

# The Differences of Parathyroid Hormone, Vitamin D, and Calcium Ion Between Patients With Controlled and Uncontrolled Diabetes Mellitus

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## ABSTRACT

**Background:** Uncontrolled diabetes mellitus (DM) may induce inflammation and the likelihood of bone fracture. The accumulation of final products from glycation and oxidative stress may cause changes in bone strength, metabolism and structure. Poor glycemic control may alter the calcium homeostasis, parathyroid hormone (PTH) and vitamin D secretion.

**Aim:** To evaluate the differences between iPTH, 25(OH)D and Ca ion levels as factors that affect on bone metabolism in controlled and uncontrolled DM groups.

**Method:** A cross-sectional study was conducted on 69 patients with DM in Diponegoro National Hospital. All patients were divided into two groups based on HbA1c levels. Intact parathyroid hormone (iPTH) and 25(OH)D levels were measured with enzyme-linked immunoassay (ELISA), while calcium (Ca) ion was measured with an ion selective electrode (ISE). T test and Mann-Whitney test were applied for data analysis and  $p < 0.05$  was considered as statistically significant.

**Results:** There were differences in iPTH level ( $p = 0.031$ ) and Ca ions ( $p = 0.033$ ) between group with controlled and that with uncontrolled DM, but there was no difference in 25(OH)D level ( $p = 0.051$ ) between both groups.

**Conclusion:** There were difference in iPTH and Ca ion levels between both groups. Both iPTH and Ca ion might impact on therapeutic monitoring in patients with DM.

**Keywords:** Diabetes mellitus, parathyroid hormone, iPTH, 25(OH)D, Ca ion

## INTRODUCTION

Diabetes mellitus (DM) is a chronic progressive metabolic disease condition characterized by hyperglycemia due to abnormal insulin secretion, abnormal insulin action, or both<sup>1,2</sup>. Type 2 DM is the most common cause of DM in Indonesia<sup>2,3</sup>. The International Diabetes Federation (IDF) has been predicting increases type 2 DM in Indonesia into 14.1 million in 2035.<sup>(4)</sup> Prevalence of DM in patients of >15 years old was increasing from 6.9% in 2013 into 8.5% in 2018<sup>3</sup>. It was also mostly in population of 55–64 years old<sup>3</sup>.

The diagnostic criteria of DM are when the fasting plasma glucose (FPG)  $\geq 126$  mg/dL, or 2 hours plasma glucose (2h-PG)  $\geq 200$  mg/dL, or random plasma glucose  $\geq 200$  mg/dL with symptoms of polydipsia, polyuria, and polyphagia, accompanied by weight loss.<sup>(1,4,5,6)</sup> Uncontrolled DM may induce inflammatory state associated with vascular endothelial dysfunction. It is an important procedure to do a regular glucose control for patients with DM, such as HbA1c that shows plasma glucose levels in 8–12 weeks before.<sup>(4-7)</sup> Good glycemic control is achieved if HbA1c level is  $\leq 7.0$ <sup>1</sup>.

The increased risk of bone disorder in patients with DM is multifactorial in pathogenesis and is not yet fully understood. Urinary calcium (Ca) excretion sometimes increase in patients with uncontrolled DM. There is an accumulation of end products from glycation and oxidative stress that will lead to some changes in bone strength, metabolism and structures. Poor glycaemic control can cause alteration in Ca homeostasis and can increase PTH secretion in response to correct the decrease in Ca levels<sup>7-10</sup>.

Glucose homeostasis is associated with the role of PTH on insulin sensitivity in increasing the production of 1.25-dihydroxyvitamin D [1.25(OH)2D]. Parathyroid

hormone has a role in increasing vitamin D production in 1.25(OH)2D form. An active form of vitamin D, 25-hydroxyvitamin D (25 (OH) D) or calcidiol, is a good indicator of knowing the status of vitamin D<sup>10,11,12</sup>. It is associated with chronic diseases, such as insulin resistance, type 1 and also type 2 DM<sup>12-15</sup>.

Electrolyte disorders, including Ca levels, due to impaired kidney function, malabsorption syndrome, acid base disorders, or diabetic drugs, can occur in patients with DM.<sup>(15-18)</sup> Ca levels can affect on mechanism of PTH secretion and 25(OH)D production.<sup>(18)</sup> Some studies demonstrated that there was a decrease of Ca levels in patients with DM.<sup>(19,20)</sup> The aim of our study was to evaluate the differences between iPTH, 25(OH)D and Ca ion levels as factors that affect on bone metabolism in controlled and uncontrolled DM groups.

## METHOD

This was a cross-sectional study that recruited adult patients with type 2 DM who came to outpatient clinic of Diponegoro National Hospital during June–July 2019 and met inclusion criteria. The inclusion criteria were including patients diagnosed with DM by clinician over 6 month, age of >35 years old, normal body temperature, and serum glutamic pyruvic transaminase (SGPT) level <2x upper limit reference value (male <45 U/L and female <34 U/L)<sup>20-23</sup>. Patients with severe anemia (<8.0 g/dL)<sup>23,24</sup>, history of bone disorders, and history of vitamin D consumption were excluded. Group with controlled DM was defined as those with HbA1c level <7% and group with uncontrolled DM was those with HbA1c  $\geq 7\%$ .

Levels of iPTH and 25(OH)D were measured by enzyme-linked immunoassay (ELISA) with universal microplate reader spectrophotometer (Elx 800, Bio-Tek

Instruments Inc, USA) in laboratory of Faculty of Medicine, Diponegoro University Semarang. Intact PTH was measured with PTH intact ELISA (DE3645, Demeditec Diagnostics GmbH, Germany). 25(OH)D was measured with 25-Hydroxyvitamin D ELISA kit (CAN-VD-510, Diagnostics Biochem Canada (DBC) Inc, Canada).

Levels of Ca ion were measured by ion selective electrode (ISE) method with electrolyte automatic analyzer (Cornley-K-Lite 5, Meizhou, Cornley Hi-Tech Co Ltd, China) in laboratory of clinical pathology, Diponegoro National Hospital. HbA1c levels was measured with high performance liquid chromatography (HPLC) (Bio-Rad D-10, Bio-Rad Laboratories Inc, USA). This study also measured liver function tests (i.e. serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT)), kidney function tests (ureum, creatinine and estimated glomerular filtration rate (eGFR) with Unstandardized Modification of Diet in Renal Disease Study method).

Ethical clearance was obtained from Health Research Ethics Commission of Faculty of Medicine, Diponegoro

University/ Dr. Kariadi Hospital Semarang with number 54/EC/FK UNDIP/II/2019. All study participants were provided with written informed consent.

## RESULTS

Of the 80 patients with DM from outpatient clinic, 69 patients were analyzed for this study who were consisted of 33 (47.8%) female and 36 (52.2%) male. Eleven patients were excluded due to age (>75 years old), history of vitamin D consumption, bone disorders, severe anemia, and impaired liver function. The mean patients' age was 57.77±9.78 (range 37–75 years).

There were 21 (30.4%) patients with hyperthyroid state, 51(73.9%) patients with vitamin D deficiency (25(OH)D level <20 ng/dL), and 13 (18.8%) patients with vitamin D insufficiency (25(OH)D level 20–30 ng/dL)<sup>24</sup>. Group with controlled DM consisted of 25 (36.2%) patient and that with uncontrolled DM consisted of 44 (63.8%) patients. Table 1 shows baseline characteristic between two groups.

Table 1. Characteristic between two groups

Parameter	Controlled dm		Uncontrolled dm		P
	Mean ± sd	Median (min–max)	Mean ± sd	Median (min –max)	
Age (year)		62 (37–75)		58 (37–70)	0.076 <sup>b</sup>
HR (x/minutes)	77.48±10.23		82.09±12.24		0.100 <sup>a</sup>
RR (x/minutes)		18 (16–23)		20.50 (16–23)	0.066 <sup>b</sup>
Temperature (°C)		36.9 (36.5–37.1)		36.9 (36.5–37.2)	0.815 <sup>b</sup>
Sistole (mmHg)		130 (110–155)		140 (110–184)	0.057 <sup>b</sup>
Diastole (mmHg)	82.00±7.29		84.86±7.34		0.091 <sup>a</sup>
Height (cm)	156.99±7.15		158.23±8.06		0.513 <sup>a</sup>
Weight (kg)	60.34±9.17		66.36±12.29		0.024 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	24.78±3.64		26.16±3.97		0.148 <sup>a</sup>
Liver function test					
SGOT (mg/dL)		22 (16–57)		21 (12–49)	0.798 <sup>b</sup>
SGPT (mg/dL)		20 (11–80)		23 (8–83)	0.565 <sup>b</sup>
Albumin (g/dL)		4.44 (3.91–5.40)		4.32 (3.23–5.11)	0.080 <sup>b</sup>
Kidney function test					
Ureum (mg/dL)	34.08±8.05		33.88±13.98		0.940 <sup>a</sup>
Creatinin (mg/dL)		1.03 (0.49 – 1.73)		0.99 (0.52 – 1.73)	0.662 <sup>b</sup>
eGFR** (ml/min/1.73m <sup>2</sup> )		61.40 (41 – 187.80)		72.35 (42 – 140.3)	0.072 <sup>b</sup>
Haematology					
Hb (g/dL)	13.02±1.41		13.47±1.23		0.191 <sup>a</sup>
Ht (%)	39.24±3.66		39.97±3.28		0.411 <sup>a</sup>
Erythrocytes (x10 <sup>6</sup> /μL)	4.49±0.52		4.70±0.49		0.091 <sup>a</sup>
MCV (fL)	87.88±5.66		85.31±4.94		0.035 <sup>a</sup>
MCH (pg)	29.14±2.01		28.71±1.89		0.537 <sup>a</sup>
MCHC (g/dL)	33.16±0.98		33.68±0.91		0.034 <sup>a</sup>
RDW (%)	13.38±1.01		13.26±0.91		0.587 <sup>a</sup>
Leukocyte (x10 <sup>3</sup> /μL)		6.7 (4.2 – 8.9)		7.80 (5.4 – 13.0)	0.006 <sup>a</sup>
Platelet (x10 <sup>3</sup> /μL)		294 (197 – 455)	325.75±139.61		0.149 <sup>b</sup>
Glycaemic control					
FPG (mg/dL)		98 (76 – 152)		164.50 (96 – 369)	0.000 <sup>a</sup>
HbA1c (%)		6.2 (5 – 7)		8.75 (7.10 – 14.10)	0.000 <sup>a</sup>
Kadar iPTH, 25(OH)D dan kalsium ion					
iPTH (pg/mL)	64.26±17.79		54.36±17.90		0.031 <sup>a</sup>
25(OH)D (ng/mL)	13.20±7.64		16.61±7.88		0.051 <sup>a</sup>
Ca ion (mmol/L)		1.16 (0.96 – 1.27)		1.19 (0.95 – 1.30)	0.033 <sup>a</sup>

\* p<0.05 was considered statistically significant

<sup>a</sup> t-test analysis, <sup>b</sup> Mann-Whitney test

RR= respiratory rate; BMI= body mass index; SGOT=serum glutamic oxaloacetic transaminase; SGPT=serum glutamic pyruvic transaminase; eGFR= estimated Glomerular filtration rate; MCV= mean corpuscular volume; MCH=mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration; RDW= red cell distribution width; FPG= fasting plasma glucose; iPTH=intact parathyroid hormone; 25(OH)D= 25 hydroxyvitamin D

\*\*eGFR dengan perhitungan Unstandardized Modification of Diet in Renal Disease Study

## DISCUSSION

Mean iPTH level in patients with controlled DM was higher than those with uncontrolled DM ( $64.26 \pm 17.79$  pg/mL vs  $54.36 \pm 17.90$  pg/mL,  $p=0.031$ ). Our finding was consistent with study from Haque et al (2017) that there was an inverse correlation between HbA1c and iPTH levels<sup>26</sup>.

Paula et al, Murakami et al, Dan S et al, and Reis et al demonstrated that poor glycemic control could decrease PTH secretion<sup>9,26-29</sup>. Hypoparathyroid condition was found in 10 (40%) patients with controlled DM and 11(25%) patients with uncontrolled DM. This finding was consistent with Ca ion levels which was lower in patients with controlled DM ( $p=0.033$ ). Hypocalcemia was found in 12(48%) patients with controlled DM and in 13(29.5%) patients with uncontrolled DM. Higher hyperparathyroid and hypocalcemia conditions in patients with controlled DM could be due to higher mean age and possibility longer duration of diabetes in patients with controlled DM than those with uncontrolled DM. Diabetes causes homeostasis disorders of Ca and affects PTH secretions, this caused the differences of iPTH levels between both groups<sup>8,28,29,30</sup>. Ca ions are active free calcium levels and more useful to measure Ca function in blood<sup>30,31</sup>. Age, sex, and also pregnancy can affect Ca ion levels. Hassan et al (2016) showed differences Ca levels between patients with DM and healthy control<sup>31,32</sup>.

The transfer of calcium ions may cause opening of the voltage-gate  $\text{Ca}^{2+}$  channel and release of insulin, which activate with stimulation of blood glucose. Increase of intracellular calcium ( $\text{Ca}^{2+}$ ) can rapidly trigger insulin release. The role of  $1.25(\text{OH})_2\text{D}_3$  in insulin secretion comes from its effects on  $\text{Ca}^{2+}$  influx, mobilization, and buffering in pancreatic cells<sup>17,32,33</sup>. Ca is also an electrolyte that plays an important role in regulating PTH secretion, low Ca levels will increase PTH level production. This role also mediated by vitamin D<sup>17,33,34</sup>.

Deficiency and insufficiency 25(OH)D level were found in 54 patients with DM in both groups, which might cause comparable 25(OH)D levels between two groups.

This result was consistent with study by Alhumaidi et al that stated that most study population (98.5%) in Saudi Arabia showed vitamin D deficiency in both groups, DM and healthy group, however they also showed that 25(OH)D levels was lower in healthy control than in DM group.<sup>(34,35)</sup> Study by Indra TA et al in Indonesia showed that 49% patients with DM were presenting with vitamin D deficiency<sup>35,36</sup>. Meanwhile, study by Kumar et al showed that there was no difference in 25(OH)D levels between healthy control and DM patients<sup>34,36,37</sup>.

Another possible conditions affecting on 25(OH) levels are poor intake of vitamin D, old age, and lack of UV exposure in all study subjects. All patients were >1 year duration of DM and with age range between 37–75 years old. Older age in patients with controlled DM could represent on the possibility of longer duration of DM that resulted on the lower 25(OH)D levels in this group. Another possible reason was that HbA1c levels represented blood glucose control within 3 months, while vitamin D deficiency was also process that required a longer period.

Another condition can such as hypertension in both groups (80% in patients with controlled DM and 90.9% in those with uncontrolled DM) might cause deficiency in both groups.

Our study did not explore further about duration of DM, exposure of UV or sunlight, physical activity, food and beverages intake containing vitamin D or calcium, and microvascular or macrovascular complication of DM. Our study included wide range of age (37–75 years). The presence of other diseases were not explored further.

## CONCLUSION

Higher iPTH levels and lower Ca ion levels were found in controlled DM group. Both iPTH and Ca ion might impact on therapeutic monitoring in patients with DM.

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