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Differences in VEGF (*Vascular Endothelial Growth Factor*) Levels AND IGF-1 (*Insulin like Growth Factor-1*) in Controlled and Uncontrolled Diabetes Mellites TIP 2 Patients at Puskesmas Padang Bulan Medan City

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Abstract: Diabetes mellitus is a metabolic disorder characterized by an increase in blood sugar due to decreased insulin secretion by pancreatic beta cells or impaired insulin function (insulin resistance) that can lead to microvascular and macrovascular complications. The results of previous studies state that plasma levels of VEGF and IGF-1 are associated with the incidence of diabetic retinopathy and diabetic nephropathy. The examinations carried out include HbA1C using the HPLC method, measurement of VEGF and IGF-1 levels using Bioassay Technology Laboratory ELISA Human IGF-1 and VEGF. Of the 52 patients examined, it was found that 10 type 2 DM were controlled and 42 type 2 DM were uncontrolled. Uncontrolled type 2 DM had a higher VEGF level of 409 (ng/ml) than controlled 391.8 (ng/ml). IGF-1 levels of uncontrolled type 2 DM were higher at 8.8 (ng/ml) than controlled 6.6 (ng/ml). The conclusion obtained from this study is that there is no significant difference in VEGF and IGF-1 levels in controlled and uncontrolled type 2 DM. Controlled type 2 DM VEGF levels are lower than uncontrolled, while controlled IGF-1 levels are lower than uncontrolled. There was no significant difference in IGF-1 levels with obese and non-obese patients based on Body Mass Index (BMI). IGF-1 levels of obese type 2 DM are lower than those who are not obese.

Keywords: IGF-1, VEGF, Type 2 Diabetes Mellitus.

1. Introduction

Endothelial dysfunction in Type 2 DM patients is associated with insulin resistance, suggesting its role as an *early* cause of the development of *atherosclerosis (early atherosclerotic cardiovascular disease)*. Endothelial cells also play an important role in muscle cell growth and endothelial cells are involved in the production of various molecules *that* play a role in the inflammatory process (Adrianti, 2017). Endothelial cells are also involved in the production of various molecules that play a role in the inflammatory process (Adrianti, 2017). Damage and dysfunction of vascular endothelium, long-term chronic inflammatory response mediated by inflammatory factors and other reasons are important mechanisms for the occurrence and progression of atherosclerosis in Type 2 DM. Among them, *Vascular Endothelial Growth Factor* (VEGF) and transforming growth factor- β 1 (TGF- β 1) are hot topics discussed in recent years (Benjamin *et al*, 2022). *Vascular Endothelial Growth Factor* (VEGF) is a proangiogenesis factor that increases vascular permeability and activates endothelial cells where its biological effects are mediated by VEGF binding to its receptors, namely receptors 1 and 2



(VEGFR-1 and VEGFR-2) (Hanjani *et al*, 2016). Therefore, VEGF plays an important role in the incidence of endothelial dysfunction in Type 2 DM patients which will lead to microvascular complications (Zhang *et al*, 2018). Insulin like growth factor (IGF-1) can also be found in patients with Type 2 DM and as a marker for severity and complications in patients with Type 2 DM. Insulin like growth factor (IGF-1) is a potentially compound growth factor that plays an important role in tissue energy metabolism and growth. Similar to insulin, IGF-1 increases insulin sensitivity and peripheral glucose uptake, which reduces hepatic glucose production (Liu, *et al* 2021). IGF-1 also plays a potential role in the pathogenesis of diabetes-related complications, and low IGF-1 levels contribute to the development of diabetic complications, including diabetic neuropathy, diabetic nephropathy, and diabetic retinopathy. IGF-I shares nearly 50% amino acid sequence homology with insulin and elicits nearly the same hypoglycemic response. Studies show that low and high levels of IGF-I are associated with impaired glucose tolerance and a higher risk of Type 2 DM (Angela *et al*, 2012).

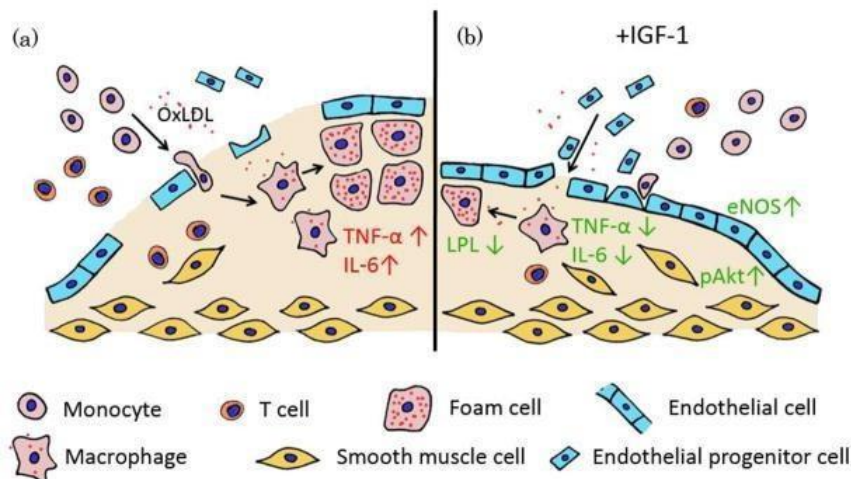
1.1 VEGF mechanism in patients with type 2 DM

There is a significant relationship between increased VEGF content in the vitreous body and the severity of Diabetes Retinopathy (Liu *et al*, 2013). Besides endothelial cells, other retinal cells can also produce VEGF when activated or stimulated continuously by high glucose levels (Lin *et al*, 2013). VEGF is essential for the modulation of ocular angiogenesis and vascular permeability. In the vitreous humor and fibrovascular tissue of PDR eyes, increased levels of VEGF can be observed. VEGF levels in serum and vitreous are related to blood glucose control in diabetic patients (Zha *et al*, 2022). Experiments have revealed that intravitreal injection of VEGF can produce various symptoms of NPDR and PDR: nonperfused capillaries, vasodilation, and tortuous arterioles characterized by endothelial hyperplasia and microaneurysms. The extent of *External Limiting Membrane* (ELM) damage was positively correlated with serum VEGF levels, indicating that VEGF levels are related to the severity of Diabetes Retinopathy and the extent of external limiting membrane damage. VEGF regulates Diabetes Retinopathy-related inflammation in the early phase (Zhang *et al*, 2018).

1.2 IGF-1 linkage in type 2 DM

IGF-1 is a potent mitogen and antiapoptotic factor in vascular cells, including (VSMC) *Vascular Smooth Muscle Cell* and endothelial cells, and also promotes migration. IGF-1 decreases the size of atherosclerotic lesions in the aorta. Increased circulating IGF-1 reduces *Oil Red-positive* foam cells, decreases plaque macrophage infiltration and downregulates TNF- α and IL-6 levels. IGF-1 also decreased *Lipoprotein Lipase* (LPL) expression in macrophages (LPL facilitates the uptake of modified LDL) and increased the expression of *endothelial Nitric Oxide Synthase* (eNOS) and phosphorylated Akt. IGF-1 increases the number of *Endothelial Progenitor Cell* (EPC) in the circulation, potentially facilitating endothelial repair (Higashi *et al*, 2010).

2. Materials and Methods



This study is an observational *study* with a *cross-sectional study* where Blood samples were taken and HbA1c levels were measured at the same time and then analyzed for VEGF and IGF-1 levels in one examination. The study was conducted on all Type 2 DM patients who met the inclusion criteria and conducted examination and treatment at the Integrated Service Unit of the Padang Bulan Health Center, Medan City, North Sumatra, as many as 52 people and divided into two groups, namely the controlled Type 2 DM group and the uncontrolled Type 2 DM group.

2.1 Sampling method

Each patient who meets the inclusion criteria is recorded, then the patient is given an explanation of the purpose and objectives of the study, as well as the examination that the subject will undergo, if the patient agrees then the patient is asked for a letter of *consent* (*informed consent*).

1. The patient was interviewed including age and history of disease suffered, then the measurement of Body Mass Index (BMI) which includes height, weight and abdominal circumference.
2. Blood samples were taken at the Integrated Service Unit Hall of the Padang Bulan Health Center, Medan City, North Sumatra by the Laborian of Bunda Thamrin Hospital Medan, then the blood samples that had been taken were taken to be examined for HbA1c levels at the Clinical Pathology Laboratory of Bunda Thamrin Hospital Medan.
3. Blood samples were then taken to the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra for VEGF and IGF-1 examination.

2.2 IGF-1 Level Check

Sample Preparation

1. The sample is serum obtained from diabetic patients stabilized at 2-8⁰C.
2. Serum or plasma samples require a 20-fold dilution. The recommended 20-fold dilution is 20 μ L of sample +380 μ L of RD5-7 calibrator diluent.

Tools and Materials

1. Bioassay Technology Laboratory ELISA Human IGF-1 Catalog Number E0103Hu (Shanghai Korain Biotech Co.), Human IGF-1 microplate, Human IGF-1 conjugate, Human IGF-1 standard, Calibrator Diluent RDS-7, Wash Buffer Concentrate, Color Reagent A (Chromogen solution A), Color Reagent B



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(Chromogen tetramethylbenzidine), Stop solutio, Plate sealers, Human IGF-1 control, Microplate reader, Incubator, Micropipettes and tips **and** Distilled water.

How it works

1. Prepare all reagents, standard solutions and samples as directed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the test. Insert the strips into the frame for use. Unused strips should be stored at 2-8°C.
3. Add 50ul standard to the standard well. Notes: Do not add antibodies to the standard wells as the standard solution contains biotinylated antibodies.
4. Add 40ul of sample to the well then add 10ul of Human IGF1 antibody to the sample well, then add 50ul of streptavidin-HRP to the sample well and standard well (Not blank control well). Mix well. Cover the plate with sealer. Incubate for 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak the wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automatic washing, aspirate or decant each well and wash 5 times with wash buffer. Blot plate onto paper towels or other absorbent material.
6. Add 50ul of substrate solution A to each well and then add 50ul of substrate solution B to each well. Incubate the plate covered with fresh sealer for 10 minutes at 37°C in the dark.
7. Add 50ul Stop Solution to each well, the blue color will immediately turn yellow.
8. Immediately determine the optical density (OD value) of each well using a microplate reader set to 450 nm within 10 minutes of adding the stop solution.

2.3 IGF-1 examination Sample preparation

1. The sample is serum obtained from diabetic patients stabilized at 2-8°C.
2. Serum or plasma samples require 20-fold dilution. The recommended 20-fold dilution is 20 µL of sample + 380 µL of RD5-7 calibrator diluent.

Tools and Materials

Technology Laboratory ELISA Human IGF-1 Catalog Number E0103Hu (Shanghai Korain Biotech Co.), Human IGF-1 microplate, Human IGF-1 conjugate, Human IGF-1 standard, Calibrator Diluent RDS-7, Wash Buffer Concentrate, Color Reagent A (Chromogen solution A), Color Reagent B (Chromogen tetramethylbenzidine), Stop solutio, Plate sealers, Human IGF-1 control, Microplate reader, Incubator, Micropipettes and tips **and** Distilled water.

How it works

Prepare all reagents, standard solutions and samples as directed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips into the frame for use. Unused strips should be stored at 2-8°C. Add 50ul standard to the standard well. Notes: Do not add antibodies to the standard wells as the standard solution contains biotinylated antibodies. Add 40ul of sample to the sample wells then add 10ul of Human VEGF antibody to the sample wells, then add 50ul of streptavidin-HRP to the sample wells and standard wells (Not blank control wells). Mix well. Cover the plate with sealer. Incubate for 60 minutes at 37°C.- 5. Remove the sealer and wash the plate 5 times with wash buffer. Soak the wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automatic washing, aspirate or decant each well and wash 5 times with wash

buffer. Blot the plate onto paper towels or other absorbent material. Add 50ul of substrate solution A to each well and then add 50ul of substrate solution B to each well. Incubate the plate covered with fresh sealer for 10 minutes at 37°C in the dark. Add 50ul of Stop Solution to each well, the blue color will immediately turn yellow. Immediately determine the optical density (OD value) of each well using a microplate reader set to 450 nm within 10 minutes of adding the stop solution.

3. Results and Discussion

The number of samples in this study were 52 patients with type 2 DM from a population of 82 patients with type 2 DM, divided into two groups, namely the controlled DM group if the HbA1C results were <7% (n=10) and the uncontrolled DM group if the HbA1C examination results were \geq 7% (n=42).

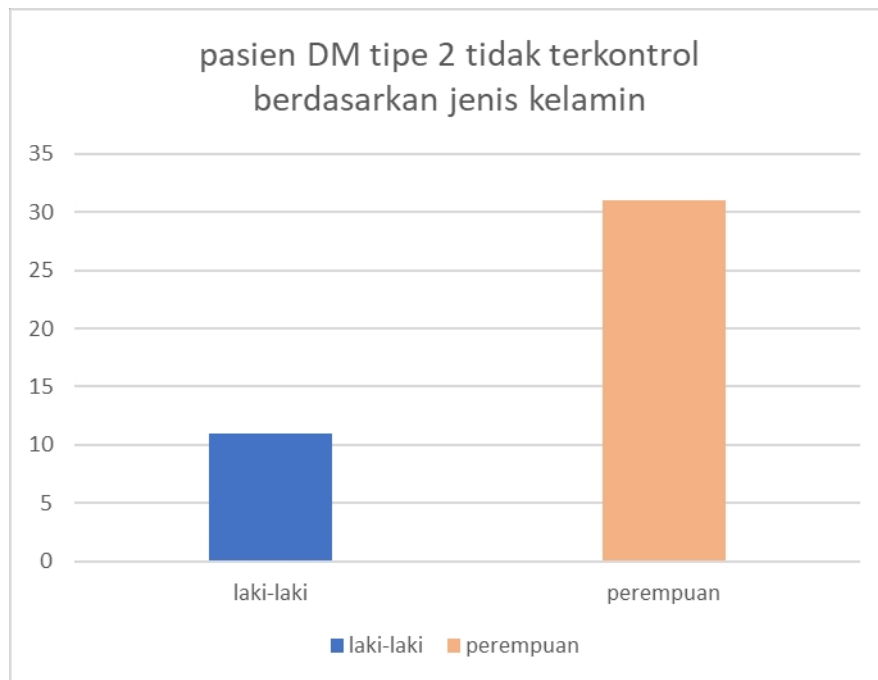


Figure 1: diagram of uncontrolled type 2 DM patients by gender

Number of patients with type 2 DM based on gender

Gender	Number of Patients
Male	12
Female	40
Total	52

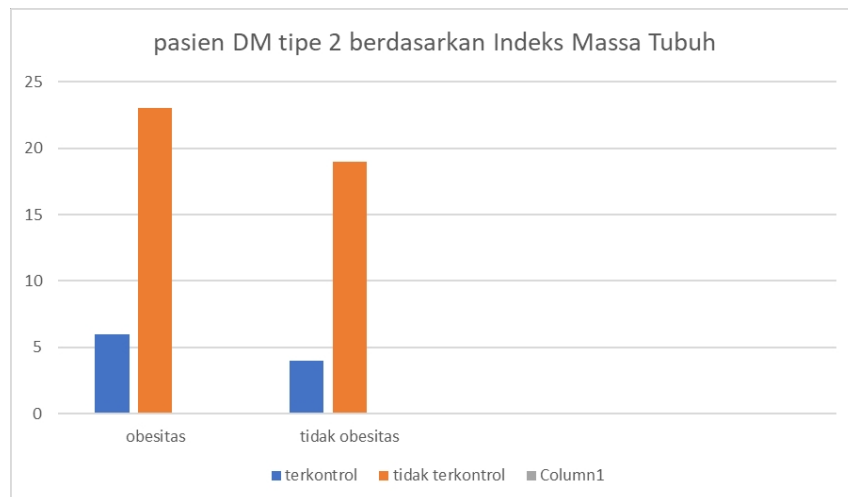


Figure 2: Type 2 DM patients by body mass index

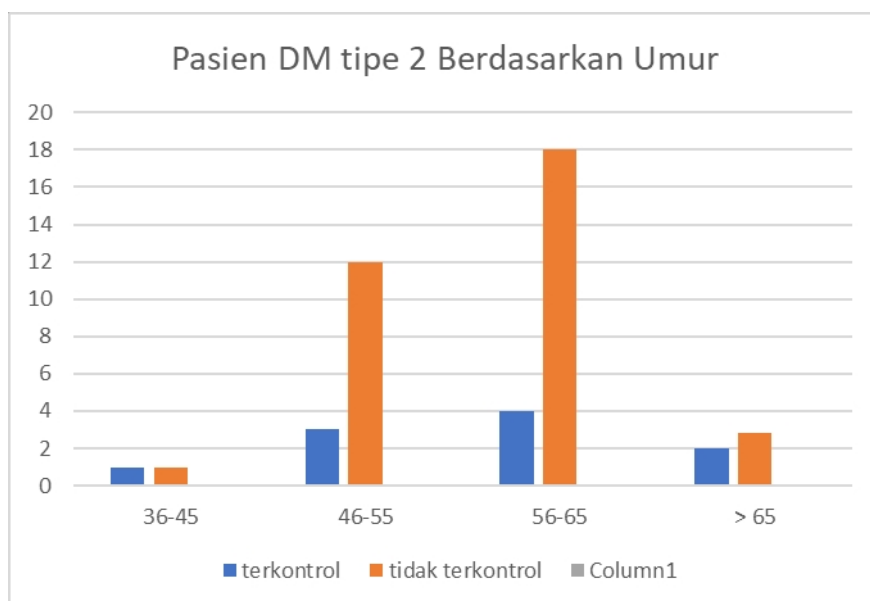


Table 2: In the table and diagram above shows the number of patients with type 2 DM female gender 40 people (76.9%) more than the male 12 people (23.1%). with the highest total age range of 56-65 years as many as 22 people (82.). The number of HbA1C levels of uncontrolled type 2 DM patients (80.8%) is higher than those controlled (19.). The number of BMI with normal category was 29 people and obese 23 people.

3.1 Comparative Analysis of Mean Vascular Endothelial Growth Factor and Insulin Growth Factor Levels in Controlled and Uncontrolled Type 2 DM Patients

Calculation of Vascular Endothelial Growth Factor and Insulin Growth Factor levels was carried out by the Elisa method. Based on the Kolmogorov Smirnov test, the data was found to be normally distributed and continued with the Independent Sample T-Test.

Table 2 Comparison of mean levels of Vascular Endothelial Growth Hormon (VEGF) and Insulin Growth Factor 1 (IGF) in DM patients

Variables	Type 2DM Controlled (n=10)	Uncontrolled Type 2 DM (n=42)	p
	Mean± SD	Mean± SD	
VEGF (ng/ml)	391,8 ±179,2	409.0± 509.6	0,04
IGF-1 (mg/dl)	6,6 ± 1,2	8,4 ± 5,3	0,9

Table 2 shows the comparison of VEGF levels in the uncontrolled DM group (409 ng/ml) was higher than the controlled DM group (391 ng/ml). *Independent T Test* statistical test showed this difference was statistically significant ($p = 0.04$). Meanwhile, the comparison of IGF-1 levels in the uncontrolled DM group (8.89 ng/ml) was higher than that in the DM group (6.62 ng/ml). *Independent T Test* statistical test showed the difference was not statistically significant ($p = 0.9$).

Table 3 Comparison of mean Insulin Growth Factor 1 (IGF) levels in type 2 DM patients with BMI (Body Mass Index) levels

Variable s	BMI Not Obese (n=29)	BMI Obese (n=23)	p
	Mean± SD	Mean± SD	
IGF-1 (ng/ml)	8.2± 5.1	7.0± 1.5	0,02

Table 3 shows the comparison of IGF-1 levels in the group of Type 2 DM patients who have normal BMI (Body Mass Index) status (9.2 ng/ml) is higher than the group of Type 2 DM patients who have obese BMI status (8.8 ng/ml). *Independent T Test* statistical test showed a significant difference statistically ($p= 0.02$).

Conclusion

VEGF levels in uncontrolled Type 2 DM patients are higher than ones, the same thing also occurs in uncontrolled Type 2 DM IGF-1 levels higher than ones. In this study also obtained a difference in VEGF levels in controlled and Type 2 DM patients, while IGF-1 levels had no difference in controlled and uncontrolled Type 2 DM patients but there was a significant difference in IGF-1 levels with Body Mass Index of controlled and uncontrolled Type 2 DM patients.



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