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Correlation between transforming growth factor-β1 (TGF- β1) with premature atherosclerosis in type 1 diabetes

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Original Article

Abstract

BACKGROUND: Type 1 diabetes (T1D) carries a significant risk of atherosclerosis as the main driver for cardiovascular events. Atherosclerosis is initiated by the activation of the endothelium by various risk factors through the inflammation process. The anti-inflammatory cytokine TGF-β1 may inhibit the development of atherosclerosis.

METHODS: In a cross-sectional study, a total of 40 patients aged 14.5±3.16 years old with T1D and 40 healthy controls aged 14.7±0.99 years old were involved. Common carotid artery IMT (cIMT) was measured by real-time M-echocardiography mode (Affinity 50G Philips) and Flow Mediated Dilatation (FMD), using high-resolution ultrasonography and Doppler flow characteristics. The TGF-β1 level was measured by indirect ELISA at Saiful Anwar Hospital Laboratory.

RESULTS: There were no differences in age, gender, Body Mass Index (BMI), duration of diabetes, renal function, or nutritional status between the T1D and healthy groups (p>0.05). A significant difference in cIMT was observed between the T1D group and the healthy group (0.567 ± 0.87 mm vs. 0.387 ± 0.57 mm, p = 0.000), FMD (7.17 ± 3.98 mm vs. 11.22 ± 5.48 mm, p = 0.000), and the level of TGF-β1 cytokine (39.83±13.51 vs. 73.67±15.34 pg/ml, p = 0.000). A significantly negative correlation between TGF-β1 and cIMT (p = 0.000; r = -0.685) and a significantly positive correlation between TGF-β1 and FMD (p = 0.000; r = +0.55) were found.

CONCLUSION: Atherosclerosis is an inflammatory disease accelerated by diabetes. The inflammation process is more prominent in T1D patients. T1D patients show a decreased level of TGF-β1, increased measurement of cIMT (>0.5 mm), and a decreased measurement of FMD.

Keywords: T1D, Premature Atherosclerosis, TGF-β1, cIMT, FMD

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Introduction

Atherosclerosis is one of the long-term complications of type 1 diabetes mellitus (T1D). Long-term complications, such as cardiovascular complications, can increase the risk of mortality and morbidity in diabetes mellitus. The incidence of cardiovascular disease in patients with diabetes mellitus is two to four times higher than in the non-diabetic group, which cannot be explained by traditional risk factors alone[1].

Various pro-inflammatory and anti-inflammatory cytokines influence the occurrence of endothelial dysfunction and atherosclerosis in type 1 diabetes patients through various mechanisms. One of the important cytokines in the pathogenesis of atherosclerosis is TGF-β1 (Transforming Growth Factor-β1). Low TGF-β1 levels correlate with an increased incidence of atherosclerosis[2].

The development of endothelium vascular clinical examination has made it possible to evaluate

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both normal endothelial function and endothelial dysfunction. Increased tunica intima-media thickness of the internal carotid artery, as measured by carotid artery intima-media thickness (cIMT), is an early marker of atherosclerosis associated with vascular risk factors and the severity of coronary artery disease[3,4]. The response of dilation mediated by flow-mediated dilatation (FMD) in the brachial artery is a marker of endothelial dysfunction, assessed by an arterial diameter response that increases flow[4,5]. High-resolution ultrasound examination can detect early changes in vascular structure and arterial walls.

Materials and methods

This study was an observational analytic cross-sectional design, measuring TGF-β1, the thickness of the internal carotid artery intima-media (cIMT), and flow-mediated response (FMD) dilatation.

The study population included all children aged 10-18 years. The accessible population for this study comprised pediatric patients diagnosed with T1D who underwent outpatient care at the pediatric endocrinology clinic of RSUD Dr. Saiful Anwar Malang. The healthy control group was selected from schools and declared healthy.

Sample inclusion criteria were children aged 10 to 18 years, whose parents provided informed consent after the study was explained to them. Sample exclusion criteria included diagnoses of systemic/sepsis infection, liver disorder, kidney dysfunction, malignancy/cancer, anemia with a hemoglobin level less than 11 g/dL, or treatment with Amlodipine, Valsartan, and Statin.

TGF-β1 Assay by ELISA

Enzyme-linked immunosorbent assay (ELISA) for cytokines. TGF-β1 (R&D Systems) was measured by ELISA kits according to the manufacturer's instructions.

cIMT measurement

cIMT was measured by determining the diameter of the left and right common carotid arteries using M-echocardiography mode to assess carotid stiffness. We measured only the common carotid artery IMT because our patients were mostly children. Measurements were taken from both the right and left sides, and the maximum value was recorded. Internal carotid and carotid bulb measurements depend on the anatomical topography of the patient and are difficult to perform in children. Premature atherosclerotic plaque was defined as intima-media thickening over 0.5 mm.

FMD measurement

Evaluate the function of the artery by FMD. Measure the diameter of the brachial artery in the right arm while the patient is positioned relaxed and supine, so that the ultrasound examination is performed on the brachial artery 5-10 cm above the antecubital fossa. Inflate the cuff to supra-systolic pressure (40-50 mmHg above systolic pressure) and occlude for 5 minutes, then quickly deflate the cuff to allow rapid blood flow. Measure the arterial diameter up to 5 minutes after the cuff is deflated and determine the highest diameter. Formula: FMD% = (peak diameter - baseline diameter) / baseline diameter. The baseline diameter is the diameter of the artery before the stimulation of the inflation sphygmomanometer cuff. The peak diameter is the largest diameter after reactive hyperemia or after the cuff is suddenly deflated, measured up to the 5th minute after reactive hyperemia.

Research subjects were recruited using the consecutive sampling method, where each patient who met the inclusion criteria was included in the study from January to July 2019 until the minimum sample size was met. Additionally, a healthy control group that met the inclusion and exclusion criteria and was willing to participate in the study signed an informed consent form.

Statistical analysis was performed using SPSS for Windows software version 24.0. Patient demographic data, including age, sex, BMI, and laboratory examination results, are displayed as descriptive data. TGF-β1, cIMT, and FMD data were tested for normality distribution using the Kolmogorov-Smirnov test and for variance homogeneity (to determine if the data variants were the same). For different tests, if the variables were normally distributed and homogeneous, the Independent T-test was used. However, if the variables were not normally distributed and not homogeneous, the Mann-Whitney test was used. To see the correlation between TGF-β1 with cIMT and FMD, the Pearson correlation test was performed. A value of $p < 0.05$ indicates a statistically significant difference.

Result

Data collection conducted from January to July 2019 found 80 study subjects consisting of 40 T1D patients and 40 healthy control patients. The study subjects had an average age of 14.5 ± 3.16 years in the T1D group and 14.7 ± 0.99 years in the control group, which did not differ significantly between the two groups. Gender characteristics in the T1D group included 17 males (42.5%) and 23 females (57.5%), whereas the control group included 14 males (35%) and 26 females (65%), which also did not differ significantly between the two groups. The average BMI (Body Mass Index) of the T1D group was 18.76 ± 3.88 kg/m², whereas the control group had an average BMI of 20.76 ± 4.40 kg/m², with no significant differences between the two groups. The mean HbA1C levels in the T1D group were significantly higher than in the control group (9.53 ± 2.33% vs. 4.69 ± 0.251%, p = 0.000)(Table 1).

Lipid profile measurement results in this study included total cholesterol, triglycerides, HDL, and LDL. The total cholesterol level of the T1D group was significantly higher than that of the control group (181.38 ± 36.582 mg/dl vs. 131.58 ± 27.599 mg/dl, p = 0.000). Likewise, the mean HDL level in the T1D group was significantly higher than in the

Table 1. Characteristics data of Research Subjects

Characteristics	Type 1 Diabetes Mellitus (n=40)	Control (n=40)	p-value
Age (year) (means)	14,5±3,16	14,7±0.99	0.236
Sex, n(%)			0.491
Male	17 (42,5%)	14(35%)	
Female	23 (57,5%)	26(65%)	
HbA1C (%) (mean±SD)	9,53±2,33	4,69±0,251	0.000*
BMI (Body Mass Index) (kg/m²)	20.76 ± 4.40	20.76 ± 4.40	0,078
Total Cholesterol (mg/dl) (means)	181.38±36.582	131.58±27.59	0.000*
Triglyceride (mg/dl) (means)	108.98±41.759	99.40±58.049	0.087
HDL (mg/dl) (means)	56.78±13.903	43.63±9.434	0.000*
LDL (mg/dl) (means)	127.05±32.963	90.20±25.139	0.000*
IL-10 (pg/ml)	3.19 ± 0.85	9.39 ± 1.07	0.000*
cIMT (mm)	0,567±0,87	0,387±0,57	0.000*
FMD (%)	7.17 ± 3.98	11.22 ± 5,48	0.000*
- Baseline diameter (mm)	2.86 ± 0.46	3.07 ± 0.44	0.000*
- Peak diameter (mm)	3.14 ± 0.46	3.65 ± 0.51	0.000*

* p < 0.005 significant

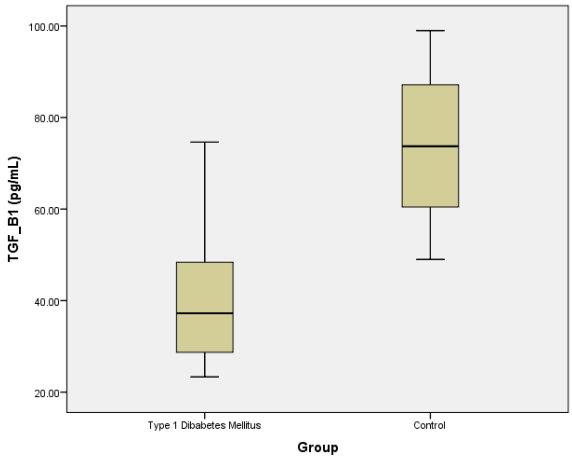

Figure 1. Comparation TGF-β1 T1D and Healthy control group

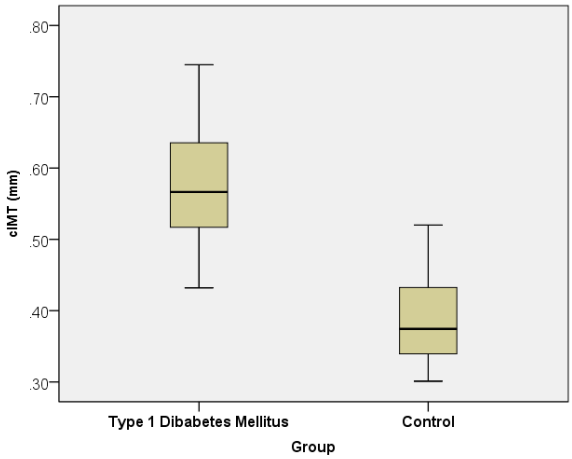

Figure 2. Comparation cIMT between T1D and Healthy control group

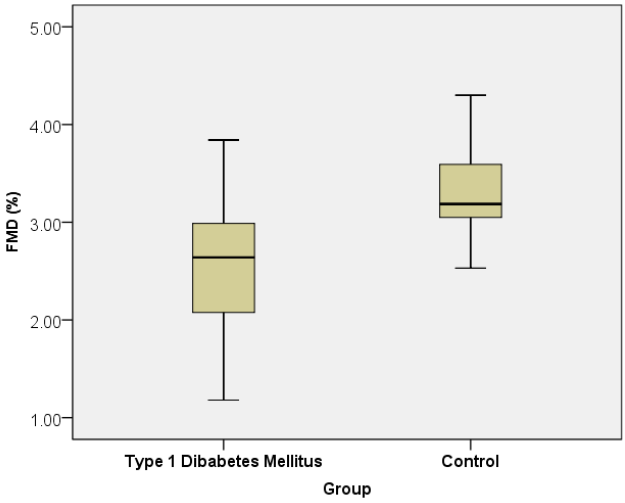

Figure 3. Comparation cIMT between T1D and Healthy control group

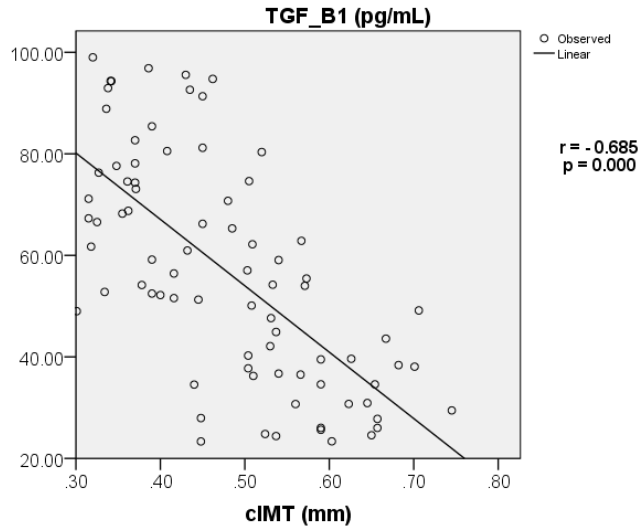

Figure 4. Correlation TGF-β1 and cIMT

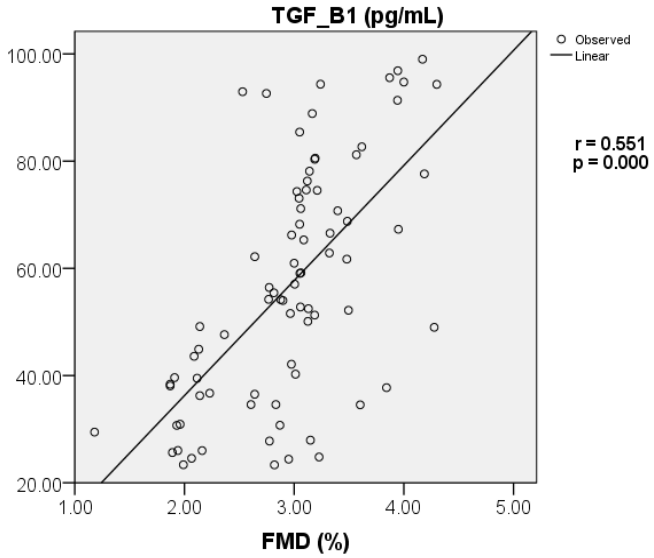

Figure 5. Correlation TGF-β1 and FMD

control group (56.78 ± 13.903 mg/dl vs. 43.63 ± 9.434 mg/dl, p = 0.000). The mean LDL level in the T1D group was also significantly higher compared to the control group (127.05 ± 32.963 mg/dl vs. 90.20 ± 25.139 mg/dl, p = 0.000). However, the average triglyceride values for the T1D group and the control group did not differ significantly (108.98 ± 41.759 mg/dl vs. 99.40 ± 58.049 mg/dl, p = 0.087).

There is a significant negative correlation between TGF-β1 and cIMT (p = 0.000) with a correlation strength of r = -0.685, meaning that a strong negative correlation, the higher the TGF-β1 level, the lower the cIMT (Figs. 1-4).

There is a significant positive correlation between TGF-β1 and FMD (p = 0.000) with a correlation strength of r = 0.55 meaning that the positive correlation is strong, the higher the TGF-β1 level, the higher the FMD(Fig. 5).

Discussion

Characteristics of research subjects

In this study, 80 research subjects were found, consisting of 40 T1D patients and 40 healthy control patients. The age data of study subjects did not differ significantly between the two groups. The average

age of the T1D group was 14.5 ± 3.16 years, with a minimum age of 10 years and a maximum age of 18 years.

The research subjects in the T1D group included 23 females and 17 males. According to the ADA, in the UK, the incidence of CHD in T1D is the same for men and women under 40 years of age, whereas men over 40 years have a higher incidence than women. In the USA, the trend in the incidence of CHD in T1D is the same for both men and women. Research on mortality rates caused by CHD in Norway shows that the incidence of cardiovascular disease in T1D is higher in women than in men. The same finding was reported in the T1D cohort study at the Allegheny Registry, which found that the impact of CHD on T1D patients is much higher in women than in men (standard mortality ratio (SMR) 13.2: 5 for total mortality). The incidence of CAC (Coronary Arterial Calcification), a predictor of atherosclerosis, is higher in many women. The reason is unclear, but data suggest that sex differences in female T1D patients are likely due to lipid distribution patterns associated with insulin resistance, as measured by waist-to-hip ratio and waist circumference[6].

Comparative analysis of TGF-β1, cIMT and FMD levels between T1D and control group

Comparative test results of TGF-β1 measurement and cIMT and FMD measurements in all study subjects were performed using an independent samples t-test, where the four parameters differed significantly between the T1D group and the control group.

The measurement of cIMT in this study obtained the mean (mean ± SD) of cIMT in the T1D group as 0.567 ± 0.87 mm vs. 0.387 ± 0.57 mm in the control group, with a p-value of 0.000, indicating a significant difference between the two groups. cIMT has been recognized as a general criterion of atherosclerosis and a marker of progression in cardiovascular disease in various studies that can be measured with a high-resolution ultrasound device. Research in Iran in 2014 on the relationship between cIMT in T1D groups (n = 40) vs. control (n = 40) found a significant difference ($p < 0.001$). The T1D group was subdivided into two groups: T1D with a duration of more than 4 years diagnosed and less than 4 years of suffering from T1D. There was a significant difference between the right carotid and left carotid in T1D > 4 years and < 4 years, i.e., (0.49 vs. 0.48) and (0.52 vs. 0.51), meaning that the T1D group suffering for more than 4 years had a mean carotid intima-media thickness higher than T1D < 4 years. A German study in 2011, which measured cIMT longitudinally to determine subclinical atherosclerosis in children and adolescents who had T1D for 4 years, obtained results of (0.58 ± 0.75, $p < 0.0001$)[7]. The shortcomings in this study did not classify T1D based on the duration of years diagnosed with T1D.

The mean FMD measurement results (mean ± SD) in the T1D vs. control group in this study were 7.17 ± 3.98 mm vs. 11.22 ± 5.48 mm, with a p-value of 0.000, indicating a significant difference between the two groups. This aligns with other studies, such as the one conducted by Pillay et al. (2018), which measured FMD in T1D children compared to healthy controls and found decreased FMD in the T1D group. Children with T1D exhibited accelerated atherosclerosis with endothelial dysfunction, as measured by FMD at 60 seconds after shear stress (early FMD), showing a reduction in flow-mediated dilation. Delayed dilation can also occur with cardiovascular risk factors and may be a more sensitive marker. In this study, the mean FMD in the T1D group was lower compared to the control group, consistent with the theory. FMD was calculated by measuring the average results at 10 seconds, 30 seconds, 60 seconds, and 180 seconds. Other studies have argued that 60-second FMD is a better marker standard for identifying children at higher risk for cardiovascular disease8.

Correlation analysis of TGF- β1 levels with cIMT and FMD

This study also analyzed the correlation between TGF-β1 levels and cIMT. There was a significant negative correlation between TGF-β1 and cIMT ($p = 0.000$) with a correlation strength of $r = -0.685$, indicating that higher TGF-β1 levels were associated with lower cIMT. Additionally, there was a significant positive correlation between TGF-β1 and FMD ($p = 0.000$) with a correlation strength of $r = 0.55$, indicating that higher TGF-β1 levels were associated with higher FMD. No previous studies have investigated the relationship between TGF-β1 and both cIMT and FMD as markers of endothelial dysfunction.

TGF-β1 is a pleiotropic cytokine that plays a major role in immunoregulation. It is involved in cardiac remodeling and has a protective effect on autoimmune diseases, including T1D. In the process of atherosclerosis, TGF-β1 can act as both an atherogenic and an atheroprotective agent. TGF-β1 is known to control cell proliferation, cell migration, matrix synthesis, wound contraction, calcification, and immune response, all of which are major components of the atherosclerotic process. However, many effects of TGF-β1 are important for tissue repair, hence it is also considered atheroprotective[9]. The role of TGF-β1 in blood vessels is to inhibit endothelial and smooth muscle proliferation and to play a role in tissue repair. Clinically, low TGF-β1 levels correlate with atherosclerosis. Therefore, TGF-β1, as an anti-atherosclerosis agent, will negatively correlate with the thickness of the tunica intima carotid artery media (cIMT) and positively correlate with FMD, which illustrates the process of atherosclerosis[9,10].

Conclusion

Atherosclerosis is an inflammatory disease accelerated by diabetes. The inflammation process is more prominent in T1D patients, as indicated by decreased levels of TGF-β1. The risk of coronary heart disease is increased in the T1D group, as shown by the increasing non-invasive marker of atherosclerosis (cIMT) measured by ultrasound. There is a strong negative correlation between TGF-β1 levels, an anti-inflammatory cytokine, and cIMT, and a strong positive correlation between TGF-β1 levels and FMD.

Suggestion

Further research is carried out on the diagnostic value of TGF-β1 for gold standard atherosclerosis.

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Conflict of interests

There is no conflict of interest.

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Author's Contributions

HAT, WB, DH, and HK have contributed to the design and conception of the study. HAT contributed to data acquisition and manuscript drafting. HAT, WB, and DH contributed to data acquisition and interpretation; and critically revised the manuscript. All the authors have approved the manuscript. The study was supervised by HK.

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