Korelasi antara kadar Osteocalcin, HbA1C dan 25(OH)D pada Anak dengan Diabetes Melitus Tipe 1

The Correlation between Osteocalcin, HbA1C and 25(OH)D Levels in Type1 Diabetes Melitus Children

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ABSTRAK

Masalah yang terjadi di banyak negara salah satunya adalah Diabetes Melitus tipe 1. Prevalensi DM tipe 1 di Indonesia masih belum jelas. DM tipe 1 disebabkan berkurangnya sekresi insulin akibat kerusakan fungsi sel β pankreas oleh respon autoimun. Diabetik osteopati merupakan salah satu komplikasi DM, yaitu peningkatan terjadinya osteoporosis. Penelitian ini bertujuan mencari korelasi antara kadar 25(OH)D₃, kontrol glikemik dengan mengukur kadar HbA1c dan osteocalcin, sebagai penanda biokimia struktur tulang. Dua puluh enam anak dengan DM tipe 1 adalah sebagai subjek penelitian. Data dianalisis secara statistik dengan menggunakan Shapiro Wilk, korelasi Pearson dan Spearman. Karakteristik sampel menunjukkan jenis kelamin didominasi perempuan 58% dan didominasi dengan gizi baik (73%). Status vitamin D didominasi defisiensi (61,5%). Kadar HbA1C <7,5 % sebanyak 27%. Rerata usia pasien adalah 12,31 ± 3,069 tahun, dosis insulin 1,17±0,233 IU, kadar GDP 115,08±46,742 mg/dL, dan kadar GDS 144,65±76,365 mg/dL. Tidak didapatkan korelasi yang signifikan antara kadar HbA1c (p=0,472, r=-0,148) dan osteocalcin (p=0,407, r=0,17) dengan kadar 25(OH)D₃. Kesimpulan tidak diapatkan korelasi antara kadar osteocalcin, kadar HbA1c, dan kadar 25(OH)D₃.

Kata Kunci: DM tipe 1 anak, HbA1C, osteocalcin, vitamin D

ABSTRACT

One of problems that occurs in many countries is Diabetes Melitus type 1. The prevalence of DM type 1 is unknown. DM type 1 is caused by decreasing insulin secretion due to β cells disfunction as results of autoimmune response. Diabetic osteopathy is one of complications, i.e. osteoporosis incidence increases. This study aims to examine the relationships of 25(OH)D levels, glycemic control by measuring the HbA1c and Osteocalcin level as a biochemical marker of bone structure. Using 26 childrens with DM type 1 as the subjects, Osteocalcin, HbA1c and 25(OH)D levels were evaluated. Data were statistically analyzed using Shapiro Wilk, Pearson and Spearman correlation. Characteristics of the sample showed predominantly female 58% (15/26) and well nourished 73% (19/26). Vitamin D statuses predominantly were deficient, 61.5% (16/26). Levels of HbA1c <7.5% were found in 27% (7/26). On average, the age was 12.31±3.069 yo, dose of insulin 0.233±1.17IU, fasting blood glucose level 115.08±46.742mg/dL, and random blood glucose 144.65±76.365mg/dL. There was negative correlation between 25(OH)D, and HbA1c levels (p=0.472, r=-0.148), positive correlation between 25(OH)D, and osteocalcin levels (p=0.407, r=0.17), and negative correlation between osteocalcin and HbA1c levels (p=0.69, r=-0.082), however all was not significant statistically. As conclusions there was no correlation between osteocalcin, HbA1c and 25(OH)D levels.

Keywords: HbA1C, osteocalcin, type1 DM children, vitamin D
INTRODUCTION

Type 1 diabetes mellitus, a problem that occurs in many countries, is one of the chronic diseases that until now has remained incurable. Along with the advances in medical science, the life quality of patients with type 1 diabetes can be improved through adequate management (1). This disease is a chronic disease of carbohydrate, fat, and protein metabolism caused by insulin deficiency due to autoimmune pancreatic beta cell destruction. Type 1 DM usually starts in children aged four years or more with a peak incidence at the age of 11-13 years (adolescence and puberty) (2-4). In 2011, the number of patients with type 1 DM was as many as 720 in Indonesia, consistent with the source of the Child Endocrinology Coordination Unit of the Indonesian Medical Association Center (1). Saiful Anwar Hospital (RSSA) Malang obtained 47 patients in 2005-2012 (5).

Management of type 1 DM in children includes glycemic control and complication prevention, as well as evaluation on child growth and development (4-6). This starts to be a consideration about the occurrence of diabetic osteopathy, which is one of the complications of the progression of diabetes mellitus characterized by an increased risk of fractures and osteoporosis, associated with impaired bone metabolism as a feedback mechanism from hyperglycemia, inflammation, and insulin deficiency (7-9). If there is a poor glucose control in type 1 DM, it also improves a poor prognosis, one of which is characterized by osteopenia and osteoporosis. Vitamin D directly stimulates osteocalcin transcription, while vitamin K regulates the carboxylation process (10,11).

Previous studies have shown the involvement of vitamin D in the modulation of the immune system and bone turnover in type 1 DM (7,10). This prompted the researchers to conduct this experimental research. Research to assess the levels of osteocalcin, 25(OH)D$_3$, and HbA1c in children with type 1 DM and the their relationship is important to do.

METHOD

Research design

This research was a cross-sectional study to assess the levels of osteocalcin, 25(OH)D$_3$, and HbA1c in children with type 1 diabetes mellitus. The study was conducted in the Department of Child Health Science at Saiful Anwar Hospital Malang, Clinical Pathology Laboratory Saiful Anwar Hospital Malang, and Physiology Laboratory Faculty of Medicine, Brawijaya University Malang in February 2016, the study was approved by the Research Ethics Committee of Saiful Anwar Hospital Malang. In addition, approval from parents of patients or guardians was also obtained for involving in the research (informed consent).

This study involved 26 samples, the research subjects were children diagnosed with type 1 diabetes mellitus and were undergoing outpatient care at the Endocrinology Unit Saiful Anwar Hospital Malang during the study period, as well as meeting the inclusion and exclusion criteria. Inclusion criteria were type 1 DM classified, aged 3 to 18 years, no contraindications in blood collection, patient parents’ permission to allow their children to get involved in the research (informed consent). Exclusion criteria were the presence of other autoimmune diseases such as severe infections, liver disorders, impaired renal function, and clinical and simple laboratory anemia.

The criteria of type 1 DM were as follows: (1) symptoms of hyperglycemia including polyuria, polydipsia, weight loss and random blood plasma glucose level > 200 mg/dL (11.1 mmol/L), (2) fasting blood glucose (> 8 hours) ≥126 mg/dL (≥7.0 mmol/L), (3) in patients with asymptomatic, random blood glucose level was found >200 mg/dL or the fasting blood glucose level was higher than normal using more than one examination of impaired glucose tolerance test (12-14).

Osteocalcin was measured from plasma using the ELISA method. The results of osteocalcin measurements were expressed in levels (quantitative) in ng/mL unit. The kit used was a product of Elabscience catalog number E-EL-H1343. Normal osteocalcin level was expressed using 1.25-80 ng/mL. The examination was carried out at Malang Physiology Laboratory. HbA1c was glucose bound to hemoglobin (glycated hemoglobin) to see the average blood glucose level over a period of 6 months, in percentage, it was said to be normal if <7.5%. The examination was measured by using Bio-Rad D-10. The level of 25(OH)D$_3$ was measured in plasma using the Enzyme-linked Immuno Assay (ELISA) method. Using the Alegria Human Vitamin D kit catalog number ORG 270 in units of ng/mL. Normal vitamin D level was expressed by serum 25(OH)D$_3$ level >30ng/ml, insufficiency if 21-29 ng/ml, and deficiency if <20 ng/ml. Both were carried out at Pathology Central Laboratory of Dr. Saiful Anwar General Hospital Malang.

Statistical analysis (SPSS ver 17.0) was performed using the Shapiro-Wilk test, followed by the correlation test. Because the data were not normally distributed, the Spearman test was used.

RESULTS

Characteristics of Research Sample

The characteristics of the study sample are presented in Table 1, which includes age, gender, vitamin D status, insulin dose, HbA1c level, osteocalcin level, fasting sugar level, random sugar level, and nutritional status.

Table 1 shows that the gender was dominated by girls as much as 58% (15/26), mostly in good nutrition as much as 73% (19/26). Vitamin D status was dominated by deficiency and efficiency (61%) of children. HbA1c level <7.5% was as many as 7 children, while> 7.5% was as many as 19 children. The average of age, insulin dose, GDP level, and GDS levels were 12.31 ± 3.069 years, 1, 17±0.233 IU, 115.08±46,742 mg/dL, and 144.65 ± 76,365 mg/dL, respectively.

Table 1. Characteristics of the study sample

<table>
<thead>
<tr>
<th>Sample characteristics</th>
<th>Total</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
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<tr>
<td>Male</td>
<td>42%</td>
</tr>
<tr>
<td>Female</td>
<td>58%</td>
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</tbody>
</table>
Correlation between $25(OH)D_3$ level and HbA1c level

Data normality test using the Shapiro Wilk showed that the data were normally distributed with each significance value $\alpha$ greater than 0.05. Pearson correlation test was conducted and obtained a significance value greater than $\alpha$ ($p = 0.472, r = -0.148$). This showed that there was no significant correlation between $25(OH)D_3$ level and HbA1c level. The correlation graph in Figure 1 showed a negative or reversing and weak correlation between $25(OH)D_3$ level and HbA1c level.

Correlation between $25(OH)D_3$ level and Osteocalcin level

The results of the normality test with the Shapiro Wilk showed that the data of HbA1c level variable were normally distributed with a significance value greater than 0.05. The data of osteocalcin level variable were not normally distributed with a significance value smaller than 0.05. Then, Spearman correlation replacement test was carried out and obtained a significance value greater than $\alpha$ ($p = 0.690, r = -0.082$). There was no significant correlation between HbA1c level and osteocalcin level. The correlation graph in Figure 3 shows no correlation between HbA1c level and Osteocalcin level.

DISCUSSION

This cross-sectional study was conducted to assess the levels of osteocalcin, $25(OH)D_3$, and HbA1c in children with type 1 DM. The study involved 26 type-1 DM children as research subjects. Vitamin D is recognized as a hormone synthesized in the human body and is active in other organs through the vitamin D receptor (VDR). Circulating vitamin D2 (VD2) or $25(OH)D_3$ is an indicator of vitamin D status. In this study, $25(OH)D_3$ levels were used to determine vitamin D status, also in type 1 DM children (12).

The basic characteristics of the subject showed more females (15/26). In accordance with previous studies, the ratio of male was fewer than female 45:55 in the incidence of type 1 DM in Indonesia (1). In 2011 data from IDF...
contradicted this study, there were more men than women as much as 1.5 times. Based on ISPAD data in the same year, gender differences in the incidence of type 1 DM did not occur in the whole worldwide (5). The existence of this difference is caused by differences in population, race, and the number of research subjects (13,14). This result was similar to epidemiological data that type 1 DM has a gender prevalence between women and men 1:1 (15).

Our study was dominated with good nutrition, as many as 19 people. Type 1 DM is caused by a decrease in insulin secretion that occurs due to autoimmune processes rather than due to a decrease in insulin sensitivity as occurs in other types of DM, so nutritional status does not have a direct effect (16). Various complex factors can affect puberty, resulting in physical and psychological changes due to sequential and regular changes in endocrine activity. In the United States, most girls will experience puberty at the age of 8-13 years, while boys at 9-14 years (17,18). To achieve optimal metabolic control, it is necessary to adjust the right dose of insulin, so the risk of hypoglycemia can be avoided and the patient’s quality of life can be increased. At the age of puberty, it is affected by the "honeymoon" period of daily insulin doses of <0.5IU/kgBW/day in this period, children before puberty (before the "honeymoon" period) the dose range is 0.7-1.0IU/kgBW/day. During puberty, the need increases above 1 IU up to 2IU/kgBW/day (1,17,18). This is a consideration for the research subjects so that the mean age of 12.31 ± 3.069 years becomes the age of puberty, and an average insulin dose of 1.17 ± 0.233IU/kgBW/day is needed.

The study showed that 25(OH)D levels were dominated, both deficiency and insufficiency, vitamin D deficiency as much as 38.5% (<20 ng/mL) and vitamin D insufficiency as much as 23.1% (21-29 ng/mL) (19,20). This study was in accordance with a cross-sectional study in the Netherlands, vitamin D deficiency happened among 60-84% of type 1 DM patients (21). A cross-sectional study in Saudi Arabia found a low level of 25(OH)D, in 77% of type 1 DM children (20). Likewise, case-control research in North India, 58% of patients with type 1 DM and 32% of healthy controls showed 25(OH)D deficiency (22). A cross-sectional study in America showed 15% of patients with 25(OH)D deficiency, 61% with insufficient level of 25(OH)D, and 24% sufficient that 77% in type 1 DM children (23).

The difference in the value of vitamin D deficiency in type 1 diabetes mellitus was caused by various reasons, including those that can affect daily diet or lack of sun exposure. In children, the most common cause of vitamin D deficiency is breastfeeding without vitamin D supplementation. This explains the reason for differences in vitamin D levels that cause malabsorption in type 1 DM. Vitamin D can prevent islet cell damage, which is related directly to beta cell dysfunction, hypoavitaminosis vitamin D, and insulin feedback resistance. Increased catabolism of vitamin D can be caused by the consumption of drugs such as anticonvulsants, glucocorticoids, and HAART (AIDS therapy). Furthermore, there can be a relationship between HbA1c and vitamin D because of its effect on beta cells, the inflammatory system, and insulin activity (20,24).

Locus HLA-DR and HLA-DQ in class II region are considered to have a risk of type 1 DM occurrence (25). In addition, certain genetic influences and different environmental influences can affect vitamin D level (20,26). From several other studies, poor nutrition and ineffective condition of children with type 1 diabetes are directly related to blood sugar levels. Glycated hemoglobin (HbA1c) is glycemic control, describing the history of glucose control in the last 90-120 days which corresponds to the mean age of erythrocytes (27). Our study showed that most HbA1c levels with a value of >7.5%, a mean score of 73.1% of our study subjects, follow poor glycemic control. This can affect the occurrence of complications in children with type 1 diabetes mellitus. In a certain period, poor glycemic control directly affects the inhibition of osteoblast activity and function. This causes diabetic complications of osteopathy, namely osteoporosis or an increased risk of fractures through the consequences of hyperglycemia, inflammation, and insulin deficiency (8,28).

Our study showed that 25(OH)D level did not significantly influence HbA1c (p = 0.472, r = -0.148). The results of our study were in accordance with other study conducted on 68 children of type 1 DM aged 4-21 years by Branco et al. that proved an insignificant correlation between HbA1c level and serum 25(OH)D level (29). Research conducted by Magee et al. on the relationship between 25(OH)D level and HbA1c level found a negative correlation. This means that the higher the level of 25(OH)D, the lower the level of HbA1c. A similar study was also in accordance with Tunc et al. that proved the inverse relationship between 25(OH)D level and insulin requirements in patients with type 1 DM (30). The mean score of HbA1c level in our study with 1.17 ± 0.233U/day insulin therapy showed that glycemic control was still poor. Osteocalcin level in our study with an average of 49.17ng/mL, with a mean age range of 12.31 ± 3.069 years, was still fairly normal. In accordance with the study of Seydewitz et al. compared with the levels of osteocalcin in healthy children, the levels in children with type 1 diabetes mellitus were lower according to their age. This is also influenced by the duration of insulin use. Osteocalcin is used as a biomarker of bone mineral levels, an indicator of decreased bone structure or turnover in diabetes (10).

The journey of diabetes is associated with long-term complications that can affect quality and life expectancy. Several recent studies have shown levels of osteocalcin, which consists of an array of 49 amino acids, bone protein matrix, are a biochemical marker of bone structure. The results of this study indicate that 25(OH)D level has no significant effect on osteocalcin level. This is caused by vitamin D through vitamin D receptors in osteoblasts that play a role in the formation of osteocalcin. Similarly, in the study of Napoli et al. in 27 children with type 1 DM, where no significant correlation was found between vitamin D and osteocalcin (31).

The results of our study also showed no significant correlation between HbA1c level and osteocalcin level in type 1 DM children. This is because in the population of children with type 1 DM, undercarboxylated osteocalcin (uc-Oc) levels were associated with increased insulin secretion and high leptin concentration, if the level of
osteocalcin is low, it causes disruption of fasting blood sugar and a decrease in glucose tolerance (31). Research by Maddaloni et al. on 93 samples, with low osteocalcin level with an average of 21 ± 13.3ng/mL, obtained a negative correlation between osteocalcin and HbA1c levels but had a significant correlation between osteocalcin and HbA1c levels. This is related to the duration of illness in type 1 DM and poor glucose control that affects osteoblast activity and bone metabolism (8).

The limitations of this study were that the number of subjects was small due to the limitations of the sample so that it is unable to describe vitamin D status, levels of osteocalcin and HbA1c in type 1 DM patients. This study used a cross-sectional design with sample taken and analyzed at one time thus could not determine the interaction of these three parameters (causal relationship), considering type 1 DM is an autoimmune disease and is chronic. The vitamin D variable used in this study was not an active form of vitamin D, which affects the activity of the autoimmune process which must be preceded by metabolism in the kidneys. In addition to exposure to sunlight, ethnicity/race, body surface area, and low vitamin D diet, drugs can affect vitamin D status. The complicating factors that may arise are still not as controlled as possible, such as, for example, HbA1c level that is influenced mostly by several factors, including levels of iron, vitamin B12, folic acid. The osteocalcin variable that we measured was only total osteocalcin instead of uc-Oc, where uc-Oc described the active metabolism of osteocalcin in the regulation of energy metabolism, besides that it was also influenced by Body Mass Index (BMI) and calcium levels in the regulation of osteocalcin production. This study did not classify deficiencies and insensitivity from vitamin D levels. For the future studies, to determine the prognosis of diabetic osteopathy in addition to the measurement of chemical biomarkers, it is necessary to develop a measurement of Bone Mineral Density (BMD), BMI, and increase in population. Several other factors that can influence the results but have not been carried out research include Treg and Th17.

This study concludes that no significant correlation is obtained from the three parameters, namely HbA1c, osteocalcin, and 25(OH)D levels in type 1 DM children.

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