Gingival Crevicular Fluid Levels of Resistin and Adiponectin in Chronic Periodontitis with Type 2 Diabetes Mellitus

*Natalina[1,2], Sri Lelyati C Masulili[2], Nadhia Anindhita Harsas[2], Imam Subekti[3], Elza Ibrahim Auerkari[4]**

1. Doctoral Program, Faculty of Dentistry Universitas Indonesia, Jakarta, Indonesia.
2. Department of Periodontics, Faculty of Dentistry Universitas Indonesia, Jakarta, Indonesia.
3. Division of Metabolism and Endocrinology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
4. Department of Biology Oral, Faculty of Dentistry Universitas Indonesia; Jakarta, Indonesia.

Abstract

Resistin and adiponectin are adipokines that secreted from adipose tissue that postulated opposing functions in insulin resistance and inflammation. The oral cavity serves as a continuous source of infectious agents that could worsen the diabetic status of the patient and serve as an important risk factor deterioration of diabetes mellitus.

The aim of this study was to clarify gingival crevicular fluids (GCF) adipokine levels in chronic periodontitis (CP) suffering type-2 diabetes mellitus (T2DM).

Twenty-four subjects with CP (pocket depth ≥5mm), consist of 12 subjects T2DM and 12 subject control NDM (32-65 years old) were selected. The level of resistin and adiponectin was analyzed using ELISA. Resistin level of T2DM compared to NDM was 21.45±10.5 pg/mL and 27.0±13.1 pg/mL respectively. Resistin T2DM was lower than NDM. Adiponectin level T2DM is 20.1±14.9 ng/mL and control group 17.28±6.9 ng/mL. The level of resistin and adiponectin between T2DM and NDM with did not reach statistical significance.

Resitin level was lower in T2DM compared to NDM. The adiponectin level was increased in deep pocket.

Clinical article (J Int Dent Med Res 2016; 9: (Special Issue), pp. 329-333)

Keywords: Chronic periodontitis, type-2 diabetes mellitus, resistin, adiponectin, gingival crevicular fluids.

Received date: 28 September 2016 **Accept date:** 29 October 2016

Introduction

Ministry of Health Republic Indonesia stated that life expectancy of Indonesian people on 2010 was 69 years old.[1] This long lifetime span should be beneficial for individual and communities. Human healthiness including oral health should be maintained to keep their life qualities.

The oral cavity is an infection agent source, but its condition often a reflection of systemic pathologies progressivity. In the past, oral disease defined as a local pathologic event, but now oral health status is considered to affect systemic health condition.[2] Periodontal tissues are an important dental structure that supports teeth function.[3]

Periodontopathogen bacteria caused periodontitis which is chronic and complex, with several clinical features. Chronic periodontitis (CP) is the most common periodontal disease found in society.[4] Indonesian mouth and dental diseases national prevalence is 25.9%, but unfortunately, there are no data on periodontitis prevalence in Indonesia.[1] World Health Organization (WHO) stated that 15-20% of world population (age 35-44 years) suffered from advanced periodontitis.[5] Individual that have advanced CP will get teeth loss if not treated, and this will affect aesthetic, speech and mastication function.[6]

Heavy chronic inflammation of periodontitis will produce a systemic response to bacteria and their products because of the degradation of periodontal tissue. Periodontitis will aggravate glycemic control, and increase the risk of diabetic complication and initiate insulin

Corresponding author:
Prof. Elza Ibrahim Auerkari, DDS, M.Biomed., Ph.D., For.Odont
Department of Biology Oral, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Jakarta, Indonesia
E-mail: eauerkari@yahoo.com

resistance, and diabetes mellitus will increase the severity of gingivitis and periodontitis.[7]

Oral cavities inflammation increase cytokine production and acute phase protein synthesis activation that caused insulin resistance in the tissue. Insulin resistance will change pathogen process and caused T2DM.[3] T2DM usually affected an individual in their middle and late age, lately, the number of cases in younger patients is increasing. WHO predicted that DM patients on 2030 in America would be 30 million, Egypt 6.8 million, India 80 million, Nigeria 4.8 million and Indonesia 23 million.[8] This increased in prevalence connected with urbanization that changed people's lifestyle that less in exercising and consumed high calories food. Riskesdas 2007 reported that DM causing 4.2% of death in age group 15-44 years old in city area, and included in the number 6 (six) highest cause of death in the world.[1] DM is affecting individual's health, and not only causing damage or even death, but also give an economic burden for health service system.[9]

Adipose tissue produced adipokine inflammatory factor (adiponectin, resistin, leptin, and visfatin) and also cytokines (TNF-α, IL-6), monocyte, and chemoattractant protein-1. These factors and cytokine affect insulin resistance and have the role in inflammation and immune response.[7] These markers can be an objective measuring instrument and evaluated as a biologic indicator of pathogenesis.[10] Gingival crevicular fluid (GCF) contained cytokine in a significant amount and used as the diagnostic marker or periodontal destruction prognosis.[11]

Pischon et al. proved that resistin production by adipose was very limited, but resistin in the large amount was produced by inflammation cell systems like PMNs, monocyte, and makrofag.[12] Resistin level increase in every chronic inflammation condition and associated with severity of inflammation and DM status.[10] Hiroshima et al. were the only researcher that conducted a research resistin level in GCF of CP and DM patients.[13] Resistin can be used as one of a marker that links between periodontitis-obesity-DM.[14]

Adiponectin serum has an important role in insulin sensitivity, and also have a negative correlation with inflammation markers, such as TNF-α and C-reactive protein. Adiponectin serum function is to correct insulin resistance through suppression system of TNF-α which will reduce systemic inflammation.[15] Osteoclast production which was caused by lipopolysaccharide of *Aggregatibacter actinomycetemcomitans* will regulate adiponectin.[16]

Based on writer knowledge, in Indonesia has never been conducted research about resistin level (pro-inflammatory) and adiponectin (anti-inflammatory) on CP and T2DM patients. The researcher wants to find out and evaluate the effect of periodontitis and T2DM on resistin and adiponectin level. Dental Hospital of Faculty of Dentistry, Universitas Indonesia (RSKGM FKG UI) is a reference hospital in Jakarta and cities nearby (Jakarta, Bogor, Depok, and Bekasi).

Materials and methods

Subjects. Twenty-four CP patients in RSKGM FKG UI include in research criteria in periods of September to December 2015, with periodontal pocket ≥5mm. CP is inflammation in periodontal tissue on adult age, with an alveolar bone loss, and periodontal pockets. Research subjects were patients of CP and T2DM (had been diagnosed by doctor or specialist), non-smoking, not pregnant, don't have any other systemic diseases besides T2DM, not consuming antibiotic in the last 3 (three) months, approved and signed the informed consent. This research is a case-control study and consecutive sampling.

Ethics. Ethics committee has approved this study at Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia (No: 36/Ethical Approval/ FKGUI/VIII/2015).

Clinical examination was performed using mouth mirror (Medesy, Italy), twizer (Medesy, Italy), halfmoon explorer (Crown, Japan), periodontal probe (Medesy, Italy). The periodontal pocket, gingival bleeding index, and loss of attachment of each patient were examined. Alveolar bone loss was evaluated using radiography to determine diagnoses of chronic periodontitis. Based on pocket depth, chronic periodontitis was divided to moderate pocket (5-7 mm) and deep pocket (> 7mm).

The collection of gingival crevicular fluids. Gingival crevicular fluids (GCF) were collected after supragingival scaling, 3-5 days later, about 2-3μL using micropipette (BIO-RAD, volume 0.1-2μL). The teeth will be isolated with cotton roll, then tips of micropipette were inserted gently into gingival margin (±0.5mm), and the sample cannot get contaminated with saliva, plaque, and

blood. The sample then inserted into sterilized plastic vial contained 100µL Phosphate-buffered saline and kept in temperature -80°C until further examination.

Adipokine Analysis. A laboratory test of adipocytokine level (resistin and adipokine) was conducted in the Laboratory of Biology Oral, Faculty of Dentistry, Universitas Indonesia. All the samples were thawed at room temperature, then tested using Bradford assay (Thermo Fisher Scientific, Pierce TM Coomassie (Bradford) Protein Assay kit. Catalog No. 23200) to assess total protein concentrations followed by ELISA assay to measure resistin level (Elabscience human RETN. Catalog No. E-EL-H1213, China) and adiponectin level (Elabscience human ADP. Catalog No. E-EL-H0004, China). This ELISA Kit is sandwich-ELISA method whereas the plate had been covered with a specific antibody. Optical density (OD) measurement was done using spectrophotometric with wavelength 450±2nm to value resistin or adiponectin level.

Statistics analysis. Description research sample consists of an average value, deviation standard, the frequency was done on the data of age, pocket depth, resistin and adiponectin level on a group with T2DM or control NDM. All the data were analyzed using statistic software SPSS.

Results

Twenty-four subjects, age average, is 48.58 years old were included in the statistics analysis. Table 1 showed CP with T2DM frequency on the moderate pocket (20.8%), and deep pocket (29.2%), and the control group equals with the tested group.

Chronic Periodontitis	T2DM N (%)	NDM N (%)	Total
Moderate	5 (20.8)	5 (20.8)	10 (41.6)
Severe	7 (29.2)	7 (29.2)	14 (58.4)
Total	12 (50.0)	12 (50.0)	24 (100.0)

T2DM: Type-2 Diabetes Mellitus; NDM: Non Diabetes Mellitus

Table 1. Distribution of Type-2 Diabetes Mellitus and Control Based on the Chronic Periodontitis Severity.

Table 2 showed a comparison of age average, resistin and adiponectin level between test and control groups; these samples were homogeneous. Resistin level on chronic periodontitis with T2DM group (21.45±10.5 pg/mL) and control (27.0±13.1 pg/mL), and the adiponectin level on the test and control group (20.1±14.9 ng/mL and 17.28±6.9 ng/mL, consecutively) with no significant differences (p=0.568).

	Chronic Periodontitis		
	T2DM Mean (SD)	NDM Mean (SD)	*p*
Age (years)	49.5 (7.5)	47.67 (13.53)	0.687
GCF Resistin (pg/mL)	21.45 (10.5)	27.0 (13.1)	0.264
GCF Adiponectin (ng/mL)	20.1 (14.9)	17.28 (6.9)	0.568

Independent T-test; *p < 0.05 significant differences; GCF = Gingival Crevicular Fluid; T2DM = Type-2 Diabetes Mellitus; NDM = Non Diabetes Mellitus.

Table 2. Differences of Age Average, Resistinand Adiponectin Level in Gingival Crevicular Fluid on Chronic Periodontitis, Type-2 Diabetes Mellitus and Non Diabetes Mellitus.

		N	Mean (SD)	*p*
Resistin (pg/mL)	T2DM CP moderate	5	18.74 (13.40)	< 0.05
	T2DMCP severe	7	23.38 (8.42)	
	NDM CP moderate	5	16.98 (4.30)	
	NDM CP severe	7	34.16 (12.62)	
Adiponectin (ng/mL)	T2DM CP moderate	5	13.47 (11.80)	< 0.05
	T2DM CP severe	7	24.75 (15.90)	
	NDM CP moderate	5	13.80 (3.60)	
	NDM CP severe	7	19.77 (7.80)	

T2DM = Type-2 Diabetes Mellitus; NDM = Non Diabetes Mellitus
Note: Resistin level. One-way Anova test. Post-hoc LSD test: T2DM-CP moderate vs T2DM-CP severe p=0.457; T2DM-CP moderate vs NDM–CP moderate p=0.792; T2DM-CP moderate vs NDM–CP severe*p=0.02; T2DM-CP severe vs NDM–CP moderate p=0.307; T2DM-CP severe vs NDM–CP severe p=0.457; NDM-CP moderate vs NDM–CP severe *p=0.011(*p<0.05 = significant differences)
Adiponectin level. Kruskal-Wallis test: p>0.05 means no different between grups(*p<0.05 = significant differences)

Table 3. Mean and Standard Deviation of Resistin and Adiponectin Level in Gingival Crevicular Fluid on The Severity of Chronic Periodontitis with Type-2 Diabetes Mellitus.

Comparison between resistin and adiponectin level based on periodontitis severity shown in Table 3. Resistin level on test groups with moderate pocket is 18.74±13.40 pg/mL, deep pocket is 23.38±8.42pg/mL, and control consecutively are 16.98±4.30 pg/mL and 34.16±12.62 pg/mL. Adiponectin level in moderate pocket 13.47±11.80 ng/mL, deep pocket 24.75±15.90 ng/mL, whereas control groups are 13.80±3.60 ng/mL and 19.77±7.80 ng/mL consecutively. There are no significant differences in adiponectin level in both groups.

Discussion

The results of this study show that there is an influence of T2DM against resistin levels with a weak negative correlation, which means T2DM group resistin levels are lower than the control group. These results were same as the results from Hiroshima et al. and Pradeep and Raju.[17,18]

Adiponectin levels in T2DM group is higher than in the control group but was not statistically significant. These results are inconsistent with previous studies that levels of adiponectin decreased on increasing pocket depth and T2DM.[15]

Based on the pocket depth or severity of chronic periodontitis, resistin levels showed a positive correlation with pocket depth; this is in line with research of Al-Shahwani.[19] Meanwhile, adiponectin levels with pocket depths have a positive correlation, and even it is not statistically significant. It is known that periodontal pathogen-infected individual will respond by producing inflammatory mediators at a very high level.[20] Pischon et al. showed that resistin production by adipocytes very tiny, whereas resistin in large quantities are producing by inflammatory system cells such as PMNs, monocytes, and makrofag.[12] Resistin also plays a role in the inflammatory process. Pro-inflammatory agents such as TNF-α, IL-6 and LPS can regulate gene expression resistin.[10] Periodontitis is an infection of local periodontal tissues so that the GCF sample can be a very practical choice for biochemical analysis of the host response in periodontal disease as an inflammatory exudate out of the periodontal tissues.[18] Local inflammatory marker levels can be measured by the level of local inflammation that occurs. Deep pockets will contain many markers as well as local inflammatory cells that will increase resistin and decrease adiponectin as a result of immune regulation markers and inflammatory cells.[21] This explains why the resistin levels equal with periodontitis severity. Local conditions strongly influence the content of the GCF than the individual systemic conditions (T2DM)[22] maybe this can explain why resistin in T2DM was lower than in NDM. In all groups adiponectin level was higher indeep pockets compare tomoderate pockets; this result was inconsistant from the previous research which was adiponectin has a negative corelation with severity of periodontitis.

Conclusions

The findings from this study were the level of resist in was decreased in T2DM compared to NDM. The resist in levels were positively correlated with pocket depth, but negative correlated with T2DM. The adiponectin level was inconsisten between groups, and severity of periodontal disease. Further studies involving a larger samples are needed to confirm the findings of the present research, to have a better understand the role of these cytokines in periodontal disease progression.

Acknowledgements

All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission. Founding source of this study was from Universitas Indonesia Grand 2015 (Hibah Riset Awal) and no conflicts of interest. The publication of this manuscript is supported by the Directorate of Research and Community Engagement of the Universitas Indonesia.

Declaration of Interest

The authors report no conflict of interest.

References

1. Litbang K. Profil Kesehatan Indonesia Tahun 2011. Jakarta: Kemenkes RI; 2012:220,421.
2. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and Diabetes: A Two-Way Relationship. Diabetologia2012;55(1):21-31.
3. Taiyeb-Ali T, Raman R, Vaithilingam R. Relationship between Periodontal Disease and Diabetes Mellitus: An Asian Perspective. Periodontol 2000 2011;56:256-268.
4. Bullon P, Newman H, Battino M. Obesity, Diabetes Mellitus, Atherosclerosis and Chronic Periodontitis: A Shared Pathology via Oxidative Stress and Mitochondrial Dysfunction? Periodontol 2000 2014;64:139-53.
5. WHO Oral Health Fact Sheet 2012;1. http://www.who.int/mediacentre/factsheets/fs318/en/
6. Díaz-romero RM, Ovadía R. Diabetes and Periodontal Disease: A Bidirectional Relationship effects of Diabetes Mellitus on the Periodontal State Effects of Periodontal Disease on the State of Diabetes. Med Biol 2007;14(1):6-9.
7. Kumari M, Naik SB, Shanbhag N, Pradeep AR. Diabetes and Periodontitis: A Casual Link or aTwo Way Street? Arch Oral Sci Res 2011;1(4):221-227.
8. Eldarrat A. Diabetics ' Awareness of Oral Disorders Associated with Diabetes Mellitus. London; 2012:1-3.
9. Pusat Data dan Informasi Kemkes R. Situasi dan Analisis Diabetes. In: Waspada Diabetes. 2014:1-6.
10. Devanoorkar A, Kathariya R, Guttiganur N, Gopalakrishnan D, Bagchi P. Resistin : A Potential Biomarker for Periodontitis Influenced Diabetes Mellitus and Diabetes Induced Periodontitis. Dis Marker 2014:1-7.
11. Raghavendra NM, Pradeep A, Katharity R, Sharma A, Rao NS, Naik SB. Effect of Non Surgical Periodontal Therapy on Gingival Crevicular Fluid and Serum Visfatin Concentration in Periodontal Health and Disease. Dis Marker 2012;32:233-8.
12. Pischon N, Heng N, Bernimoulin J, Kleber B, Willich S, Pischon T. Obesity, Inflammation, And Periodontal Disease. J Den Res 2007;86(5):400-409.
13. Hirosima Y, Bando M, Inagaki Y, et al. Resisin in Gingival Crevicular Fluid and Induction of Resistin Release by Porphyromonas gingivalis Lipopolysaccharide in Human Neutrophils. J Period Res 2012;47(5):554-62.

14. Javed F, Ahmed A. Proinflammatory Cytokines in the Saliva, Gingival Crevicular Fluid and Serum of Diabetic Patients with Periodontal Disease. J Res Pract Dent 2013;2013:1-10.
15. Matsumoto S, Ogawa H, Soda S, Hiramaya S, Amarasena N, Aizawa Y, et al. Effect of Antimicrobial Periodontal Treatment and Maintenance on Serum Adiponectin in Type 2 Diabetes Mellitus. J Clin Period 2009;36:142-8.
16. Furugen R, Hayashida H, Yamaguchi N, Yoshihara A, Ogawa H, Miyazaki H. The Relationship between Periodontal Condition and Serum Levels of Resistin and Adiponectin in Elderly Japanese. J Period Res 2008;43:556-62.
17. Hiroshima Y, Bando M, Inagaki Y, Mihara C, Kataoka M, Murata H. Resistin in Gingival Crevicular Fluid and Induction of Resistin Release by Porphyromonas gingivalis Lipopolysaccharide in Human Neutrophils. J Period Res 2012;47(5):554-62.
18. Patel SP, Raju PA. Gingival Crevicular Fluid and Serum Levels of Resistin in Obese and Non - Obese Subjects with and without Periodontitis and Association with Single Nucleotide Polymorphism. J Indian Soc Period 2014;18(5):555-560.
19. Al-shahwani RMS. The Role of Resistin as a Mediator of Cross-susceptibility between Periodontal Disease and Type 2 Diabetes Melitus. Dissertation. Newcastle University, Newcastle Country, England. 2012;(8):32-70.
20. Gemmell E, Seymour G. Immunoregulatory Control Of Th1/ Th2 Cytokine Profiles In Periodontal Disease. Periodontol 2000 2001;35:21-41.
21. Raghavendra NM, Pradeep AR, Katharity R, Sharma A, Rao NS. Effect of Non Surgical Periodontal Therapy on Gingival Crevicular Fluid and Serum Visfatin Concentration in Periodontal Health and Disease. Dis Markers 2012;32:383-8.
22. Pradeep A, Raghavendra NM, Sharma A, Pradeep PS, Raju AR, Rao NS, Naik SB. Association of Serum and Crevicular Visfatin Levels in Periodontal Health and Disease With Type 2 Diabetes Mellitus. J Periodontol 2012;83(5):629-34.