA1c on the FMD% in type 1 DM group was negative and insignificant and it is the same of the results done before in Brazil in 2017 and shows moderately negative correlation between FMD%, and HbA1c.[29], the imbalance between the production of endothelium-derived factors crack the physiological properties of the vascular endothelium and vascular tones [30],enhancing vasoconstriction of blood vessels [31],and an inflammatory process [32],perpetuated by the long-lasting hyperglycemia [33], and in our research, we didn't concentrate on the duration of the type 1 DM and marker of inflammation like CRP and the other risk factors which can affect the FMD like lipid.

4.9. Relationship between vitamin D3 deficiency and cIMT through IFN- γ

In this study, we found that the effect of vitamin D deficiency on cIMT through IFN- γ in type 1 DM group was an insignificant effect because vitamin D deficiency have negative and insignificant effect on IFN- γ and that's opposite the theory which mentioned that vitamin D can inhibit Th1 response through vitamin D response elements on the IFN- γ gene or through the indirectly inhibit IFN- γ response through inhibition of differentiation of monocytes to antigen-presenting dendritic cells.[4, 34]and that's happen because that's research done as cell line and cell line culture and the review in vitro and in vivo and not as our research which do it directly via measurement of both vitamin D and IFN- γ in many children with type 1 DM. Also the effect of vitamin D deficiency negatively and insignificantly, Which is the same results done in Denmark in type 2 DM and shows that the carotid intima media thickness (cIMT) not associated with Vitamin D deficiency in patients with type 2 diabetes.[35]

Other research shows the opposite the results like research done in patients with type 2DM to see the role of vitamin D on the carotid intima media thickness, which shows that there is a strong association between the two variable.[36], because of this research only measurement the cIMT in type 2 DM and not in the control group not like our research which measured the cIMT in both groups.

Because the direct effect of vitamin D deficiency was insignificant in both IFN- γ and cIMT so when we did the path analysis to see the indirect effect of vitamin D deficiency on cIMT through IFN- γ it became insignificant.

4.10. Relationship between vitamin D3 deficiency and FMD% through IFN- γ

In this study, we found that the effect of vitamin D deficiency on FMD% through IFN- γ in type 1 DM group was insignificant effect, because vitamin D deficiency have positive and insignificant effect on FMD% Which is the same results done in type 2 DM patients to know the effects of improved glycemic control on endothelial function and shows that the decrease of HbA1c levels did not affect endothelial function in patients with type 2 DM and previously poor controlled HbA1c.[37].

Other research shows the opposite of our results, like the research done on 57 overweight American and African adults, people which have vitamin D3 deficiency and treated with vitamin D for 16 weeks and shows significant effect of FMD after vitamin D3 supplementation.[38].and this against our result may be because the sample in this research was adult and obese and not diabetic and so no hormonal changes can occur to affect the results not like our sample which was children and type 1 DM, and also vitamin D deficiency had negative and insignificant effect on IFN- γ and that's opposite the theory which mentioned that vitamin D can inhibit Th1 response through vitamin D response elements on the IFN- γ gene or through the indirectly inhibit IFN- γ response through inhibition of differentiation of monocytes to antigen-presenting dendritic cells.[34].and that's happen because that's research done as cell line and cell line culture and not in diabetic children and not as our research which do it directly via measurement of both vitamin D and IFN- γ in many children with type 1 DM. and because the direct effect of vitamin D deficiency was insignificant in both INF γ and FMD% so when we did the path analysis to see the indirect effect of vitamin D deficiency on FMD% through IFN- γ it became insignificant.

5. Conclusion

Based on the results of the research on the relationship between vitamin D deficiency and premature atherosclerosis in adolescents with type 1 DM through inflammatory IFN- γ , it can be concluded that The HbA1c has a significant effect on the cIMT in patients with type 1 DM also conducted that there is no relationship between vitamin D deficiency and premature atherosclerosis in adolescents with type 1 DM through INF γ .

Compliance with ethical standards

Acknowledgments

We would like to thank the department of Biomedical Sciences, Faculty of Medicine, Brawijaya University and Pediatrics department in Saiful Anwar General Hospital, Malang, Indonesia to provide all the support until the completion of this research.

Disclosure of conflict of interest

We have no conflicts of interest to disclose.

Statement of ethical approval

The present research work obtained approval from the Rumah Sakit Umum Daerah of Indonesia.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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How to cite this article

Aldaefi MFA and Tjahjono HA. (2020). Relationship between vitamin D deficiency and premature atherosclerosis in an adolescents with type 1 DM through inflammatory IFN- γ . *GSC Biological and Pharmaceutical Sciences*, 11(1), 55-68.
