HbA1c and Plasma Transforming Growth Factor-Beta 1 in Type-2 Diabetes Mellitus Patients

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HBA1C AND PLASMA TRANSFORMING GROWTH FACTOR-BETA 1 IN TYPE-2 DIABETES MELLITUS PATIENTS

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Abstract

Background: *The diabetic microvascular pathophysiology is associated with many chronic inflammatory processes triggered by some cytokines and growth factors. Transforming Growth Factor Beta-1 (TGFβ-1) is considered to be a key mediator of the pathogenesis of microvascular complications associated with chronic hyperglycemia. We analyzed the correlation between HbA1c and TGFβ-1 plasma levels in type-2 diabetes mellitus (T2DM) patients.*

Methods: *We enrolled T2DM patients over the age of 18, then HbA1c level from venous blood samples supplemented with anticoagulants was measured by using HPLC (High-Performance Liquid Chromatography). TGFβ-1 examination was performed by using ELISA (Enzyme-Linked Immunosorbent Assay) and Human TGFβ-1 quantizing ELISA (R&D) reagent. Data were analyzed using Spearman correlation test.*

Results: *The number of research subjects was 30 patients. The median of HbA1c levels were 7.15% (4.7-13.6%). Median TGFβ-1 plasma levels were 150.8 pg/mL (23.6-2089.2 pg / mL). Spearman's correlation test showed a strong and significant positive correlation between HbA1c and TGFβ-1 plasma levels in patients with T2DM (rs = 0.637; p <0.001).*

Conclusion: *There was a strong and significant positive correlation between HbA1c and TGFβ-1 plasma levels in T2DM patients.*

Keywords: HbA1c, transforming growth factor beta-1, type-2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a global problem with increasing prevalence worldwide. Morbidity and mortality of DM caused by microvascular and macrovascular complications are also found higher. Control of blood sugar levels plays an important role to prevent those complications. The condition of chronic hyperglycemia is a triggering factor in the initiation and progression of diabetic microvascular complications, but the mechanisms of hyperglycemia underlying pathological changes of microvascular complications are complex and not fully elucidated. Recent research suggested that the pathophysiology of diabetic microvascular complications was associated with a chronic inflammatory process triggered by some cytokines and growth factors. Transforming Growth Factor Beta-1 (TGFβ-1) was considered to be a key mediator of the pathogenesis of diabetic microvascular complications associated with chronic hyperglycemia. However, studies on the correlation of TGFβ-1 with chronic hyperglycemia conditions showed controversial results (1-3).

Epidemiological data showed an increase in incidence rates and the prevalence of diabetes exponentially across the world. Controversy results of studies linked the condition of chronic hyperglycemia to and TGFβ-1 which is one of the factors triggering diabetic microvascular complications that clearly will have an lead to the delay of recognition and treatment of diabetes complications. This will contribute to the ongoing progress of the complications of microvascular complications and the higher morbidity and morbidity rates associated with diabetes complications (2). The data in 2012 in the United States showed that 29.1 million

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Americans suffered from diabetes with total of 69,071 deaths each year. Data on diabetic microvascular complications showed 28.5% diabetic retinopathy, 44% diabetic nephropathy, and 60-70% diabetic neuropathy cases, which costed about 245 billion US dollars annually, creating significant socioeconomic burden (4-6). Thus the progression of diabetic vascular complications is not something that can be underestimated.

In vitro and in vivo studies performed on the effect of TGFβ-1 on the progressive complications of microvascular conditions in chronic hyperglycemia showed different results. In 1995 by using a renal biopsy specimen from 29 patients with type-2 diabetes mellitus (T2DM), significant positive correlation between TGFβ-1 mRNA and Hemoglobin A1c (HbA1c) positive intraglomerular with (7). In 2014 case-control study designs in 102 subjects showed that TGFβ-1 was significantly correlated with HbA1c in macroalbuminuria and microalbuminuria group (8). A case-control study by Azar et al. (2000) in 70 subjects the study showed a weak positive correlation between TGFβ-1 and HbA1c levels (9). A case-control study of 51 DMT1 and T2DM study subjects showed no correlation between TGFβ-1 and HbA1c levels in diabetes (10).

Based on the description above, it was necessary to conduct research to identify the relationship of HbA1c and TGFβ-1 levels in T2DM patients, by controlling the factors that influence the TGFβ-1 and HbA1c levels. To prove that HbA1c was associated with elevated levels of TGFβ-1, the study subjects were selected with the exclusion of massive proteinuria, hypertension and chronic renal failure with glomerular filtration rate (GFR) of <60 mL/min/1.73m².

METHOD

The subjects in this study were T2DM patients who fulfilled inclusion but not exclusion criteria in Polyclinic Metabolic Endocrinloogy and Diabetes, Internal Disease Outpatient Installation of Dr. Soetomo General Hospital Surabaya Indonesia. Subject inclusion criteria were new and old T2DM patients who have received oral antidiabetic medications and age over 18. Subject exclusion criteria were as follows : subjects involved in other studies, subjects suffering from chronic kidney disease with LFG ≤60 ml/min/1.73m², subjects with albumin creatinine ratio (ACR) >300 mg/g, subjects with a history of hypertension, subjects with or antihypertensive medication either anti-hypertensive drug or anti-proteinuria, subjects with a history of cerebrocardiovascular disease (stroke, coronary syndrome, myocardial infarction, heart failure or other cardiovascular diseases), subjects with history of chronic liver disease, history of malignancy, anemia, polycythemia, history of hemoglobinopathy, smoking, alcohol, pregnancy, and drugs use that affect the erythrocyte turnover.

Before starting the research subject first filled out the informed consent sheet and the researchers asked for ethical approval from Dr. Soetomo Teaching Hospital Surabaya, Indonesia. This research useed cross-sectional analytic observational design and sampling were performed by quota samples of 30 subjects. TGFβ-1 examination was performed using ELIZA (Enzyme-Linked Immunosorbent Assay). TGFβ-1 examination takes ±1 hour starting from blood withdrawal until result. Blood was withdrawn using a syringe inserted into the vacutainer tube and labeled. Blood was measured the poor plasma platelet by using with centrifugation twice, at 3000 rpm for 10 minutes and the second at 10,000 rpm for 10 minutes. Plasma was inserted into several Eppendorf tubes (volume 1.5 ml) labeled and stored at 70 oC. HbA1C examination was performed by inserting tube containing blood sample into tool Cobas c 501 for 30 minutes and the results come out in the form of percentage.

The data were presented in the form of frequency distribution table. The tools used to prove the correlation of HbA1c and TGFβ-1 plasma levels are SPSS 17.0 (SPSS, Inc., Chicago, IL). The Pearson correlation test was used to determine the correlation between HbA1c and TGFβ-1 plasma levels.

RESULTS

Subject Characteristics

The number of subjects was 30 patients that obtained the most female patients by 76.67% and average age 53.93 years. The mean BMI of the subjects was 24.53 kg/m2, serum creatinine was 0.88 mg/dL, and hemoglobin was 14.08 g/dL. ACR levels of subjects in the range 2.64 - 88.06 mg/g (table 1).

HbA1c Level

The result of measurement of the HbA1c content of subjects obtained abnormal HbA1c value with median 7.15% with the lowest value range of 4.7% and the highest score of 13.6% (table 2).

TABLE 1.

Characteristics of subjects

Characteristics	Category	n (%)	Median (min - max)	Mean ± SD
Sex	Male	7 (23.33)		
	Female	23 (76.67)		
Age	41-50 years	7 (23.33)		53.93±6.62
	51-60 years	17 (56.67)		
	61-70 years	6 (20.00)		
Body Mass Index	18.5-22.9 kg/m²	10 (33.33)		24.53±3.48
	23-24.9 kg/m²	5 (16.67)		
	25-29.9 kg/m²	13 (43.33)		
	≥30 kg/m²	2 (6.67)		
ACR	<30 mg/g	25 (83.33)	13.32 (2.64-88.06)	
	≥30 mg/g	5 (16.67)		
Fasting Glucosa			130.50 (76.00-333.00)	
Glucosa 2j PP				196.70±60.31
Serum Creatinine				0.88±0.23
Hemoglobin				14.08±1.01

TABLE 2.

Results of Rank Spearman
HbA1c and TGFβ-1 plasma analysis

Variable	Median (Min-Max)	r	p
HbA1c	7.15 (4.7-13.6)		
TGFβ-1	150.80 (23.60-2089.20)	0.637	0.000

When the normality test was obtained p = 0.006 (p <0.05) so it concluded that the data was not normally distributed.

TGFβ-1 Plasma Level

The result of the measurement of TGFβ-1 plasma of the subjects obtained abnormal plasma TGFβ-1 value with the median of 150.80 pg/mL with the lowest range of 23.60 pg/mL and the highest value of 2089.20 pg/mL (table 2). When the normality test obtained p = 0.000 (p <0.05) so it is concluded that the data is not normally distributed.

Correlation of HbA1c Levels and TGFβ-1 Plasma

The result of correlation analysis between HbA1c and TGFβ-1 plasma in this study using Rank Spearman obtained Spearman r-value of 0.637 with p-value 0.000 (table 2). The r value in this study was 0.637 which showed a strong correlation. The correlation of HbA1c and TGFβ-1 plasma levels in this study was positive or a significant direction with increasing HbA1c levels, plasma TGF levels will be higher. The correlation between HbA1c and plasma TGFβ-1 levels appears in the scatter diagram of Figure 1.

DISCUSSION

The correlation between HbA1c and TGFβ-1 plasma levels in this study is in line with some previous studies. Research on 29 subjects T2DM with normal creatinine clearance level which then performed renal biopsy, obtained r = 0.65 with P <0.001(7). Research on 72 T2DM patients divided into 3 groups of 19 subjects T2DM with normoalbuminuria, 27 subjects T2DM with microalbuminuria and 26 subjects T2DM with macroalbuminuria, obtained statistically significant results in macroalbuminuria and microalbuminuria groups with r-values of 0.82 and 0 respectively, 67 with P <0.001. The study shows a relationship with a strong positive correlation strength (8). Research on 60 patients with various levels of T2DM diabetic nephropathy conditions obtained a moderate

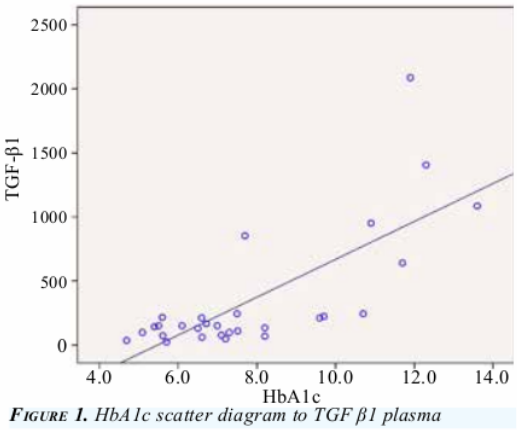

FIGURE 1. *HbA1c scatter diagram to TGF β1 plasma*

positive correlation with the value of r = 0.40 with p <0.01. In this study, exclusion was not performed on advanced stage of chronic kidney disease, hypertension condition or proteinuria condition that differentiated with this study so that this study obtained a stronger correlation between HbA1c and TGFβ-1 plasma levels, which means the risk of progressive complications of microvascular will be greater as a worsening of chronic hyperglycemia conditions (9).

Several other studies showed a non-significant correlation between HbA1c and TGFβ-1 plasma levels. A study of 25 patients with T2DM exclusively excluded from albuminuria resulted in a statistically non-significant correlation (10). Roopakala et al., study on 73 T2DM patients with various levels of albuminuria and did not exclude high hypertensive or creatinine conditions, found statistically significant correlation (15).

The HbA1c examination is a standardized examination that is used worldwide as a diabetes diagnostic tool as well as control monitoring of chronic hyperglycemia conditions. HbA1c levels can be affected by the accuracy of several conditions including anemia, age, polycythemia vera, pregnancy, chronic kidney disease, hemoglobinopathy, excessive alcohol consumption, hemoglobin-induced drug use, gastrointestinal bleeding, liver disease, and HIV infection (11, 12).

TGFβ-1 is a pleiotropic cytokine that plays a role in the pathogenesis of microvascular complications resulting in extracellular matrix protein deposition and thinning of the renal glomerular basement membrane. The role of TGFβ-1 as an etiologic mediator of microvascular complications has been investigated in several studies. Examination of TGFβ-1 levels can be performed to predict the course of microangiopathic complications in T2DM disease (1, 13).

Recent research has shown that the pathophysiology of diabetic microvascular complications is associated with a chronic inflammatory process, one of which is played by TGFβ-1. Chronic hyperglycemia plays an important role in the initiation and progression of diabetic microvascular. The progression of microvascular complications is caused by impaired glucose metabolism and intracellular signaling abnormalities through polyol pathways, protein kinase C pathways, hexosamine pathways, AGE formation, and activation of renin-angiotensin pathways. These pathways are interconnected with each other and result in elevated levels of TGFβ under conditions of chronic hyperglycemia (2, 14).

CONCLUSION

There was strong correlation was between HbA1c and TGFβ-1 plasma in T2DM patients. There is a tendency of elevated plasma TGFβ-1 levels along with the elevated HbA1c levels in T2DM patients. Thus, elevated HbA1c levels needs to be considered as a marker of elevated plasma TGFβ-1 levels.

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