

EXAMINATION OF VITAMIN D RECEPTOR (VDR) PROTEIN LEVELS IN TYPE II DIABETES MELLITUS PATIENTS IN MAKASSAR

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ABSTRACT

Type II diabetes mellitus is a disease characterized by hyperglycemia resulting from the body's resistance to the effects of insulin produced by pancreatic cells. Type II diabetes mellitus can be associated with a deficiency of Vitamin D receptors (VDR). This study aims to determine the levels of Vitamin D receptor (VDR) protein in type II diabetes mellitus patients. This type of research is a type of laboratory observation research. This study used the serum of 15 samples of type II diabetes mellitus patients and then measured the levels of vitamin D receptor protein (VDR) using the ELISA method (Enzyme linked Immunosorbent Assay). The results showed that there was a decrease in vitamin D receptor (VDR) levels in 12 samples (60%). So it can be concluded that the decrease in vitamin D receptor (VDR) levels in type II diabetes mellitus patients did not show a significant difference ($p = 0.386$) and ($p = 0.710$).

Keywords: *Diabetes Mellitus Type II; ELISA; VDR*

INTRODUCTION

Type II diabetes mellitus is a disease characterized by hyperglycemia caused by the body's resistance to the action of insulin produced by pancreatic cells. Type II diabetes is diabetes mellitus that occurs in adults, in around 80% of diabetes mellitus sufferers. The incidence of chemical type II diabetes is increasing and the increase in the number of type II diabetes sufferers is caused by several factors, namely: genetics, lifestyle and obesity (Ainun et al. 2021).

According to data from the International Diabetes Federation (IDF) in 2021, there are more than half a billion diabetes sufferers worldwide, more precisely 537 million diabetes sufferers, and this number is predicted to increase to 643 million. Diabetes mellitus results in a very high death rate for diabetes mellitus sufferers, estimated at more than 6.7 million in the adult group or around the age of 20 to 79 years. (International Diabetes Federation 2021).

The number of diabetes mellitus sufferers is 10.7 million people. Of the ten countries, Indonesia is ranked seventh in terms of diabetes mellitus sufferers. Data collection for diabetes sufferers defines age ≥ 15 years according to the new criteria applied when fasting blood glucose levels ≥ 126 mg/dl and postprandial blood glucose ≥ 200 mg/dl or blood glucose ≥ 200 mg during treatment and there are various symptoms such as wasting urination, frequent thirst, frequent urination and many more. As well as weight loss (Lihu, Botutihe, and Napu 2020).

Cases of diabetes mellitus in Indonesia have increased in various provinces, one of which is the province of South Sulawesi with diabetes mellitus sufferers with a percentage of 66%, then ranked 4th as a non-communicable disease in South Sulawesi and South Sulawesi ranked 5th as a cause death. Diabetes mellitus as well as in the city of Makassar which is ranked 5th out of 10 cases of death, diabetes mellitus is one of the causes of the most deaths with the number of diabetes mellitus sufferers having reached 5700 cases and there has been an increase of 1300 cases of diabetes mellitus (Irwansyah 2020).

The large increase in type II diabetes mellitus may be partly related to various factors including modifiable factors, namely obesity, lack of physical activity, dyslipidemia (high blood fat levels) and unhealthy eating patterns. Risk factors that cannot be changed include

a family history of diabetes mellitus, age ≥ 45 years, birth of a baby with a weight of >4000 grams or history of a baby with a birth weight of <2.5 kg (Bhatt, Saklani, and Upadhayay 2016).

Vitamin D functions to maintain normal levels of calcium and phosphate in the blood, thus supporting the bone mineralization process, metabolic function and neuromuscular function. Vitamin D has been suggested to contribute to the process of muscle protein synthesis through both genomic and non-genomic mechanisms. The vitamin D receptor (VDR) is a mediator in the muscle cell signaling process which is required for the activation process of the role of vitamin D (Caniago, Ngestiningsih, and Fulyani 2019).

Vitamin D is needed in the body to maintain insulin levels in the bloodstream, the vitamin D receptor (VDR) is found in the pancreas where insulin is produced. Vitamin D supplementation has been proven to be successful in increasing insulin levels in type 2 diabetes mellitus. The dose of vitamin D for diabetes is not yet known. If an excess dose can cause toxicity (Teguh Sutanto 2013).

Vitamin D deficiency can occur if exposure to sunlight and daily intake is very low, although there is no consensus regarding serum 25(OH)D levels, deficiency is defined if serum 25(OH)D levels are less than 20 ng/ml. This level is in contrast to the thyroid hormone level which can reach 30-40 ng/ml. At that time, thyroid hormone levels will stop (Dr.dr.Dina Keumala Sari, M, Giz 2557).

Vitamin D receptors (VDR) are a group of nuclear receptors. This receptor functions as a protein that regulates transcription which is activated by ligands. VDR selectively binds the hormone 1,25-dihydroxyvitamin D₃ (1,25(OH)₂ D₃) and controls the expression of selected genes in target cells (Ainun et al., 2021). VDR as a transcription factor in muscle protein synthesis (actin and myosin). VDR activity decreases with age. Low circulating vitamin D levels (<50 nmol/L) can be defined as vitamin D deficiency (Caniago, Ngestiningsih, and Fulyani 2019).

The aim of this study was to determine vitamin D receptor (VDR) protein levels in type II diabetes mellitus sufferers in Makassar.

METHOD

Study Design

This research is a laboratory observational study using a cross sectional study (Nisak 2021).

Population, Samples, and Sampling

The study population was type II diabetes mellitus sufferers at Labuang Baji Regional Hospital and Bhayangkara Hospital, Makassar City who met the inclusion and exclusion criteria. The samples used were 3 ml venous blood samples totaling 15 samples. Examination of VDR protein levels was carried out at the Hasanuddin University Medical Research Center (HUM-RC) laboratory.

Instruments

The tools used are ELISA sandwiches, tube racks, centrifuges, micropipettes, tourniquets, pellets or wel, tips, pipettes, cooler bags, tubes and tubing. The specifications need to be written down and the materials used are: 3cc spoon, vacuum tube, alcohol cotton. Type II DM Serum Samples, Plasters , Biohenm Canada Diagnostics Kit (DBC) ELISA Reagents.

Procedure

Serum samples were examined by sandwich ELISA. Pipet 25 µl of serum into each microplet, add 150 µl of incubation buffer, then incubate for 60 minutes in the dark at room temperature, wash using wash buffer 3 times, then add 150 µl of working conjugate solution, then incubate for 30 minutes at room temperature, then washed using wash buffer 3 times, pipetting 150 TMB (tetramethyl benzidine) substrate into each microplets, then incubate for 10-15 minutes at room temperature, add 50 µl of reaction buffer to all plates, add 50 µl of stopping solution to each plate, reducing reagent to each well plate. Then read using sandwich ELISA with a wavelength of 450 nm.

Data Analysis

Test result data is presented in table form accompanied by an explanation.

RESULTS

1. Characteristics of Research Subjects

Several characteristics of research subjects in people with type II diabetes mellitus illustrate how vulnerable age and gender are. The number of respondents who were examined based on age 30 - 40 years was 1 person with a percentage of 6%, age 41-50 years was 6 people with a percentage of 40%, age 51-60 years was 4 people with a percentage of 27% and age 61-70 years as many as 4 people with a percentage of 27%. The number of female respondents was 9 people with a percentage of 60%, while the number of male respondents was 6 people with a percentage of 40% of the total research sample of 15. Based on table 1, type 2 diabetes mellitus patients based on age and gender showed no differences. significant with a p value >0.05 , namely $p = 0.386$ and $p = 0.710$.

Table 1. Characteristics of Respondents Based on Age and Gender of Research Subjects with Type II Diabetes Mellitus Cases

Characteristics	N	%	<i>p value</i>
Age			0.386
30-40	1	6%	
41-50	6	40%	
51-60	4	27%	
61-70	4	27%	
Total	15	100%	
Gender			0.710
Laki-Laki	6	40%	
Perempuan	9	60%	
Total	15	100%	

2. Results of examination of vitamin D receptor protein levels in diabetes mellitus sufferers in type II diabetes mellitus sufferers.

Based on the data in table 2, the results of examining vitamin D receptor (VDR) levels using the ELISA method showed that 3 people (40%) had vitamin D receptor (VDR) protein levels with a normal value of 50-70 ng/ml ($p = 0.386$), while vitamin D receptor (VDR) protein was sufficient at 30-40 ng/ml in type II diabetes mellitus sufferers and as many as 12 people (60%) had VDR levels of less than 30 ng/ml ($p = 0.710$).

Table 2. Results of examination of Vitamin D receptor levels in type II diabetes mellitus sufferers.

Mark (ng/ml)	N	%	<i>p value</i>
50-70	-	-	
30-40	3	40%	0.386
<30	12	60%	0.710
Total	15	100%	

The value is significant if $p < 0.05$

Based on the data in table 3, it shows that when examining vitamin D receptor (VDR) protein levels in patients with type II diabetes mellitus, based on the age of 30-40 years of respondents, 1 person with a percentage of 6% was obtained from the results of examining sufficient vitamin D receptor levels, in Respondents aged 41-50 years were 2 people with a percentage of 13%, obtained sufficient results and low results were obtained as many as 4 people with a percentage of 27%, at the age of 51-60 years there were 4 respondents with a percentage of 27% obtained low results and at the age of 61-70 years as many as 4 people with a percentage of 27% obtained low results, based on male gender, 1 person had sufficient vitamin D receptor protein levels with a percentage of 6% and 5 respondents experienced Vitamin D protein levels were low and in women 2 respondents had normal vitamin D protein levels and 7 respondents had low vitamin D protein levels, with a sample size of 15.

Table 3. Distribution of Vitamin D Receptor (VDR) Levels in Type II Diabetes Mellitus Patients.

Group	Normal		sufficient value		less value		<i>p value</i>
	N	%	N	%	N	%	
Age							0.386
30-40	-	-	1	6%	-	-	
41-50	-	-	2	13%	4	27%	
51-60	-		-	-	4	27%	
61-70	-	-	-	-	4	27%	
Gender							0.710
Female			2	13%	7	47%	
Male			1	6%	5	34%	

DISCUSSIONS

In this study, all research subjects, both case and control groups, underwent an ELISA examination to determine vitamin D receptor protein levels in type II diabetes mellitus sufferers. The ELISA (Enzyme linked Immunosorbent Assay) method is a quantitative analysis showing antigen-antibody reactions with color changes achieved with enzyme-linked conjugates and enzyme substrates. The ELISA method is used to identify the presence and concentration of molecules in biological fluids. The ELISA kit used to identify a particular molecule must be specific for that antigen (Ipandi, Sa'adi, and Sudjarwo 2019).

Based on the results of research using the ELISA method, there were 3 samples with sufficient vitamin D levels and 12 samples lacking vitamin D levels. At the age factor of 30-40 years, 1 person with a percentage of 6% obtained adequate results. aged 41-50 years, 6 people with a percentage of 40% got results, 2 people had adequate results with a percentage of 13%, while 4 people got vitamin D deficiency results with a percentage of 27%, aged 51-60 years, 4 people with a percentage of 27% got vitamin D deficiency results. D and in the

61-70 year group, 4 people with a percentage of 27% had vitamin D deficiency results (Table 2).

According to Ikhwan Rinaldi et.al (2007), based on his study, 77.4% of elderly people have normal vitamin D status (>50 nmol/liter). Based on the results of research by Lihu et al., (2020), it shows that of the 38 respondents, the majority of elderly people were at the level of severe cognitive function, namely 17 people (44.8%). Of this number, 15 people experienced a Vitamin D deficit (68.2%) and 2 people (12.5%) had good vitamin D intake. In the elderly, memory is one of the cognitive functions that often declines, in the age period 60-74 years there is a decline in several abilities with wide differences between individuals due to the formation of abnormal proteins in the brain, for example amyloid- β and protein or which can cause nerve tangles, attacking axons thereby affecting the transmission of memory to the brain (Rinaldi et al. 2007) (Lihu, Botutihe, and Napu 2020).

Based on data on gender distribution in type II diabetes mellitus sufferers, in the research sample the gender predominates were 9 people with a percentage of 60%, while there were 6 people who were male with a percentage of 40% (Table 2). . Al Ghadeer et al., (2022) reported that 98.4% of type 2 diabetes patients had vitamin D deficiency. Nearly 75% of female diabetes patients had vitamin D deficiency compared to less than half of male diabetes patients (46.9%), but 50.8% and 25.6% had vitamin D deficiency, vitamin D deficiency (<20 ng/ml) was detected in 59.5% of type II diabetes patients (Al Ghadeer et al. 2022).

Based on the results of statistical tests using the chi-square test, it was obtained that the p value was > 0.05 or there was no relationship between gender and age from the results of examining vitamin D receptor (VDR) levels in diabetes mellitus sufferers which could be associated with vitamin D deficiency. VDR can also influence diabetes. There is sufficient evidence that vitamin D can help prevent type 1 and type 2 diabetes. Vitamin D deficiency impairs insulin secretion. Pancreatic beta cells that secrete insulin were found to contain VDR as well as the enzyme alphahydroxylase (Fiannisa 2019).

According to Caniago et al., (2019), research on elderly men and women showed that 80.2% of elderly people had vitamin D deficiency. Differences in the results of vitamin D status in this study and previous studies may be due to factors such as age, gender and place of residence.

The most accurate measurement of vitamin D levels in the body is to measure levels of 25-OH vitamin D, the active form of vitamin D that is distributed throughout the body. Serum vitamin D-25-OH levels of 30-100 ng/m³ are recommended. Low levels of vitamin D in the body decrease insulin secretion, and higher serum 25(OH)D levels may reduce the incidence of some types of cancer. Adequate vitamin D levels can prevent various malignant cancers.

Vitamin D is a fat-soluble micronutrient that is involved in calcium and phosphate metabolism, calcium homeostasis, blood vessel health, and cell differentiation and reproduction. Vitamin D, also known as the "sunshine vitamin", is often associated with various diseases, from degenerative diseases to malignant tumors. There is evidence that vitamin D levels higher than those required to maintain calcium homeostasis may reduce the risk of insulin resistance, obesity, metabolic syndrome, and malignancy (Iskita Fiannisa, 2019).

Apart from exposure to sunlight, the main source of vitamin D is intake of vitamin D from food. Natural sources of vitamin D include salmon, mackerel, tuna, cod liver oil, mushrooms and egg yolks. Fish oil at least 3-4 times a week can help optimize the body's need for vitamins (Iskita Fiannisa, 2019).

In this study, it can be concluded that when examining vitamin D receptor (VDR) levels in type II diabetes mellitus sufferers, 12 people were found to have vitamin D receptor (VDR) deficiency with a percentage of 60% with a P value of 0.710 and sufficient vitamin D receptor levels. 3 people with a percentage of 40% with a P value of 0.386, with a total of 15 respondents. In this study there were several weaknesses in the results of the SPSS chi-square test so that there was no significance between gender and age because the type II diabetes mellitus sample used was very small and the SPSS test could have an effect.

CONCLUSION

Based on the research results, it can be concluded that the research results showed a decrease in vitamin D receptor (VDR) levels in 12 samples (60%), but did not show a significant difference ($p = 0.386$) and ($p = 0.710$).

CONFLICT OF INTEREST

The Author(s) declare(s) that there is no conflict of interest.

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