INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 157
2021 March; 27(2): 157-163
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Plasma Osteopontin Correlates with Glycemic Control in Type 2 Diabetes Mellitus Patients

Maria Immakulata Diah Pramudianti, Briggite Rina Aninda Sidharta, Josua Sinambela, Medityas Winda Krissinta

Department of Clinical Pathology, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi General Hospital, Surakarta, Indonesia. E-mail: mi_diahp@yahoo.co.id

ABSTRACT

Diabetes Mellitus (DM) is a metabolic disease characterized by hyperglycemia due to abnormal secretions and/or insulin activity. Osteopontin (OPN) is an important component of inflammation and insulin resistance, and vitamin D decreases insulin resistance. This study aimed to analyze the correlation between OPN and glycemic control and total 25-OH vitamin D in type 2 DM. An observational analytic study with a cross-sectional approach was performed in Dr. Moewardi Hospital, Surakarta, from May to September 2018. Plasma OPN levels were measured by a sandwich enzyme immunoassay kit from Elabscience 96T Human OPN (USA), and a total of 25-OH vitamin D was evaluated using the ELFA method from Biomerieux SA (France). Data were tested by Pearson correlation (r). Type 2 DM subjects consisted of 45 (54.2%) males and 38 (45.8%) females, 36 (43.45%) well- and 47 (56.65%) poorly-controlled. The average age was 56.81±9.76 years old. The mean of OPN level in poorly-controlled cases was significantly higher (20.27±3.20 ng/mL) than well-controlled ones (15.04±3.34 ng/mL) with p=0.001. There was no significant difference in total 25-OH vitamin D between well- and poorly-controlled groups (19.84±6.65 vs. 17.24±6.78 ng/mL, respectively, p=0.085). The correlation of OPN with glycemic control (fasting glucose, 2-hour post-prandial glucose, HbA1c) and total 25-OH vitamin D in all subjects with type 2 DM were r=0.241 (p=0.028), r=0.378 (p=0.0001) r=0.529 (p=0.0001) and r=-0.151 (p=0.173), respectively. This study suggested that plasma OPN level was correlated with glycemic control but not with serum total 25-OH vitamin D in type 2 DM. Further research was needed in populations of other types of DM and other research variables related to inflammation or insulin resistance.

Keywords: Type 2 DM, osteopontin, total 25-OH vitamin D

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic diseases with hyperglycemia characteristics due to abnormal secretion and/or activity of insulin with multifactorial causes, either various genetic and environmental factors leading to progressive loss of beta-cell mass and or function.[1] The prevalence of type 2 DM worldwide remains to escalate. The primary health study in 2018 conducted by the Indonesian Ministry of Health reported that the prevalence of DM in people over 20 years old in urban areas was 14.7% and in rural area was 7.2%.[2]

The pathogenesis of type 2 DM is very complex and involving the interactions of genetic and environmental factors. The genetic factors consist of polygenic and monogenic, whereas the environmental factors include excessive caloric intake, obesity, and lack of physical activities. The clinical symptoms of type 2 DM are heterogeneous with a wide range of age, severe clinical conditions relevant to the hyperglycemic level, and the degree of obesity. Type 2 diabetic patients demonstrate three significant abnormalities, i.e.: Peripheral tissues insulin resistance, mainly in muscle, fat, and liver; Insulin secretion disorder, particularly in response to glucose stimulation; Increased glucose production by the liver. Other abnormalities are lipolysis of fat adipocytes, deficiency and resistance of incretin hormones, hyperglucagonemia, increased reabsorption of renal tubular, and metabolic regulation of the central nervous system.[3]

Chronic hyperglycemia in type 2 DM will cause oxidative stress, mitochondrial dysfunction, and insulin resistance through Protein Kinase C (PKC), Advanced Glycation Endproduct (AGE), hexosamine, and polyol or Aldose Reductase (AR) pathways. Chronic hyperglycemia directly increases Reactive Oxygen Species (ROS), Tumor Necrosis Factor (TNF)-α, Nuclear Factor (NF)-κB and decreases Nitric Oxide (NO), leading to β-cell dysfunction, and stimulate microvascular and macrovascular complications, which can increase the mortality and morbidity of patients. Based on Perkeni's (2019) criteria, DM is controlled by laboratory tests of

glucose level, HbA1c (< 7%), and lipid profiles.[1,2,4,5] Fracture risk was higher in subjects with DM compared with those without DM. It is necessary to assess older patients with DM for fracture risk history and recommend examination of Bone Mineral Density (BMD). When eGFR is < 60 mL/min/1.73 m^2, screening for complication of metabolic bone disease in Chronic Kidney Disease (CKD) is indicated by laboratory test of serum 25-OH vitamin D. Fractures prevention strategies for DM patients are similar to that of the common population and include vitamin D supplementation.[1]

Osteopontin (OPN) or also known as secreted phosphoprotein-1 (SPP1), is a specific phosphoprotein transformation with the molecular weight of 60 kiloDalton (kDa) and bound with ~314 amino acid residue. The experimental study reported that OPN was an essential component in the development of insulin resistance. In human fat cells, OPN destructs the differentiation and sensitivity of primary fat cells insulin as expressed by the peroxisomal proliferator-activated receptor-gamma gene, adiponectin, and insulin, which stimulates glucose uptake. However, further study is needed to analyze OPN involvement in the pathogenesis of type 2 DM.[6-10] As a bone-bridging protein related to bone remodeling and tissue calcification, OPN is a calcium-binding site, which plays a role in the adhesion and resorption of osteoclast. Osteopontin expression is regulated by vitamin D [1,25 (OH)2D3], which can increase OPN synthesis through osteoblast cells by compressing collagen production; this vitamin D can enhance OPN and osteocalcin secretion for bone mineralization. Osteopontin binds integrin receptors in osteoclasts by the arginine-glycine-aspartate (RGD) sequencing, activating the pathway of phospholipase C osteoclast and increasing intracellular calcium. Osteopontin deficiency results in a 30% decline in bone fracture, suggesting the vital role of OPN in the prevention of bone fractures.[11-14]

Vitamin D is a pro steroid hormone dissolving in fat. There are two forms of vitamin D, i.e. vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Total 25-OH vitamin D3 is a primary element of vitamin D circulating in the blood. Once vitamin D gets into circulation through skin or lymph, it will be cleaned by heart or storage tissue in a few hours. In the liver, precalciferol is quickly hydroxylated by 25-hydroxylase, a cytochrome P450 enzyme (mainly CYP2R1), so that 25-hydroxyvitamin D [25(OH)2D; calcidiol] through an unregulated process. Once synthesized, DBP-bound 25-OH vitamin D is secreted into the blood, and it needs a renal hydroxylation to obtain the active form 1α, 25 dihydroxy vitamin D (calcitriol). 25-OH vitamin D metabolite is a primary circulating type of vitamin D, and it is the last metabolite converted to be an active form. Serum 25-OH vitamin D level resembles a reserve in the skin, diet, and an essential biomarker of vitamin D status. The half-life of plasma 25-OH vitamin D is about three weeks; it indicates that serum 25-OH vitamin D level reflects the reserve and vitamin D status in the body. The level of vitamin D are divided into four categories, i.e. deficiency (< 20 ng/mL), insufficiency (21-29 ng/mL), normal (30-150 ng/mL) and excess (> 150 ng/mL) of 25-OH vitamin D.[15-17]

Vitamin D decreases insulin resistance and increases insulin sensitivity. Vitamin D deficiency is also a risk factor for Impaired Glucose Tolerance (IGT).[17] Prospective observational study reported that vitamin D played a role in preventing cancer, immunity, diabetes, cardiovascular and muscle disorders. 1,25(OH)2D can induce human insulin receptor gene transcription in U-937 human promote cells. The activation of vitamin D [as 1α, 25(OH)2D3] may take part in pancreatic beta-cell function, insulin sensitivity, peripheral cell targets, and indirectly involved in systemic inflammation.[18]

This study aimed to investigate the correlation between plasma OPN and total serum 25-OH vitamin D in type 2 DM patients with poor and well glycemic control.

METHODS

This observational analytical study with a cross-sectional approach was conducted in patients with type 2 DM who underwent laboratory tests in the Clinical Pathology Department of Clinical Pathology at Dr. Moewardi Hospital, Surakarta, from May to September 2018. Only adult patients with type 2 DM were included in the study. Type 2 DM patients who presented bone disorder or malignancy, received vitamin D therapy, liver and kidney disease were also excluded from the study. The study subjects were chosen consecutively, and 83 patients were obtained.

Fasting glucose, 2-hour post-prandial glucose, HbA1c, plasma OPN, and total serum 25-OH vitamin D levels were measured with ADVIA 1800 chemical analyzer, High-Performance Liquid Chromatography (HPLC) with ADAMS TM A1c instrument, a human OPN Sandwich Enzyme Immunoassay (ELISA) kit (Elabscience 96T, USA) read with Rayto RT-2100C, and an ELFA method using Vidas (Biomerieux SA, France).

The subjects were divided into two groups, such as well-controlled and poorly-controlled type 2 DM (HbA1c < 7% and ≥ 7%, respectively). The continuous data were presented in the mean and Standard Deviation (SD). The categorical data were presented in percentage (%). The normality of the data was evaluated using the Kolmogorov-Smirnov test (p > 0.05 was significant). The mean comparison of two variables was analyzed with independent-samples T-test for normally-distributed data, whereas the Mann-Whitney test was used for data with a normal distribution. The correlation was analyzed with Pearson correlation test (r), a p-value of < 0.05 was considered significant with a confidence interval of 95%. This study was approved by the local Ethics Committee of Dr. Moewardi Hospital with number 550/IV/HREC/2018, and written informed consent was obtained from all of the study subjects.

RESULTS AND DISCUSSIONS

In total, eighty-three patients with type 2 DM were included in this study, consisting of 45 (54.2%) males and 38 (45.8%) females. Normality test showed normal distribution for the variables of age, height, weight, body mass index, waist circumference, fasting blood glucose, 2-hour post-prandial blood glucose, and HbA1c. In contrast, the duration of DM, systolic, and diastolic blood pressure were not normally distributed.

Among the subjects, 47 (56.65 %) were in poorly-controlled, and 36 (43.45 %) were in the well-controlled group. The mean age was 56.81±9.76 years old. The median duration of DM was 8 (5-19) years. The means of height, weight, BMI, and waist circumference were 158,87±6.47 cm, 66.25±10.82 kg, 26.24±3.96 kg/m^2, and 85.81±9.94 cm, respectively. The mean±SD of BMI in poorly-controlled type 2 DM subjects was significantly higher than in well-controlled ones (27.07±4.26 vs. 25.15±3.28 kg/m^2, p=0.027). The means of systolic and diastolic blood pressure were 120 (100-160) mmHg and 80 (60-100) mmHg, respectively. The means of fasting blood glucose, 2-hour post-prandial blood glucose, and HbA1c levels were 167,22±52.38 mg/dL, 196,82±61.74 mg/dL, and 7.74±1.67%, respectively.

In subjects with poorly-controlled type 2 DM, the levels of fasting blood glucose, 2-hour post-prandial blood glucose and HbA1c were significantly higher than well-controlled type 2 DM subjects (182,51±58.05 vs. 147,25±35.50 mg/dL, p=0.002; 220,91±67.06 vs. 165,36±34.97 mg/dL, p=0.001; and 8.84±1.43 vs. 6.32±0.41 mg/dL, p=0.001; respectively). The characteristics of all subjects were shown in Table 1.

Table 1. Basic characteristics of subjects

Variables	Type 2 DM			p
	Total n=83 (100%)	Well-Controlled n=36 (43.4%)	Poorly-Controlled n=47 (56.6%)	
Age (years)*	56.81±9.76	57.94±9.77	55.94±6.76	0.356
Gender, n (%)$				0.227
Male	45 (54.2)	23 (64.9)	22 (46.8)	0.881
Female	38 (45.8)	13 (36.1)	25 (53.2)	0.052
The duration of DM (years)$	8 (5–19)	8 (5–16)	8 (5–19)	0.499
Height (cm)*	158,87±6.47	159,42±6.22	158,45±6.70	0.502
Weight (kg)*	66.25±10.82	64.03±9.85	67.96±11.33	0.101
BMI (kg/m^2)*	26.24±3.96	25.15±3.28	27.07±4.26	**0.027**‡
Waist circumference (cm)*	85.81±9.94	85.19±9.85	86.29±10.09	0.623
Blood pressure (mmHg)$				
Systolic	120 (100–160)	120 (100–160)	130 (100–150)	0.054
Diastolic	80 (60–100)	80 (67–100)	80 (60–95)	0.710
Fasting blood glucose (mg/dL)*	167,22±52.38	147,25±35.50	182.51±58.05	**0.002**‡
2-hour post-prandial blood glucose (mg/dL)*	196.82 ± 61.74	165,36±34.97	220.91±67.06	**0.001**‡
HbA1c (%)*	7.74 ± 1.67	6.32±0.41	8.84±1.43	**0.001**‡

* Data were normally distributed, Mean±SD, independent-samples T-test. $ Data were not normally distributed, median (minimum-maximum), The Mann-Whitney test. $ Chi-Square. ‡Significant if p < 0.05, confidence interval 95%.

Well-controlled= HbA1c < 7%, poorly controlled= HbA1c ≥ 7, n= number, %= percentage, SD= Standard Deviation, DM= Diabetes Mellitus, BMI= Body Mass Index, cm= centimeter, kg= kilogram, m^2= cubic meter, mmHg= milimeter mercury, mg= miligram, dL= deciliter

Table 2. Comparison of research variables

Variables	Type 2 DM			p
	Total	Well-Controlled	Poorly-Controlled	
Osteopontin (ng/mL)	18.00±4.16	15.04±3.34	20.27±3.20	**0.001**‡
Total 25-OH Vit D (ng/mL)	18.37±6.81	19.84±6.65	17.24±6.78	0.085

‡ Data were normally distributed, mean±SD, the comparison of well-and poorly- controlled type 2 DM was tested with independent-samples T-test, significant if p < 0.05.
Well-controlled=HbA1c < 7%, poorly-controlled=HbA1c ≥ 7, SD=Standard Deviation, ng=nanogram, mL=mililiter

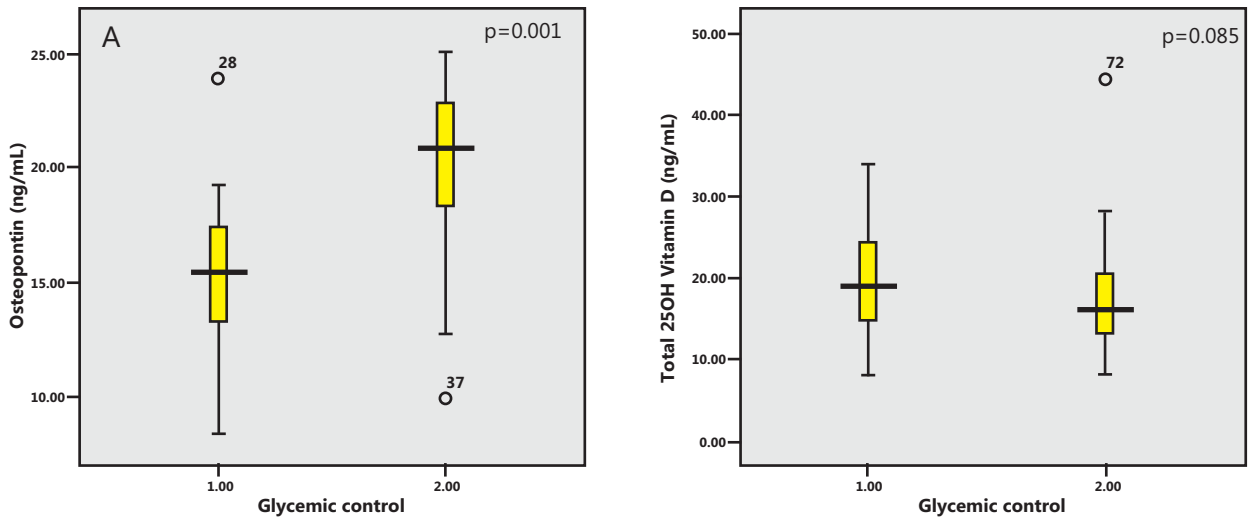

Figure 1. Box plot representing the comparison of plasma OPN (A) and total 25-OH vitamin D (B) in type 2 DM according to glycemic control, 1.00=well-controlled, 2.00=poorly-controlled type 2 DM

Table 3. Correlation between plasma osteopontin and research variables in type 2 DM according to glycemic control

Variable	Type 2 DM					
	Total		Well-Controlled		Poorly-Controlled	
	r	p	r	p	r	p
Total 25-OH vitamin D	- 0.151	0.173	- 0.087	0.612	- 0.006	0.970
Fasting glucose	0.241	**0.028**‡	-0.219	0.200	0.170	0.253
2-hour post-prandial glucose	0.378	**0.0001**‡	-0.249	0.143	**0.309**	**0.035**‡
HbA1c	0.529	**0.0001**‡	-0.204	0.234	0.204	0.169

‡ Pearson correlation, significant if p < 0.05

The means of plasma OPN and total 25-OH vitamin D levels were 18.00±4.16 ng/mL and 18.37±6.81 ng/mL, respectively. In poorly-controlled type 2 DM subjects, the plasma OPN level was significantly higher than the well-controlled group (20.27±3.20 vs. 15.04±3.34 ng/mL, respectively, p=0.001). The level of total 25-OH vitamin D was not significantly higher in the well-controlled group (19.84±6.65 ng/mL) than the poorly-controlled group (17.24±6.78 ng/mL) with p=0.085 and most of the subjects with vitamin D deficiency (Table 2).

The means comparison of plasma OPN level with serum total 25-OH vitamin D in well-controlled and poorly-controlled type 2 DM were shown in Figures 1A and 1B. Graph correlation of plasma OPN levels and research variable in total subject with type 2 DM were shown in Figure 2 (A, B, and C). There was a correlation between OPN and 2-hour post-prandial glucose in poorly-controlled subjects [r=0.309 (p=0.035)]. This finding was shown in Figure 2D.

Type 2 DM is a group of metabolic diseases involving genetic and environmental factors,

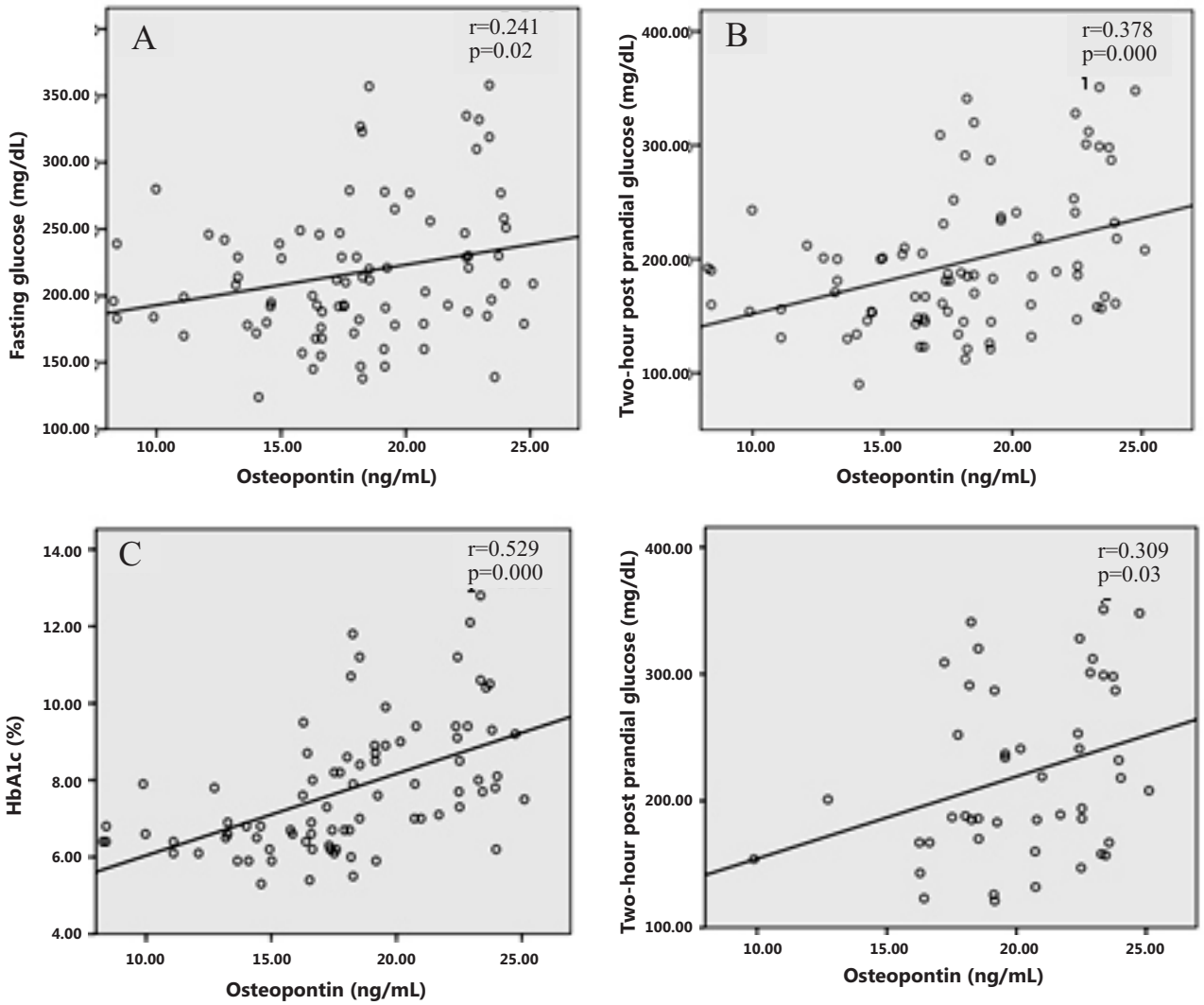

Figure 2. Graph correlation of plasma OPN levels in total subject with type 2 DM (A=fasting glucose, B=2-hour post-prandial glucose, C=HbA1c), and graph correlation of plasma OPN levels and 2-hour post-prandial glucose in poorly-controlled type 2 DM (D).

demonstrating insulin resistance and disorder of its secretion.[3] This study showed that the mean±SD of age in type 2 DM subjects was 56.81±9.76 years old. This result was in line with a study by Berezin and Kremzer, which observed the role of OPN as a coronary vascular calcification marker of type 2 DM patients and found that the mean age of patients with type 2 DM was 59.10±2.80 years old.[19]

Osteopontin is also called early T-lymphocyte cell activation gene-1 (Eta-1) or sialoprotein-1, which is part of small integrin-binding ligand N-linked glycoprotein (SIBLING) family, mainly involved in bone metabolism and plays a role in inflammation by increasing the production of Interferon Gamma (IFN-γ), Interleukin (IL)-12, IL-17, and inhibition of IL-10 expression.[11-14] The mean±SD of plasma OPN level in this study was 18.00±4.16 ng/mL, and most of the subjects with OPN deficiency (reference value of OPN is 49.2-175 ng/mL). The mean±SD level of plasma OPN in poorly-controlled type 2 DM subjects (20.27±3.20 ng/mL) was significantly higher than that in the well-controlled ones (15.04±3.34 ng/mL) with p=0.001. Berezin and Kremzer found the mean OPN level was higher in the type 2 DM subjects compared to the non-type 2 DM [52.63 ng/mL (95% CI=47.11-58.15 ng/mL) vs. 36.54 ng/mL (95% CI=31.77-41.31 ng/mL), respectively, p<0.0001].[19] No correlation was found between plasma OPN with total serum 25-OH vitamin D level in all of type 2 DM subjects (Table 3), with well and poor glycemic control [r=-0.151 (p=0.173), r=-0.087 (p=0.612), r=-0.006 (p=0.970), respectively]. However, there was a correlation between OPN and glycemic control (fasting glucose, 2-hour post-prandial glucose,

HbA1c) in all subjects with type 2 DM [r=0.241 (p=0.028), r=0.378 (p=0.0001) r=0.529 (p=0.0001), respectively].

Studies show the role of OPN in insulin resistance, type 2 DM, bone destruction, and osteoporosis. Osteopontin is ubiquitously found in bone. It mediates osteoclast adhesion to the bone matrix as OPN cytokine mediates cell-matrix and interacts among cells through the surface αvβ3 integrin and Cluster Differentiation (CD)44. The deficiency of OPN results in osteoclast dysfunction due to a decrease of CD44. It is proved in OPN-/- mice with bone resorption delay and disorder.[6,7,9,20]

There was no correlation between OPN and total 25-OH vitamin D in type 2 DM patients (r=-0.151 and p=0.173), as well as in well-controlled or poorly-controlled type 2 DM (r=-0.087; p=0.612 vs. r=-0.006; p=0.970, respectively). A study by Berezin and Kremzer revealed correlations between OPN and Agatston index score (r=0.418; p=0.009) and OPN with hs-CRP (r=0.368; p=0.008).[19] A study suggested that vitamin D level was significantly lower in patients with type 2 DM receiving oral therapy and insulin than in the control group. There was a positive correlation between vitamin D level and insulin sensitivity in normal-weight individuals with normal glucose tolerance. This finding indicated that vitamin D deficiency correlated with DM and glucose intolerance.[17] This activity is related to insulin sensitivity, which indirectly reduces cardiovascular risk, particularly in patients with poor glycemic control.[18]

A study by Maser *et al*., which investigated OPN and osteoprotegerin (OPG) levels in type 2 DM in correlation with autonomic cardiovascular function showed the correlation of OPN with systolic blood pressure (r=0.33; p=0.018), calcium (r=0.31; p=0.027) and OPG (r=0.29; p=0.039).[21] Increased OPN levels were observed in various chronic inflammatory diseases, like obesity, type 2 DM, and others. Osteopontin is largely distributed in various human body tissues located in and around inflammatory cells.[21] Osteopontin is known to be an important component in the development of insulin resistance. As shown in this study, there was a correlation between OPN and glycemic control (fasting glucose, 2-hour post-prandial glucose, HbA1c).[13] The absence of the correlation between plasma OPN and total serum 25-OH vitamin D in this study was probably due to the mean duration of DM in subjects (8 years) was shorter than Maser *et al*. study (13±8 years).[21]

The limitations of this study were caused by the use of an observational analytic study with a cross-sectional approach with no control of subjects and its limited variables such as OPN, and vitamin D. Further studies were needed to determine whether OPN and vitamin D are related to insulin resistance and inflammation by using markers such as hsCRP, interleukin or TNF-alpha to define the specific function of OPN and vitamin D in the development of type 2 DM.

CONCLUSION AND SUGGESTION

As a conclusion, this study revealed the correlation between plasma OPN and glycemic control (fasting glucose, 2-hour post-prandial glucose, HbA1c) but not serum total 25-OH vitamin D in patients with type 2 DM. Further study was needed in populations of other types of DM and other research variables related to inflammation or insulin resistance.

ACKNOWLEDGMENT

This funding research was supported by Dr. Moewardi General Hospital, Surakarta, Central Java, Indonesia.

REFERENCES

1. American Diabetes Association (ADA). Standards of medical care in diabetes-2018. Diabetes Care, 2018; 41(1): S13-S27.
2. Perkeni. Pedoman pengelolaan dan pencegahan diabetes melitus tipe 2 dewasa di Indonesia 2019. Jakarta, PB Perkeni, 2019; 1-50.
3. Polonsky KS, Burant CF. Type 2 diabetes mellitus. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM. Williams textbook of endocrinology. 13th Ed., Philadelphia, Elsevier, 2016; 1386-1450.
4. Brownlee M, Aiello LP, Cooper ME, Vinik AI, Plutzky J, Boulton AJM. Complications of diabetes mellitus. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM. Williams textbook of endocrinology. 13th Ed., Philadelphia, Elsevier, 2016; 1484-1581.
5. Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: A systematic review. Eur J Clin Nutr, 2011; 65(9): 1005–1015.
6. Gravallese EM. Osteopontin: A bridge between bone and the immune system. J. Clin. Invest, 2003; 112: 147-149.
7. de Fusco C, Messina A, Monda V, Viggiano E, Moscatelli F, *et al*. Osteopontin: Relation between adipose tissue and bone homeostasis. Stem Cells International, 2017; 2017: 1-6.

8. Sase SP, Ganu JV, Nagano N. Osteopontin: A novel protein molecule. Indian Medical Gazette, 2012; 62-66.
9. Agah E, Zardui A, Saghazadeh A, Ahmadi M, Tafakhori A, Rezaei N. Osteopontin (OPN) as a CSF and blood biomarker for multiple sclerosis: A systematic review and meta-analysis. PLoS One, 2018; 13(1): e0190252.
10. Van Driel M, Pols HAP, van Leeuwen JPTM. Osteoblast differentiation and control by vitamin D and vitamin D metabolites. Current Pharmaceutical Design, 2004; 10(00): 1-21.
11. Al-qtaitat AI, Aldalaen SM. A review of non-collagenous proteins; their role in bone. AJLS, 2014; 2(6): 351-355.
12. Thurner PJ, Chen CG, Lonova-Martin S, Sun L, Harman A, *et a*l. Osteopontin deficiency increases bone fragility but preserves bone mass. Bone, 2010; 46: 1564–1573.
13. Kahles F, Finfeisen HM, Bruemmer D. Osteopontin: A novel regulator at the cross-roads of inflammation, obesity, and diabetes. Molecular Metabolism, 2014; 3: 384-393.
14. Musialik K, Skrypnik D, Dogdanski P, Szulinska M. Osteopontin-a multifunctional protein and its impact on an insulin resistance development. J Med Sci, 2014; 2(83): 173-176.
15. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev, 2016; 96: 365-408.
16. Gil A, Plaza-Diaz J, Mesa MD. Vitamin D: Classic and novel actions. Ann Nutr Metab, 2018; 72: 87-95.
17. Battault S, Whiting SJ, Peltier SL, Sadrin S, Gerber G, Maixent JM. Vitamin D metabolism, functions and needs: From science to health claims. Eur J Nutr, 2012; 52(2): 1-14.
18. Boyraz I, Bilge U, Unalacak M, Bilgin M. A comparison of 25(OH) vitamin D levels in patients with type 2 diabetes on oral hypoglycemic agents and insulin treatment. Biomed Res, 2016; 27(1): 1-5.
19. Berezin AE, Kremzer AA. Circulating osteopontin as a marker of early coronary vascular calcification in type two diabetes mellitus patients with known asymptomatic coronary artery disease. Atherosclerosis, 2013; 229: 475-481.
20. Chellaiah MA, Kizer N, Biswas R, Alvarez U, Strauss-Schoenberger J, *et al*. Osteopontin deficiency produces osteoclast dysfunction due to reduced CD44 surface expression. Mol. Biol. Cell, 2003; 14: 173-189.
21. Maser RE, Lenhard MJ, Pohlig RT, Balagopal PB. Osteopontin and osteoprotegerin levels in type 2 diabetes and their association with cardiovascular autonomic function. J Diabetes Complications, 2016; 30(3):507-510.