INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 138
2021 March; 27(2): 138-142
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Correlation between Serum Endocan and HbA1c in Type 1 Diabetes Mellitus Patients

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ABSTRACT

Endothelial dysfunction is a key mechanism in the pathogenesis of complications of cardiovascular disease in Diabetes Mellitus (DM) patients. One of the new biomarkers for inflammatory conditions and endothelial dysfunction is endocan. This study aimed to determine the correlation between endocan levels and HbA1c in type 1 DM patients. This study was an analytical observational study with a cross-sectional approach performed at the Dr. Saiful Anwar Hospital, Malang from May to August 2019. The research subjects were children aged 10-18 years with a diagnosis of type 1 DM who met the inclusion criteria. Students who underwent routine health checks participated as the control group. In both groups, serum endocan levels were measured using the ELISA method and HbA1c levels were measured by the HPLC method. Independent T-test analysis was used to determine the differences between both groups and the Pearson test was used to determine the correlation between serum endocan and HbA1c with SPSS version 23. In this study, there were 40 type 1 DM patients and 40 healthy controls with a mean age of 14.5 (3.16) years in the type 1 DM group and 14.7 (0.99) years in the healthy control group. There was a higher number of female subjects in both the type 1 DM group (57.5%) and the healthy control group (65%). The mean endocan level in the type 1 DM group was higher than the control group and was statistically significant with 1090.61 (150.84) pg/mL vs. 775.56 (8.91) pg/mL, p=0.000). The mean value for HbA1c levels in the type 1 DM group was also significantly higher compared to the control group 9.63 (2.22%) vs. 4.69 (0.251%), p <0.001), respectively. There was a significant positive correlation between endocan levels and HbA1c in DM patients (p=0.025, r=0.354). This study showed a correlation between serum endocan levels and HbA1c in patients with type 1 DM.

Keywords: Type 1 diabetes mellitus, endocan, HbA1c, endothelial dysfunction

INTRODUCTION

Type 1 diabetes mellitus is a type of Diabetes Mellitus (DM), which is frequently found in children and adolescents with an increasing incidence every year.[1] The International Diabetes Federation (IDF) estimates that 1.1 million children and adolescents globally are affected by type 1 DM and 133 children and adolescents are diagnosed as new cases of type 1 DM each year. Across Europe, there was an average annual increase of 3.4% in the incidence of type 1 DM in children under 15 years, and the highest incidence was reported in children under 5 years of age.[2,3] Based on data from the Indonesian Pediatrician Association (IDAI) in 2012, the incidence of type 1 DM in Indonesia ranged from 0.2 to 0.42 per 100,000 children per year. Also, it was reported that there were 35 T1DM patients aged 1-18 years at the Dr. Saiful Anwar Hospital (RSSA), Malang between 2005-2013.[4]

According to the 2019 ADA, the diagnosis of DM is confirmed by the presence of classic symptoms, such as polyuria, polydipsia, polyphagia, and unexplained weight loss. In addition, several laboratory criteria, which are used as diagnosis of DM are as follows: HbA1c levels ≥ 6.5% or fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/l), 2-hour post-prandial plasma glucose ≥ 200 mg/dL (11.1 mmol/l) or random plasma glucose ≥ 200 mg/dL (11.1 mmol/l).[5] Hemoglobin A1c (HbA1c) is one of the tests used for diagnosis and monitoring of DM. HbA1c is the Hb glycosylated at 1 or 2 N-terminal valine from β chains of tetramer Hb molecules that represent mean blood glucose levels in the last three months.[6] Several studies have reported that HbA1c levels correlated with arterial narrowing and endothelial dysfunction in diabetes mellitus patients.[7,8]

Type 1 diabetes mellitus is caused by the damage of pancreatic beta cells due to autoimmune or

idiopathic processes, causing absolute insulin deficiency and hyperglycemic state. Hyperglycemia in type 1 DM patients triggers a chronic inflammatory process that causes endothelial dysfunction and impaired blood vessel integrity, causing microvascular and macrovascular complications.[9] Endocan is a newly developed biomarker of endothelial dysfunction. Endocan or also known as molecular-specific endothelial cells (ESM-1) is a small Dermatan Sulfate Proteoglycan (DSPG) mainly expressed by endothelial cells.[10] A research reported that endocan levels were higher in diabetic patients and were correlated with oxidative stress and inflammation.[11]

Another study measuring endocan levels in type 1 DM with ACS and a group of non-diabetic patients with ACS showed that endocan levels were higher in type 1 DM with Acute Coronary Syndrome (ACS) than in a group of non-diabetic patients with ACS, suggesting a more severe endothelial dysfunction of blood vessels in type 1 DM patients.[12] Research on endocan and endothelial dysfunction in pediatric patients with type 1 DM and its correlation with HbA1c remains highly limited. This study aimed to determine the correlation between endocan levels and HbA1c in type 1 DM patients.

METHODS

This study was an analytical observational study with a cross-sectional approach performed at Dr. Saiful Anwar Hospital, Malang from May to August 2019. The research subjects were children aged 10-18 years with a diagnosis of type 1 DM who met the inclusion criteria. Patients who were suffering from local and systemic infections, patients with malignancy and anemia with hemoglobin levels less than 11 g/dL measured by a hematology analyzer Sysmex XN1000 (Japan) were excluded from the study. Student-age patients who underwent voluntary health checks with glucose level < 200 mg/dL and HbA1c < 6.5% were involved as the control group.

The diagnosis of type 1 DM was confirmed by clinical symptoms of polydipsia, polyuria, polyphagia, weight loss, and laboratory parameters of fasting blood glucose ≥ 126 mg/dL, or two-hour post-prandial blood glucose after glucose tolerance test ≥ 200 mg/dL or HbA1c ≥ 6.5% or the classic symptoms followed by random blood glucose ≥ 200 mg/dL. An autoantibody test, Glutamic Acid Decarboxylase 65 kD (GAD65) test was carried out based on the method of Immunochromatography/ICT GAD65 antibody produced by Biopharma Bioscience UB. INA to confirm type 1 DM patients.

Without prior fasting, a total of 5 mL of blood was drawn from the peripheral vein and was divided into two tubes, an EDTA anticoagulant tube and a tube without an anticoagulant. Three mL EDTA blood was used for the HbA1c test and 2 mL blood was prepared into serum for measurement of serum endocan levels. HbA1c levels, which represented the mean value of blood glucose levels in the last three months, were measured using a High-Performance Liquid Chromatography (HPLC) method using a reagent of D10 Biorad, USA. Meanwhile, the endocan level as a marker of endothelial dysfunction was measured from serum using the Enzyme-Linked Immuno Assay (ELISA) kit from Cohesion Biosciences ElisaKit (Singapore Company) with the catalog number CEK1144 and the results were reported in units of pg/mL.

Statistical analysis was performed using SPSS for Windows software version 23.0 with a p-value < 0.05 as the significant level. Patient demographic data including age, gender, Body Mass Index (BMI), and laboratory test results were displayed as descriptive data. The mean levels of endocan and HbA1c in both groups were tested differently by using the independent T-test. Meanwhile, the correlation between endocan and HbA1c was determined by using the Pearson correlation test. This research was carried out after approval from the Research Ethics Committee of the Faculty of Medicine, Brawijaya University/Dr. Saiful Anwar Hospital, Malang with number 400/214/K.3/302/2018.

RESULTS AND DISCUSSIONS

A total of 80 subjects in this study consisted of 40 type 1 DM patients and 40 healthy patients as control were tested. The characteristics of the research subjects can be seen in Table 1.

There was no significant difference between the mean age of the type 1 DM group of 14.5 (3.16) years and the control group of 14.7 (0.99) years. Based on the gender of research subjects, the type 1 DM group consisted of 17 (42.5%) males and 23 (57.5%) female patients, while the control group consisted of 14 (35%) males and 26 (65%) female patients. It was found in this study that there was no significant difference in gender between both groups. The mean value of BMI in the type 1 DM group was 18.76 (3.88) kg/m^2, while that of the control group was 20.76 (4.40) kg/m^2, however, no significant difference in BMI was found between both groups. All subjects in the type 1 DM group were GAD65-positive but all

Table 1. Characteristics of research subjects

Characteristics	Type 1 DM (n=40)	Control (n=40)	p-value
Age (years), mean±SD	14.5 (3.16)	14.7 (0.99)	0.236
Gender, n (%)			0.491
Male	17 (42.5%)	14 (35%)	
Female	23 (57.5%)	26 (65%)	
BMI (kg/m^2), mean±SD	18.76 (3.88)	20.76 (4.40)	0.078
Positive GAD65, n (%)	40 (100%)	0 (0%)	0.000*
Hemoglobin (g/dL), mean±SD	14.15±1.11	14.57±1.83	0.128

*p < 0.05 was significant, independent T-test

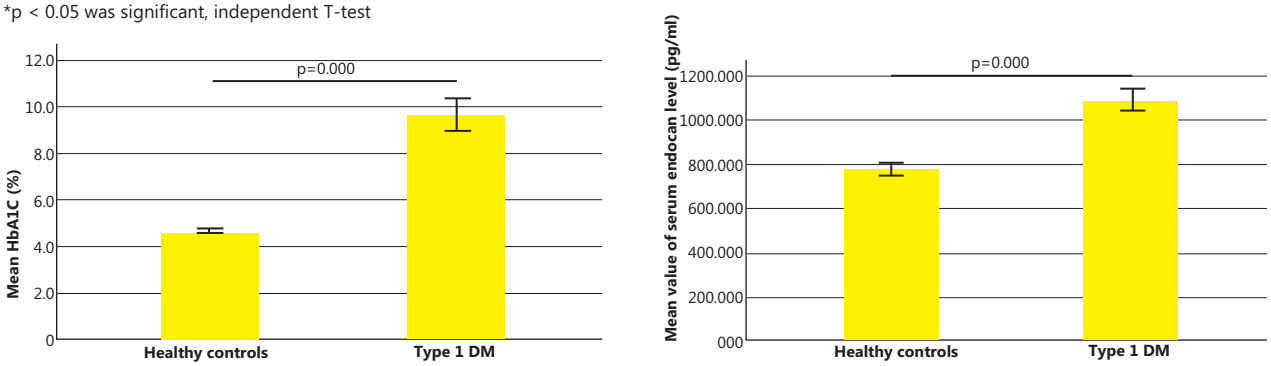

Figure 1. Mean HbA1c and endocan levels in both groups. A. The mean HbA1c levels in the type 1 DM group were significantly different compared to the control group; B. The mean endocan serum levels in the type 1 DM group were significantly different compared to the control group

healthy subjects in the control group were GAD65-negative. The mean hemoglobin values in both groups were in the normal range and no significant difference was found between both groups.

The mean HbA1c level in the type 1 DM group was significantly higher than the control group (9.63 (2.22) vs. 4.69 (0.251) p=0.000). Likewise, the mean endocan levels in the type 1 DM group were significantly different compared to the control group, showing higher endocan levels in the type 1 DM group (1090.61 (150.84) pg/mL vs. 775.56 (8.91) pg/mL, p=0.000). The following Figure 1 showed the difference in endocan and HbA1c levels in both groups.

From the results of the correlation test, it was found that there was a moderate positive correlation between HbA1c levels and endocan in type 1 DM patients (p=0.025, r=0.454), suggesting that a higher HbA1c level led to a higher endocan level. The graph of the correlation between HbA1c and endocan can be seen in Figure 2.

Type 1 diabetes mellitus is a type of DM, frequently found in pediatric and adolescent patients caused by damage of pancreatic beta-cells due to autoimmune or idiopathic processes.[1] Type 1 DM occurs with absolute insulin deficiency, which causes hyperglycemia. Hyperglycemia in type 1 DM patients triggers a chronic inflammatory process that can lead to endothelial dysfunction known as the key mechanism for cardiovascular complications in DM patients.[13,9]

This study assessed endocan as one of the markers of endothelial dysfunction in type 1 DM patients. Endocan is a proteoglycan that is specifically expressed by endothelial cells.[10] Several studies reported that endocan levels were higher in diabetic patients.[11,14] Similar results were reported in this study, which showed a higher mean endocan level in the type 1 DM group compared to the healthy control group. Research by Arman *et al.* showed that serum endocan levels increased in type 1 DM patients and decreased after anti-hyperglycemia therapy.[15,16]

Endothelial dysfunction in diabetes can be caused by decreased synthesis or inactivation of Nitric Oxide (NO) and/or increased production and release of vasoconstrictor substances. Also, a hyperglycemic state will increase the production of free radicals through the oxidation process of glucose, such as Advanced Glycosylation End Products (AGEs), which can induce NO inactivation.[17] A study by Coriello *et al.*, showed that there was a relationship between hyperglycemia and endothelial dysfunction,

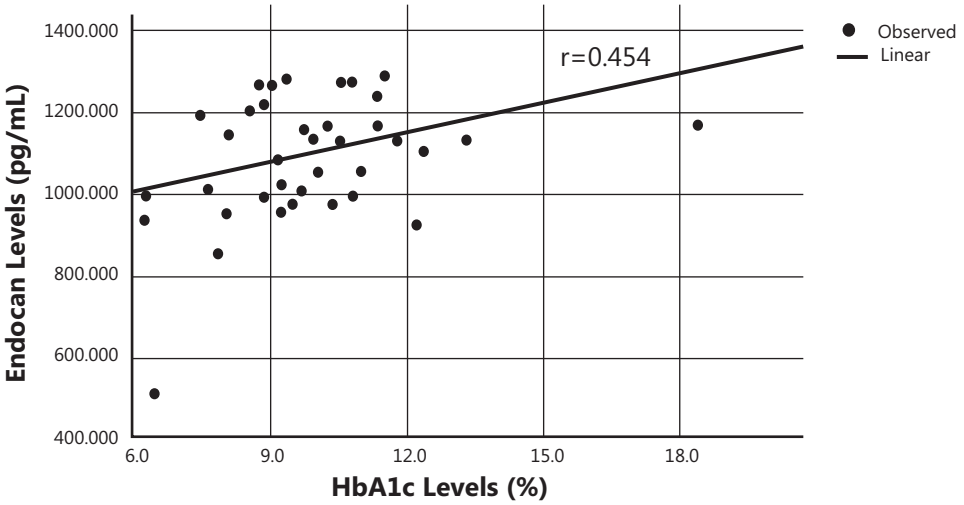

Figure 2. Graph of correlation between HbA1c and endocan

oxidative stress, and inflammation.[18] Data In this study showed that there was a significant positive correlation between endocan and HbA1c levels, suggesting that a higher HbA1c level led to a higher endocan level. HbA1c is a minor component of hemoglobin that binds to glucose, which represents the average blood glucose level in the last three months. Several studies have reported that HbA1c levels correlated with arterial narrowing and endothelial dysfunction in patients with DM.[7,8,19]

There is damage to pancreatic beta-cells in type 1 DM due to an autoimmune process that causes low insulin secretion leading to chronic hyperglycemia, which triggers a chronic inflammatory process. The inflammatory process in type 1 DM triggers macrovascular and microvascular complications. Macrovascular inflammation accelerates the process of atherosclerosis, which in turn increases the incidence of cardiovascular disease. The destruction of pancreatic beta-cell results in an insufficiency of insulin, especially in peripheral tissues such as adipocytes, resulting in abnormal lipid metabolism characterized by lipolysis in peripheral tissues and adipocytes. Triglycerides are broken down into free fatty acids in the process of lipolysis, which triggers the liver to secrete atherogenic lipoproteins. The chronic inflammatory process in persons with type 1 DM also triggers an interaction between atherogenic lipoproteins and some cells (such as monocytes and macrophages). Atherogenic lipoproteins include LDL, beta-VLDL, and lipoprotein, which develop into oxidized LDL due to inflammatory processes and are triggered by ROS. Oxidized LDL triggers the expression of several genes triggering cell adhesion, migration, and angiogenesis. For example, VEGF, which can increase endocan expression in the vascular endothelium and Monocyte Chemoattractant Protein-1 (MCP-1), which then triggers aggregation of monocytes.[20]

Due to MCP-1 aggregation and adhesion, naive T-cells differentiate into Th1, which expresses IFN-γ as proatherogenic cytokines, Th2, which expresses IL-10, Th17, expressing IL-17 as proatherogenic and Treg, which express TGF-beta 1 as an antiatherogenic cytokine. In addition, the inflammatory process itself also triggers dysfunction of the endothelium, leading to reduced secretion of NO, as a vasodilator and increased secretion of endothelin-1 as a vasoconstrictor. Endothelin-1 will stimulate VEGF together with pro-inflammatory cytokines to trigger platelet aggregation and adhesion, smooth muscle cell proliferation, increase the extracellular matrix and increase endocan expression. The three pathogenesis cause lesions in the endothelium, which promote endocan secretion. It is expected that serum endocan levels can be developed into a marker of endothelial dysfunction and a predictive value and prognostic value for vascular complications and in type 1 DM.[20,21] However, the difficulty to obtain a control serum assay for endocan as a new marker is one of the limitations of this study.

CONCLUSIONS AND SUGGESTIONS

There was a significant correlation between serum endocan levels and HbA1c in type 1 DM patients. Therefore, it is suggested that serum endocan examination can detect endothelial dysfunction in type 1 DM patients with uncontrolled HbA1c patients.

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