Level of Tumor Necrosis Factor Alpha in Elderly Patients with Periodontitis and Diabetes Mellitus

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Abstract

Periodontal diseases, such as periodontitis, have risk factors in common with other systemic and chronic inflammatory disorders. One inflammatory mediator that plays a role in the proinflammatory process is tumor necrosis factor α (TNF-α). With age, the body's inflammatory responses change, and this is one reason why periodontitis is common among elderly patients. We compared TNF-α levels of 33 elderly patients with periodontitis who did not have diabetes with those of 18 elderly patients with both periodontitis and diabetes mellitus. Patients' gingival crevicular fluid (GCF) was taken from sites with pocket depths of 5 to 6 mm, and their TNF-α levels were measured. Clinical data (pocket depth, bleeding index, and clinical attachment loss) and GCF samples were collected. Patients with diabetes had significantly higher Oral Health Information Suite indexes (3.1 ± 1.25) than did those without diabetes (2.17 ± 1.29; $p < 0.05$). Patients with diabetes had significantly higher TNF-α levels (5.04 ± 0.48) than did those without diabetes (4.67 ± 0.76; $p < 0.05$). Thus, the level of TNF-α is significantly higher in patients with diabetes than in those without.

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Introduction

The proportion of elderly adults in the population is increasing significantly. According to the World Health Organization, people aged 65 years or older are considered elderly in most developing countries.[1] A 2017 report from the United Nations, "World Population Aging," stated that in Indonesia, 22 million people were aged 60 years and older; they account for approximately 8.6% of the total population, and this proportion is expected to increase to 61 million by 2050.[1] The increase in the elderly population is a result of the increased current life expectancy. In Indonesia alone, the life expectancy for men reaches 69 years, and the life expectancy of women is higher, reaching 73 years.[2] Increased life expectancy is supported by many advances in modern medicine, but the number of elderly people with acute and chronic diseases continues to escalate. Most elderly people have at least one chronic disease.[3]

As people get older, the body's inflammatory response changes, both quantitatively and qualitatively and both adaptively and innately. The activity of proinflammatory mediators is commonly increased in elderly patients. This progressive modification of the immune system, called immunosenescence, could exaggerate risks for infection, neoplasms, or autoimmune manifestations.[3]

Periodontal disease has multifactorial causes, but Gram-negative microorganisms are known to play an important role in the initiation and development of periodontitis. Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia are considered the main bacteria that cause periodontitis. Other bacteria such as Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Prevotella intermedia, Campylobacter rectus, Peptostreptococcus migros, and Eikenella corrodens also play

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important roles in the development of periodontitis. Periodontitis is an inflammatory disease of the periodontal tissue that results in damage to the periodontal ligament and alveolar bone. Inflammation results from the release of proinflammatory mediators by the host in response to the presence of specific microorganisms. If this condition continues, the periodontal tissue becomes damaged. The body releases an anti-inflammatory mediator to prevent further damage and stop the development of periodontal disease.[3,4] Several inflammatory mediators play a role in the development of periodontal disease; one is tumor necrosis factor α (TNF-α). Research conducted by Garlet in 2010 showed that secretion of TNF-α serves as a proinflammatory mediator in periodontitis.[5,6] Inflammatory mediators such as TNF-α, interleukin-10, and interleukin-17 are produced by macrophages and lymphocytes. TNF-α is known for its role in bone damage in periodontitis. TNF-α can be found in saliva and gingival sulcus, both in healthy patients and in those with periodontitis, but in the latter, TNF-α levels are increased. Increased levels of TNF-α are associated with tissue damage and immune responses. TNF-α can induce regulation of adhesion molecules in leukocytes and endothelial cells and can also stimulate the production of chemokines, other inflammatory mediators (such as prostaglandin), and lytic enzymes (such as metalloproteinase matrix and its inhibitors). TNF-α stimulates the secretion of the metalloproteinase-3, -8, and -9 matrixes from gingival fibroblasts and the secretion of the metalloproteinase-13 matrix from osteoblasts.[5]

One of the most common chronic diseases among elderly people is diabetes mellitus.[3] Diabetes mellitus is characterized by an increase in glucose levels in the blood, which results from impaired insulin production by the pancreas. The prevalence of diabetes in Indonesia is the sixth highest in the world after China, India, the United States, Brazil, and Mexico; it is estimated that 10 million people in Indonesia have diabetes. This number is expected to continue increasing, and it is estimated that in 2045, 16 million people in Indonesia will have diabetes.[6] The most important causes of hyperglycemia are thought to be the deficiency of insulin secretion and increase in insulin resistance, which rise with age. The Baltimore Longitudinal Study of Aging demonstrated that insulin secretion after glucose load decreases with age, even after the influence of obesity and the distribution of adipose tissue is taken into account.[7]

Studies have shown a relationship between periodontal disease and glycemic control, and each disease has the potential to exacerbate the other.[8] Diabetes mellitus can affect the severity of periodontal disease conditions. According to epidemiological data, diabetes is a major risk factor for periodontal disease.[5] Diabetes mellitus also affects aspects of the immune system, such as neutrophil activity and cytokine biological activity. In patients with diabetes mellitus, blood levels of TNF-α are increased. TNF-α can interfere with the signaling process of insulin, which can cause insulin resistance.[5] Singh et al. found higher TNF-α levels in the saliva of patients with periodontitis and type 2 diabetes mellitus than in healthy patients and patients with chronic periodontitis but without diabetes.[9] According to research conducted by Qiao (2017) in China shown that serum TNF-α level in type 1 diabetes mellitus patients has significantly elevated among all age, disease duration and ethnicity groups.[10] To the best of our knowledge, among elderly patients with periodontitis, TNF-α levels in patients with diabetes mellitus have not been compared with those in patients without diabetes mellitus in Indonesia.

Materials and methods

The Ethical Committee of the Faculty of Dentistry at Universitas Indonesia approved this study (no: 36/Ethical Approval/FKGUI/V/2019). Written informed consent was obtained from each patient. In this cross-sectional study, the subjects were elderly people from Dinas Sosial Panti Werdha Budi Mulia 3 and patients who came to the Dental and Oral Educational Hospital Universitas Indonesia (RSGM-P FKG UI) between April and July 2019. The inclusion criteria were ages between 60 and 74 years, a diagnosis of periodontitis (pocket depth of 5 to 6 mm), a history of diabetes mellitus and/or a random blood glucose level of 200 mg/dL or higher, and willingness to be a research subject and to sign the informed consent form. The exclusion criteria were the use of antibiotics in the preceding month, periodontal therapy in the preceding 3 months, alcoholism, and absence of

all teeth (edentulousness). We identified 100 people who were willing to participate in this study, but only 33 without diabetes mellitus and 18 with diabetes mellitus met all the criteria.

Interviews, clinical intraoral examinations, and sample collections were conducted for all subjects. Three professionally trained dentists performed the clinical examination and sample collection. All of them were unaware of patients' assignments to interventions. All subjects underwent a full mouth examination for periodontal status with regard to pocket depth, papilla bleeding index, plaque index, calculus index, Oral Hygiene Index (**OHI**), and debris index at six sites (buccal–mesial, midbuccal, buccal–distal, palatal/lingual–mesial, midpalatal/lingual, and palatal/lingual–distal) of the teeth.

The samplings of TNF-α were obtained from the subjects' gingival crevicular fluid (GCF). GCF was obtained by gentle insertion of three #30 sterile paper points into the site with the deepest pocket. After 20 s, the three paper points were immediately placed in a microcentrifuge tube containing 1 mL of phosphate-buffered saline. All samples were stored at − 20°C immediately after collection until they were analyzed.

The obtained samples were then analyzed in Integrated Laboratory Faculty of Medicine Universitas Indonesia with the Human TNF-α enzyme-linked immunosorbent assay kit (catalog no. E-EL-H0109; Elabscience, Houston, TX, USA), according to the manufacturer's instructions. First, 100 µL of standard or sample was added to each well and incubated for 90 min at 37℃. Next, liquid was removed from each well, and 100 µL of Biotinylated Detection Ab was immediately added to the solution remaining in the well. The wells were placed in an incubator for 1 h at 37℃. The solution was aspirated or decanted from each well, and then 350 uL of wash buffer was added to each well, the solution was replaced by wash buffer. The contents of the well were allowed to soak for 1 to 2 min and then aspirated or decanted from each well; the wells were patted dry with clean absorbent paper. This wash step was performed three times. Then, 100 µL of horseradish peroxidase conjugate was placed in each well, incubated for 30 min at 37℃, aspirated, and washed five times. Next, 90 µL of substrate reagent was added to the solution, and the solution was incubated for 15 min at 37℃. Then, 50 µL Stop Solution was added to each well. We then determined the optical density value of each well at once with a microplate reader set to 450 nm. The differences in TNF-α levels were analyzed with SPSS 22.0. We performed tests to check normality of the data, and, depending on the results, we used either the Mann–Whitney *U* test or an independent *t*-test.

Results

Eighteen male and 31 female patients with an average age of 65.47 ± 5.75 years were recruited for this study. Table 1 presents the demographic data of the participants.

We compared each variable (pocket depth, bleeding index, plaque index, calculus index, debris index, OHIS index, and TNF-α level) between patients with and without diabetes. We used the Shapiro–Wilk test of normality because each patient group had fewer than 50 participants (Table 2). The bleeding index for patients without diabetes, the plaque indexes for patients with and without diabetes, the calculus index for patients with diabetes, the debris indexes for patients with and without diabetes, the OHIS index for patients with diabetes, and TNF-α levels for patients with diabetes were normally distributed and analyzed with an independent *t*-test; the other data were nonnormally distributed and analyzed with the Mann–Whitney *U* test.

The Mann–Whitney *U* test was used to analyze the differences in pocket depth, bleeding index, calculus index, OHIS index, and TNF-α levels between patients with and without diabetes. Table 3 shows that patients with diabetes had deeper pocket depths, lower bleeding indexes, and a lower calculus index than did those without diabetes, but the differences were not significant (all $p > 0.05$). Patients with diabetes had significantly higher OHIS indexes and STNF-α levels than did those without diabetes (both ps < 0.05).

An independent *t*-test was used to analyze the differences in plaque index and debris index between patients with and without diabetes. Table 3 shows that patients with diabetes had lower plaque indexes and lower debris indexes than did those without diabetes, but the differences were not significant (both ps > 0.05).

We performed normality tests based on gender in elderly patients with both diabetes and periodontitis. We used the Shapiro–Wilk test of normality because each patient group had fewer than 50 participants. As shown in Table 4, both genders were normally distributed; therefore, we used an independent *t*-test to compare TNF-α levels by gender. Table 5 shows that female patients had higher TNF-α levels than did male patients, but the difference was not significant ($p > 0.05$).

Characteristic	Data
Age (years)	65.47 ± 5.75
Sex *(n)*	
Male	18 (36.7%)
Female	31 (63.3%)
Glycemic status	
Patients with diabetes	16 (32.65%)
Patients without diabetes	33 (67.35%)

Table 1. Demographic Data.

Index	*p*
Pocket depth for patients with diabetes	0.000
Pocket depth for patients without diabetes	0.000
Bleeding index for patients with diabetes	0.025
Bleeding index for patients without diabetes	0.207
Plaque index for patients with diabetes	0.208
Plaque index for patients without diabetes	0.403
Calculus index for patients with diabetes	0.939
Calculus index for patients without diabetes	0.006
Debris index for patients with diabetes	0.525
Debris index for patients without diabetes	0.285
OHIS index for patients with diabetes	0.471
OHIS index for patients without diabetes	0.020
TNF-α level for patients with diabetes	0.423
TNF-α level for patients without diabetes	0.022

Table 2. Normality Test Results in Elderly Patients with and without Periodontitis.
Shapiro–Wilk test; *$p \geq 0.05$ for normal data distribution.
OHIS, Oral Health Information Suite; TNF-α, tumor necrosis factor α.

Index	Mean (SD)		*p*
	Diabetes (*n* = 16)	Without Diabetes (*n* = 33)	
Pocket depth	5.31 (0.48)	5.09 (0.29)	0.051[a]
Bleeding index	1.36 (0.99)	1.63 (1.09)	0.405[a]
Plaque index	1.79 (0.77)	1.89 (0.71)	0.652[b]
Calculus index	1.31 (0.74)	1.87 (1.06)	0.114[a]
Debris index	1.15 (0.59)	1.41 (0.85)	0.278[b]
OHIS index	3.1 (1.25)	2.17 (1.29)	0.018[a*]
TNF-α level	5.04 (0.48)	4.67 (0.76)	0.028[a*]

Table 3. Comparative Analysis of Indexes in Elderly Patients with Periodontitis and With and without Diabetes.
[a]Mann–Whitney *U* test; *$p < 0.05$ indicates a significant difference.
[b]Independent *t*-test; *$p < 0.05$ indicates a significant difference.
OHIS, Oral Health Information Suite; SD, standard deviation; TNF-α, tumor necrosis factor α.

Gender	TNF-α Levels
Male	0.625*
Female	0.162*

Table 4. Results of Gender-Based Normality Tests of TNF-α in Elderly Patients with Periodontitis and Diabetes.
Shapiro–Wilk test; *$p \geq 0.05$ for normal data distribution.
TNF-α, tumor necrosis factor α.

TNF-α Levels (µg/mL)	Mean (SD)	*p*
Gender		0.557
Male (*n* = 6)	4.71 (0.5)	
Female (*n* = 10)	4.84 (0.14)	

Table 5. Comparative Gender Analysis of TNF-α Levels in Elderly Patients with Periodontitis and Diabetes.
Independent *t*-test; *$p < 0.05$ indicates a significant difference.
SD, standard deviation; TNF-α, tumor necrosis factor α.

Discussion

Obtaining oral fluids such as GCF and saliva is an easy, noninvasive method for examining host molecular immunity and bacterial components commonly used as biomarkers. Biomarkers of oral fluids can reflect various physiological and pathological oral conditions. Saliva sampling is easier, practical, and noninvasive and does not require special material or expertise, and even patients can do it alone. GCF sampling requires more sampling time, and the samples can be contaminated with blood, saliva, and plaque products. However, we preferred collecting GCF samples because the biomarkers in GCF are more site-specific than are those in saliva.[9]

In this study, patients with diabetes had significantly higher TNF-α levels than did patients without diabetes. This result is similar to those obtained by Singh et al., Acharya et al., and Omneya et al.[9,11,12] and may suggest that TNF-α is potentially an inflammatory biomarker. In diabetes, advanced glycation end-products that are responsible for diabetic collagen crosslinks are formed, which could bind to macrophage receptors and induce a cycle of cytokine (IL-1 and TNF-α) upregulation.[9] Elevation of TNF-α levels may contribute to the increased prevalence and severity of periodontal diseases found in numerous studies of populations of people with diabetes.[8] In this study, male patients

with diabetes had higher TNF-α levels than did female patients, but the difference was not significant. Cartier et al. found that premenopausal women had lower plasma TNF-α concentrations than did men.[13]

We excluded patients with alcoholism in this study because Heberlein et al.'s findings supported an association between TNF-α level and alcohol consumption.[14] None of their subjects was being treated with insulin, but one was treated with metformin. These medications may exert an anti-inflammatory effect, but according to the literature, this has not been confirmed.[11] In our study, subjects with any acute and chronic systemic conditions were not excluded; however, some studies have suggested that diseases such as bronchiectasis, asthma, atherosclerosis, and inflammatory bowel disease might elevate TNF-α level.[9] Therefore, a limitation of this study was that there might have been selection bias.

We also did not analyze other factors such as body mass index, smoking, and glycemic status (degree of control of hyperglycemia). Swaroop et al.[15] demonstrated a significant correlation between TNF-α levels and body mass index (p = 0.006); the correlation was stronger in male subjects than in female subjects. Petrescu et al.[16] showed that TNF-α serum levels were significantly higher in patients who smoked than in patients who did not smoke, and the serum TNF-α concentration was higher in subjects who smoked more than one pack than in those who smoked less than one pack per day. The serum level of TNF-α was also positively correlated with exposure to tobacco smoke. Soorya et al.[17] and Jaganath and Vijayendra[18] revealed that the mean TNF-α values increased with the decline in glycemic control; that is, levels of TNF-α were highest in individuals with poorly controlled diabetes mellitus, lower in patients with moderately controlled diabetes, and lowest in patients with well-controlled diabetes. Therefore, further studies with larger sample sizes and the assessment of more confounding factors are needed to confirm the results of this study.

Conclusions

In comparison with patients who did not have diabetes, those who did had significantly higher levels of TNF-α. Thus, TNF-α level has strong potential as a biomarker; however, further studies with larger sample sizes and the assessment of more confounding factors are needed.

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Declaration of Interest

There are no conflicts of interest.

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