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RESEARCH ARTICLE

Differences in levels of bone morphogenetic protein-2 in periodontitis patients with and without type 2 diabetes mellitus

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ABSTRACT

Periodontitis is characterized by gingival bleeding, periodontal pocket formation, damage to the connective tissue attachment, and alveolar bone resorption. One of the risk factors that can increase the occurrence of periodontitis is diabetes mellitus (DM). In Indonesia, the incidence and prevalence of periodontitis are higher in patients with type 2 DM. In the present study, BMP-2 expression in periodontitis patients with and without type 2 DM was evaluated. We performed a descriptive analytical cross-sectional study in 40 respondents with stages II-III grades B-C chronic periodontitis. They were divided into a chronic periodontitis group with type 2 DM and a chronic periodontitis group without DM. Crevicular fluid of the gingival sulcus of the central maxillary anterior teeth from each respondent was taken using paper points for 5 minutes and placed in an Eppendorf tube. Each tube was labelled according to its group. All of the samples were examined by BMP-2 ELISA kit and measured by BioRad microplate reader with a wavelength of 450 nm. Data were analysed using SPSS 16.0 program. The independent sample t-test was used to compare the BMP-2 among two groups. The results of the study revealed the BMP-2 level in periodontitis patients with type 2 DM was 63.1 pg/ml, while in periodontitis patients without DM was 66.8 pg/ml. BMP-2 levels in periodontitis patients with type 2 DM were significantly lower than those in periodontitis patients without DM ($p < 0.05$).

Keywords: bone morphogenetic protein-2; diabetes mellitus; gingival crevicular fluid; periodontitis

INTRODUCTION

Periodontal infection is a disease of the supporting tissues of the teeth including gingiva, alveolar bone, cementum and periodontal ligament. The prevalence of periodontal disease in Indonesia is still high (at 74.1%) based on the Riskesdas journal in 2018.[1] In general, periodontal disease is caused by bacteria plaque on tooth surface, a soft deposit that makes up a form of a thin layer of biofilm which contains a collection of pathogenic microorganisms such as *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia* and *Fusobacterium nucleatum*. These bacteria initiate gingival inflammation, and if not treated, it can cause chronic periodontitis and the teeth may lose their supporting tissue.[2] Periodontitis is a widespread chronic disease characterized by gingival bleeding, periodontal pocket formation, damage to connective tissue attachment, and alveolar bone resorption.[3] Periodontitis can occur in all age groups (adults, children and adolescents) but most often found in adult population.[4] Increasing risk factors of periodontal disease include smoking, diabetes mellitus (DM), HIV/AIDS, family history, and certain medications. Periodontitis in people with diabetes mellitus is called diabetic periodontitis. It has been reported that diabetic periodontitis is more frequent in uncontrolled DM patients and the severity is higher than in patients without DM even though the bacteria that cause it are the same.[5] Recent study reported that the prevalence of periodontitis in DM patients in the Internal Medicine Department, Dr. Sardjito Hospital, Yogyakarta, was 88.24%.[6] An increasing severity of diabetic periodontitis can be influenced by disorders of the immune system of DM patients including the vascular changes in

the form of thickening of the capillary basement membrane due to hyperglycemia which also disrupts nutrient supply and migration of immune cells to the periodontal tissue.[6]

The prevalence of diabetes mellitus has increased in recent decades. It is estimated that around 422 million adults were diagnosed with diabetes mellitus in 2014, with the highest prevalence of DM existed in the age group of 55-64 years, and 4.6 million deaths each year.[7] Indonesia had the fourth highest number of diabetes mellitus patients in 2019.[8] According to the data of Riskesdas journal in 2018, the prevalence of DM patients in the Special Region of Yogyakarta was the second highest in Indonesia with a percentage of 2.4%.[9] Furthermore, DM was also the second leading cause of death in the age group of 45-54 years in urban areas (14.7%). This is caused by an increase in risk factors such as obesity and a sedentary lifestyle, a lifestyle with little to no physical activity.[10] In Indonesia, type 2 DM (non-insulin dependent) patients comprise more than 90% of the diabetic population, and many are accompanied by periodontal disease (periodontitis).[7] Many patients may not realize this condition; therefore, biological markers are needed to determine the severity of type 2 DM patients with periodontitis using bone morphogenetic proteins (BMPs).

BMPs are growth factors and cytokines that induce the formation of bone and cartilage. More than 12 types of BMPs have been identified including BMP-1 as a metalloproteinase, while BMPs-2, -3, and -4 are members of the transforming growth factor-β (TGF-β) family.[11] BMP-2 has been shown to have the strongest bone production activity.[12] Several studies revealed that BMP-2 is proven to influence the pathophysiological process of obesity and is related to the status of diabetes mellitus.[13] Previous research indicated lower BMP-2 levels in periodontitis patients compared with those in healthy people, but did not explain how much BMP-2 levels were in periodontitis with type 2 diabetes mellitus. BMP-2 is the focus of the current research to determine the severity of infectious diseases before and after being given treatment because of easy and non-invasive sampling. Examination of BMP-2 can be done by taking a sample from the gingival sulcus fluid.

In the present study, BMP-2 expression in periodontitis patients with and without type 2 DM was evaluated. Crevicular fluids of the gingival sulcus of the central maxillary anterior teeth were confirmed as a research subject.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Faculty of Dentistry, Universitas Gadjah Mada (project no. 196/KE/FKG-UGM/EC/2022) and conformed to the STROBES guidelines for an observational study. All participating patients were individually informed about the study, and informed consent forms were signed. Cross sectional design was carried out in this study. Screening of DM patients with chronic periodontitis with uncontrolled type 2 DM and without DM was done at Prof. Soedomo Dental Hospital UGM and the KORPAGAMA Family Physician Clinic. Screening procedures for chronic periodontitis patients were carried out to examine pocket depth and clinical attachment loss using a periodontal probe. Panoramic radiography showed the pattern of horizontal or vertical damage to the alveolar bone. If no alveolar bone damage was observed, periodontitis may not develop and it could not be used as a research sample. Therefore, screening procedures for patients with uncontrolled type 2 diabetes mellitus were confirmed for patients who had been diagnosed with type 2 diabetes mellitus by a doctor with laboratory results of an HbA1c of more than 7% within a maximum of 4 months. For patients who were suspected of having diabetes mellitus but had not had a laboratory examination, a laboratory examination of HbA1c was required.

Supragingival plaque was carefully cleaned using a cotton pellet. The tooth was isolated with a cotton roll to avoid saliva contamination. Sulcular fluid was collected from 3 sites per patient (mesial, facial, and distal) using paper points inserted into the sulcus between the teeth and the gingival margin to a depth of 2 mm. The paper points were

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left for 5 minutes in the gingival sulcus. Sulcular fluid was placed in an Eppendorf tube and it was immersed in 100 µL of PBS solution and centrifuged at 1,000 rpm for 20 minutes at 4 °C. Finally, all of samples were stored in the refrigerator at -20 °C.

BMP-2 measurements were performed according to the manufacturer's instructions of ABclonal human BMP-2 ELISA kit. All reagents and samples were placed at room temperature before use. Measurements were carried out by a BioRad microplate reader with λ = 450 nm. The following are the measurement stages. First, 350 µL/well of wash buffer was added and incubated for 40 seconds at room temperature. Then each well was aspirated, and the process was repeated twice. A hundred µL sample diluent (R1) and 100 µL sample was added to each well, then incubated for 2 hours at 37 °C and washed three times. Next, 100 µL working biotin conjugate antibody was added and incubated for 1 hour at 37 °C, then washed three times. After washing, 100 µL working streptavidin-HRP was added and incubated for 30 minutes at 37 °C, then washed three times. Furthermore, 100 µL substrate solution was added and incubated for 15-20 minutes at 37 °C in the dark. Finally, 50 µL stop solution was added and optical density was detected for 5 minutes with a 450 nm microplate reader.

The Kolmogorov-Smirnov test and the Levene's test were used to measure normally distributed and homogeneous data. The results showed that the data were normally distributed and homogeneous. Thus, the data were analysed using the independent sample t-test with a significance level of 95%.

RESULTS

Research on BMP-2 levels in chronic periodontitis stages II-III grades B-C patients with and without type 2 DM was analysed by an ELISA kit which was measured using a microplate reader with a wavelength of 450 nm. The samples were obtained from 20 gingival sulcus fluid samples from participating patients with chronic periodontitis stages II-III grades B-C with type 2 diabetes mellitus in the age range of 50-70 years, and 20 patients with periodontitis without DM in the age range of 30-50 years (Table 1). Chronic periodontitis stages II-III grades B-C with type 2 DM group consisted of 6 men and 14 women with pocket depths of 4-12 mm. Chronic periodontitis stages II-III grades B-C without DM group included 9 men and 11 women with pocket depths of 3-5 mm. All study subjects were examined for pocket depth, attachment loss,

Table 1. Clinical characteristics of samples and average levels of BMP-2

	Periodontitis with type 2 DM (n = 20)	Periodontitis without type 2 DM (n = 20)	p-value
Age (year)	50-70	30-50	
Sex (M/F)	6/14	9/11	
Periodontal pocket			
< 5 mm	5	18	
5-10 mm	14	2	
>10 mm	1	-	
Attachment loss			
< 5 mm	1	6	
5-10 mm	10	14	
>10 mm	9	-	
BMP-2 level's (pg/ml)	63.1 ± 0.0036	66.8 ± 0.0043	0.005*

*: p value shows a significantly different results

and HbA1C to filter samples according to study groups. The clinical characteristics of the study samples and the average levels of BMP-2 were shown in Table 1.

The results of the Kolmogorov-Smirnov test showed a value of p = 0.827 (p > 0.05), which means that the data was normally distributed. The results of the data homogeneity test using the Levene's test showed that the value was significant. The Levene's test for equality of variance was 0.425 (p > 0.05), which means the data was homogeneous. Based on the results of the statistical analysis, the data was normally distributed and homogeneous. Thus, the statistical analysis was carried out by the parametric independent sample t-test with a significant level of 95%. Independent sample t-test between the two groups found p = 0.005 (p < 0.05), which suggests that there was a significant difference between the means of the two groups (Table 1).

DISCUSSION

The results of this study showed that periodontal pocket depth and attachment loss were more severe in the periodontitis group with type 2 DM. The periodontitis group with type 2 DM had greater pocket depth than the periodontitis group without type 2 DM. The periodontitis group with type 2 DM had greater pocket depth in the range of 5-10 mm which occurred in 14 respondents, while attachment loss of 5-10 mm occurred in 10 respondents. The periodontitis group without type 2 DM was found to have pocket depths of less than 5 mm in 18 respondents and attachment loss of 5-10 mm in 14 respondents. These findings may indicate that the periodontal pocket may get deeper if accompanied by systemic disease like diabetes mellitus. Continuous exposure to collagen fibers in the periodontal ligament induces its non-enzymatic glycation and oxidation. This glycation causes changes in the physical properties of these molecules, reduces the solubility of collagen and increases the degradation of connective tissue. This may cause accelerated breakdown of connective tissue and bone.[5] BMP-2 levels in periodontitis patients with type 2 diabetes mellitus was 63.1 pg/ml, while in periodontitis patients without diabetes mellitus was 66.8 pg/ml.

Bone morphogenetic protein-2 is a protein that plays a role in bone and cartilage development by inducing the differentiation of mesenchymal stem cells into osteoblast cells that produce bone.[12] Most BMPs are expressed in various tissues during embryogenesis and BMP expression becomes restricted in certain tissues after birth.[11] One example is a disease that can hinder bone repair and affect BMP levels such as diabetes mellitus and periodontitis.[14] Type 2 diabetes mellitus accounts for the majority of all types of diabetes mellitus. Type 2 diabetes mellitus is caused by the body's cells which become less sensitive to insulin, causing hyperglycemia which can increase the concentration of glucose in saliva and gingival sulcus fluid.[7] This can cause a high proliferation of bacteria in the oral cavity. Hyperglycemia also has an indirect side effect such as stimulating immune system cells to release inflammatory cytokines. Increased levels of proinflammatory mediators in the periodontal pocket can lead to osteoclastic destruction. Diabetic microangiopathy, impaired immune response, and lower resistance to infection may contribute to the development of periodontitis in uncontrolled diabetics.[4]

Hyperglycemia can cause indirect damage through the end result of glucose products, that is advanced glycation end products (AGEs) mediated by RANKL, and direct cell damage from stimulation of intracellular pathways. Advanced glycation end products (AGEs) cause an increase in matrix metalloproteinases (MMPs) and a decrease in collagen synthesis resulting in collagen degradation. AGEs that bind to the AGE receptor (RAGE) can induce the production of reactive oxygen species (ROS), production of inflammatory cytokines such as tumour necrosis-alpha (TNF-a), and the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) which cause disturbances in cellular function, vascular changes, exacerbating inflammation, disturbances in bone repair, and impaired wound healing in the periodontal tissue, which eventually can cause periodontitis.[15] Several studies have shown

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that DM sufferers have decreased neutrophil and monocyte function, chemotactic power, diapedesis, and phagocytic power.[16] Diabetes mellitus affects every organ in the body and one of its oral manifestations is periodontitis which is characterized by loss of tissue attachment.

Periodontitis is characterized by increased susceptibility to infection, poor wound healing, and increased morbidity and mortality associated with disease progression. Diabetes mellitus has also been reported as an important risk factor for more severe and progressive periodontitis, an infection or lesion that results in the destruction of the supporting tissue and bone that form the attachment around the teeth. Because the periodontium has a highly vascular tissue, any inflammation can serve as a gateway to the systemic circulation for bacterial products and inflammatory mediators.[4]

Longitudinal studies have shown that the severity of periodontitis is associated with poorly controlled glycemia, higher HbA1c levels, and the development of systemic diabetes complications. Periodontitis is associated with a slight increase in HbA1c in non-diabetic subjects (periodontitis has the potential to increase the incidence of diabetes), although a clear association has not been established. It was reported that periodontal infection can impair glycemic control by increasing tissue insulin resistance.[4,7]

The results of this study revealed that bone morphogenetic protein-2 (BMP-2) levels in the periodontitis group with type 2 DM were significantly lower compared to the BMP-2 levels in the periodontitis group without type 2 DM. This may indicate that type 2 DM was likely to trigger more severe periodontal tissue damage. Bascil reported that BMP-2 levels in periodontitis patients were lower than BMP-2 levels in healthy people. When BMP-2 levels increase, the severity of periodontitis will decrease. These findings strongly support the local protective and regenerative role of BMP-2.[17] The selection of central incisor teeth was chosen to facilitate sampling. If the patient did not have central incisors, other anterior teeth could be used.

BMP-2 is included to the TGF-β superfamily, the multifunctional cytokines that have the ability to induce bone formation and bone tissue reconstruction. BMP-2 plays an important role in osteogenesis and bone metabolism. The bone matrix contains many growth factors, including BMP which is synthesized and secreted by osteoblasts and enters the matrix during bone formation. Adequate progenitor cell proliferation and differentiation is essential for bone healing. This process is regulated by growth factors such as TGF-β and BMP. BMP is capable of inducing mesenchymal cell differentiation into osteoblasts (osteoinduction), which stimulates bone formation in both the remodeling and repairing processes through the Smad and p38 mitogen-activated protein kinase (MAPK) signaling pathways by binding to their receptors (BMPR). Furthermore, diphosphorylation and active BMPR will activate R-Smad to increase osteogenesis and stem cell differentiation.[18] The BMP-BMPR complex can act on transforming growth factor kinase 1 (TAK1) via osteopontin to activate the p38-MAPK signaling pathways and increase the expression of osteocalcin, osteocytes, and the other factors that accelerate bone formation. On the other hand, hyperglycemia is associated with decreased BMP expression, inhibition of mesenchymal stem cell differentiation into osteoblasts, and decreased bone formation by encouraging adipogenesis of mesenchymal stem cells by activating peroxidase proliferator-activated receptor-gamma (PPARγ) which decreases bone formation and mass.[19]

The rules of BMP also induce heterotopic bone in soft tissue via endochondral ossification, in which mesenchymal cells differentiate into chondrocytes that secrete a cartilage-specific extracellular matrix, such as type II collagen and various proteoglycans. Osteoblasts arise in the perichondrium near mature hypertrophic chondrocytes in endochondral ossification. In intramembranous ossification, mesenchymal cells directly differentiate into osteoblasts that secrete bone-extracellular matrix specific, such as type I collagen, osteopontin, and osteocalcin. BMP levels can be affected by various diseases that result in reduced bone mass, such as diabetes mellitus, osteoporosis, osteogenesis imperfecta,

and periodontitis.[11] Various studies have shown that women are more often affected by periodontitis than men due to hormonal fluctuations that occur in the body, especially during puberty and pregnancy or during menstruation and menopause.[20] The patient's age can also affect BMP levels because as they get older, they are more susceptible to systemic diseases. Smoking, diet, and stress are risk factors for periodontitis and DM which can affect BMP levels.[10] Further research is needed on BMP-2 levels using all types of diabetes mellitus and type 2 diabetes mellitus patients who have had periodontal treatment to compare with previous results.

CONCLUSION

This study has shown that BMP-2 levels in periodontitis patients with type 2 diabetes mellitus were lower than BMP-2 levels in periodontitis patients without diabetes mellitus.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Suratri MAL, Jovina TA, Andayasari L, Edwin VA, Ayu GAK. Pengaruh Hipertensi Terhadap Kejadian Penyakit Jaringan Periodontal (Periodontitis) pada Masyarakat Indonesia (Data Riskesdas 2018). Buletin Penelitian Kesehatan. 2020; 8(4): 227-234. doi: 10.22435/bpk.v48i4.3516
2. Andriani I, Chairunisa FA. Periodontitis kronis dan penatalaksaan kasus dengan kuretase. Incisiva Dent J. 2019; 8(1): 25-30.
3. Kasuma N. Sulcular fluids. Andalas University Press: Padang; 2019. 2-26.
4. Pratiwi AI. Periodontitis as early detection of diabetes mellitus: a literature review. Inti Sains Med. 2020; 11(1): 108-111. doi: 10.15562/ism.v11i1.623
5. Madianos PN, Koromantzos PA. An Update of the evidence on the potential impact of periodontal therapy on diabetes outcomes. J Clin Periodontol. 2018; 45(2): 188-195. doi: 10.1111/jcpe.12836
6. Sari R, Herawati D, Nurcahyanti R, Wardani PK. Prevalensi periodontitis pada pasien diabetes mellitus (Studi observasional di poliklinik penyakit dalam RSUP Dr. Sardjito). Majalah Kedokteran Gigi Indonesia. 2017; 8(2): 98-104. doi: 10.22146/majkedgiind.11241.
7. Decroli E, Kam A, Efendi YP, Decroli DP, Rahmadi A. Diabetes mellitus tipe 2. FK Unand: Padang. 2019. 1-8.
8. Rizkiyah M, Oktiani BW, Wardani IK. Prevalensi dan Analisis Faktor Resiko Kejadian Gingivitis dan Periodontitis pada Pasien Diabetes mellitus (Literatur Review). Dentin. 2021; 1(1): 33-36. doi: 10.20527/dentin.v5i1.3231
9. Laporan Nasional Riset Kesehatan Dasar (RISKESDAS). Kementerian Kesehatan Republik Indonesia: Jakarta; 2018. 160-161.
10. Aliyah AN, Listyawati, Utami ND. Profil Penyakit Periodontal Pada Penderita Diabetes Melitus Berdasarkan Tipe Diabetes Melitus, Jenis Kelamin, dan Usia di RSUD dr. Kanujoso Djatiwibowo Pada Tahun 2016-2020. J Sains Kes. 2022; 4(1): 168-175.
11. Katagiri T, Watabe T. Bone morphogenetic protein. Cold Spring Harb Perspect Biol. 2016; 8(6): 1-27. doi: 10.1101/cshperspect.a021899
12. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Clinical Periodontology 13th Edition. ELSEVIER: Philadelphia; 2019. 166-167.
13. Chen Y, Ma B, Wang X, Zha X, Sheng C, Yang P, Qu S. Potential functions of the BMP family in bone, obesity, and glucose

metabolism. J Diabetes Res. 2021; 3(1): 1-10. doi: 10.1155/2021/6707464
14. Al-Qarakhli AMA. Altered Bone Cell Biology Associated with Type Two Diabetes Mellitus – Consequences for Periodontal Disease. Cardiff University: United Kingdom; 2021. 5-7.
15. Wu Y, Xiao E, Graves DT. Diabetes mellitus related bone metabolism and periodontal disease. Int J Oral Sci. 2015; 7(2): 63–72. doi: 10.1038/ijos.2015.2
16. Widyawati H, Sudibyo S, Failasufa H. Compliance relation of antidiabetic drug consumption with periodontal network health in type II diabetes mellitus prolanis patients: case study at Puskesmas Mranggen III. Magna Medika. 2018; 2(4): 1-10. doi: 10.26714/magnamed.2.4.2018.1-8
17. Bascil S, Iyidir OT, Bayraktar N, Ertorer ME, Tutuncu NB. Severe chronic periodontitis is not common in acromegaly: potential protective role of gingival BMP-2. Turk J Med Sci. 2021; 51(3): 1172-1178. doi: 10.3906/sag-2006-93
18. Sularsih S, Wahjuningsih E. Expression of bone morphogenetic protein-2 after using chitosan gel with different molecular weight on wound healing process of dental extraction. Dent J. 2015; 48(2): 53-58.
19. Wang X, Deng J, Xiong C, Chen H, Zhou Q, Xia Y, Shao X, Zou H. Treatment with a PPAR-γ agonist protects against hyperuricemic nephropathy in a rat model. Drug Des Devel Ther. 2020; 8(14): 2221-2233. doi: 10.2147/DDDT.S247091
20. Ali A, Harapan IK, Fione VR. Gambaran penyakit periodontal berdasarkan umur dan jenis kelamin pada pengunjung poliklinik gigi Puskesmas Tikala Baru Kota Manado tahun 2017. JIGIM (Jurnal Ilmiah Gigi dan Mulut). 2020; 3(1): 20-26.