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Original

IL-6 levels in gingival crevicular fluid (GCF) from patients with non-insulin dependent diabetes mellitus (NIDDM), adult periodontitis and healthy subjects

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Abstract: Cytokines play an important role in the pathology associated with chronic inflammatory diseases. One of these cytokines, interleukin 6 (IL-6) is a major mediator of the host response to tissue injury, infection and bone resorption. In the present study, gingival crevicular fluid (GCF) level of IL-6 was determined in patients with non-insulin dependent diabetes mellitus (NIDDM) with periodontitis, adult periodontitis, and healthy controls by use of an enzyme linked immunosorbent assay (ELISA). Twenty-four NIDDM patients with periodontitis, twenty-four adult periodontitis and twenty-four healthy controls were selected for the study. GCF sampling was performed on the vestibular aspects of maxillary incisors and canine teeth. Plaque index (PI), gingival index (GI), gingival bleeding time index (GBTI), probing depth (PD) and probing attachment levels (PAL) were recorded from each sampling area and also the entire dentition. NIDDM and adult periodontitis patients had numerous sites with radiographic evidence of alveolar bone resorption, loss of attachment and pocket depth greater than 3 mm. The mean GCF IL-6 level was 2.43 ± 0.97 ng/ml in NIDDM patients, 1.31 ± 0.92 ng/ml in adult periodontitis and 0.62 ± 0.58 ng/ml in healthy subjects, respectively ($p < 0.05$). GCF IL-6 levels were markedly higher in NIDDM and adult periodontitis groups compared to the healthy controls. No correlation was found between GCF IL-6 levels and all clinical parameters. These findings suggested that GCF IL-6 levels were significantly higher in the area of inflammation and periodontal destruction locally. The high IL-6 levels in NIDDM patients might be due to different microbial flora in periodontal pockets and altered immune system. Future studies are needed to evaluate the complex interaction among IL-6 GCF levels, host response and local microbial environment in the NIDDM patients. (J. Oral Sci. 41, 163-167, 1999)

Key words: gingival crevicular fluid; interleukin-6; periodontal diseases; non-insulin dependent diabetes mellitus.

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Introduction

In recent years, the etiology of periodontal tissue breakdown is primarily attributed to the interaction of bacterial antigens and inflammatory cells resulting in the production of cytokines (1,2). IL-1 alpha, IL-1 beta, IL-6 and tumor necrosis factor alpha are proinflammatory cytokines that have been identified in gingival crevicular fluid (GCF) (3-7).

IL-6 plays a major role in the terminal differentiation of B-lymphocytes to plasma cells, which are the predominant inflammatory cells in tissues involved in established and advanced periodontal disease and also enhances T cell proliferation and bone resorption (8-10). IL-6 stimulates DNA synthesis and inhibits collagen and non-collagen protein synthesis by osteoblasts, suggesting a direct role for IL-6 in regulation of bone formation (11). There is a significant correlation between tissue levels of IL-6 and the severity of the coincident inflammation (12). IL-6, synergistically with IL-1 beta, stimulates the recruitment and bone resorption by osteoclasts (4,5,10). However, the role of IL-6 in the etiology of periodontal disease remains unclear and its role in the resorption of alveolar bone to periodontitis is not specifically determined (6).

Two basic types of primary diabetes mellitus, insulin dependent (type I) and non-insulin dependent (type II), have been described. Type II, non-insulin dependent diabetes mellitus, may develop over a period of time and is often called adult-onset diabetes (13). Improved methods of assessing metabolic control in diabetics, assessing periodontal status, microbial risk factors and risk indicators in GCF, and analysis have provided new information to clarify the relationship between diabetes and periodontitis (13,14). A number of studies reported a higher incidence and severity of periodontal disease in diabetic patients as compared with non-diabetic subjects (15-17).

The aim of the present investigation was to determine IL-6 GCF levels and the possible relation between periodontal status and IL-6 GCF levels in NIDDM patients with periodontitis, adult periodontitis and healthy controls.

Materials and Methods

Patient selection

Twenty-four NIDDM patients with periodontitis (age between 37-65), 24 adult periodontitis (age between 35-60) and 24 healthy controls (age between 17-22) were selected from those referred to the Department of Periodontology of the Dentistry Faculty of Gazi University. Diabetic status of the patients is determined by the results of a modified 2-hour oral glucose tolerance test performed according to the World Health Organization criteria (18). Diabetes diagnosis is achieved if the 2-hour glucose is > 11.1 mM. Periodontal disease status was determined from clinical (attachment loss and pocket depth greater than 3mm) and radiographic records. Diabetic patients were selected from patients both with history of non-insulin dependent diabetes mellitus and adult periodontitis. Care was taken to ensure that none of the patients had received any antimicrobial agents and periodontal treatment in the previous six months. The controls were selected from periodontally-healthy subjects. The informed consent of all subjects was obtained and GCF sampling procedures had been fully explained before the study.

Clinical studies

Plaque and Gingival Indices of Löe and Sillness (19) and Bleeding Time Index (20) of Nowicki et al. were recorded in each patient. In addition to the above indices, probing depths and probing attachment levels were also measured with a Williams probe calibrated in millimeters. The indices were recorded for the GCF sampling area and also for the entire dentition after the collection of GCF.

GCF collection

GCF sampling was performed on the vestibular aspects of the maxillary incisors and the canine teeth, according to the method of Rudin et al. (21). The experimental area was isolated with cotton rolls and gently air dried. GCF was collected by use of standardized (2 × 10 mm) paper strips with a 1mm notch at their tips. The pre-weighed strips were carefully inserted at the orifice of the gingival pocket of the appropriate sites and allowed to remain until the wetness on the strip was observed. Strips contaminated by bleeding were discarded. The amount of GCF on the strips was measured by weighing the accumulated fluid. Strips from individual sites were placed into coded sealed plastic tubes and weighing was repeated immediately after collection. Tubes were then wrapped in aluminum foil and stored at -70 °C until cytokine analysis.

ELISA assay

Prior to the quantification of IL-6, paper strips were thawed and GCF was eluted from the strips by placing each strip in 300 μl of PBS containing 0.05 % Tween 20, 0.5 % BSA and 0.01 % thimerosal for 2 h at room temperature. The commercial human IL-6 ELISA kit (Bender Med System, Vienna, Austria) was used in order to measure the level of IL-6. Before this assay was performed, the microwell strips were washed twice with approximately 300 μl washing buffer (PBS with 1 % Tween 20).

After washing each sample, standard and optional control sample were assayed in duplicate. For this study, the commercial test protocol was used, and the absorbances were measured at 450 nm. A 620 nm filter was used as reference wave length. The concentrations of the samples were determined by extrapolation from a standard curve constructed using known standard concentrations. IL-6 concentrations of the GCF were calculated by dividing the ELISA concentration values by the GCF volumes. The mass of the fluid on each strip was converted to a volume in ml by assuming that the density of GCF was 1 and mass (mg) was converted to the volume (ml). The IL-6 levels were expressed as ng IL-6 / ml of GCF.

Statistical analysis

The correlation for each clinical parameter and GCF IL-6 levels for each group were calculated by one-way variance analysis. The correlation between IL-6 levels and each clinical parameter for each group (only for sampling area) was calculated by Kruskal Wallis variance analysis.

Results

Data regarding the mean GCF IL-6 levels and amount of GCF volume for each group are given in Table 1. Statistical differences were found among the three groups ($p < 0.05$) with regard to IL-6 levels and GCF volume. IL-6 levels in NIDDM patients with periodontitis group were significantly higher than for the adult periodontitis and healthy groups ($p < 0.05$).

No statistical correlation was found between the NIDDM and the adult periodontitis group for GCF volumes ($p > 0.05$). However, statistical differences were found between the control group and the other two diseased groups for GCF volumes ($p < 0.05$).

Table 1 The mean IL-6 levels and GCF volumes in NIDDM patients with periodontitis, adult periodontitis and healthy controls.

Groups	IL-6 levels (ng/ml)	GCF volumes (mg)
NIDDM patients (n=24) (age=37-65)	2.43 ± 0.97*	3.95±1.73*
Adult periodontitis (n=24) (age=35-60)	1.31 ± 0.92	4.75±2.14
Healthy controls (n=24) (age=17-22)	0.62 ± 0.58	2.59±0.63

*Mean ± standard error of the mean.

Statistical differences were found among three groups in terms of IL-6 levels ($p < 0.05$). No statistical differences were found between two diseased groups ($p > 0.05$) and statistical differences were found between healthy and diseased groups in terms of GCF volumes ($p < 0.05$).

Table 2 showed the mean clinical parameters of entire the dentition for each group. No statistical differences were found between the two diseased groups ($p > 0.05$). Statistical differences were found between the healthy and diseased groups ($p < 0.05$).

Table 2 The mean clinical parameters of three groups (entire dentition)

Groups	Probing depth (mm)	Attachment loss (mm)	Gingival index (GI)	Plaque index (PI)	Gingival bleeding time index (GBTI)
NIDDM patients (n=24)	3.96±0.59*	3.15±0.95*	1.70±0.17*	1.90±0.44*	2.17±0.31*
Adult periodontitis (n=24)	3.71±0.63	3.21±1.29	1.72±0.19	2.03±0.35	2.15±0.34
Healthy controls (n=24)	1.98±0.27	0.00±0.00	0.22±0.13	0.24±0.11	0.04±0.02

*Mean ± standard error of the mean.

No statistical differences were found between two diseased groups in terms of any of the parameters ($p > 0.05$). Statistical differences were found between diseased and healthy groups ($p < 0.05$).

The mean clinical parameters of sampling area for each group are given in table 3. No statistical differences were found between two diseased groups ($p > 0.05$). Only statistical differences were found between diseased and healthy groups ($p < 0.05$).

Table 3 The mean clinical parameters of three groups (sampling area)

Groups	Probing depth (mm)	Attachment loss (mm)	Gingival index (GI)	Plaque index (PI)	Gingival bleeding time index (GBTI)
NIDDM patients (n=24)	4.15±0.52*	3.28±1.01*	1.81±0.35*	1.97±0.42*	2.22±0.40*
Adult periodontitis (n=24)	4.01±0.47	3.31±0.86	1.75±0.22	1.95±0.44	2.19±0.52
Healthy controls (n=24)	1.90±0.34	0.00±0.00	0.22±0.11	0.23±0.10	0.05±0.05

*Mean ± standard error of the mean.

No statistical differences were found between two diseased groups in terms of any of the parameters ($p > 0.05$). Statistical differences were found between diseased and healthy groups ($p < 0.05$).

The relationship between the clinical parameters and IL-6 levels for sampling area are explained with symboles in table 4. A positive correlation was found between probing depth and plaque index, and between gingival index and bleeding time index in the NIDDM group. In the adult periodontitis group, a positive correlation was found between probing depth and probing attachment level, and between gingival index and bleeding time index. No statistical differences were found between the IL-6 levels and clinical parameters.

Table 4 Correlations between clinical parameters and IL-6 levels in three groups for sampling area

Groups	Probing depth (mm)	Attachment loss (mm)	Gingival index (GI)	Plaque index (PI)	Gingival bleeding time index (GBTI)	IL-6 levels (ng/ml)
NIDDM patients (n=24)	¶	NS	‡	¶	‡	NS
Adult periodontitis (n=24)	**	**	§	NS	§	NS
Healthy controls (n=24)	NS	NS	NS	NS	NS	NS

Same symbols show the positive correlation between two parameters.

‡ Positive correlation between probing depth and plaque index in NIDDM group ($p < 0.05$).

¶ Positive correlation between gingival index and gingival bleeding time index in NIDDM group ($p < 0.05$).

** Positive correlation between probing depth and attachment loss in adult periodontitis group ($p < 0.05$).

§ Positive correlation between gingival index and gingival bleeding time index in adult periodontitis group ($p < 0.05$).

No correlation was found between GCF IL-6 levels and the clinical parameters. NS. Not significant

Discussion

Studies have reported that diabetes clearly increases the risk of severe periodontitis and the incidence of periodontal disease progression by approximately 2-3 fold (13,22,23). Increased periodontal disease severity has been reported to occur in diabetic patients. A study of 254 Pima Indians with Type II diabetes mellitus found that they were 3 times more likely to have alveolar bone loss than non-diabetics (14).

Recently, many immunological and microbiological studies have been performed in diabetic patients to evaluate the relationship between diabetes and periodontal diseases (13,15,16,24). Several studies have demonstrated decreased chemotaxis, adherence and phagocytosis of pheripherial blood leukocytes in diabetic patients (15,24-26). A normally functioning immune system is an effective defense against any invading organisms and a defective immune system results in disease. Diabetes mellitus alters the resistance of periodontal tissues and makes them prone to invasion by microorganisms. In addition the crevicular space in patients with NIDDM may comprise a unique environment favoring an anaerobic microbial ecology (25). It has been reported that paralleling serum glucose levels and gingival crevicular fluid glucose levels are elevated in NIDDM and may be twice that of normal subjects (27). Zambon et al. reported that these biochemical changes and increased amounts of glucose levels in the crevicular fluid may cause the growth of facultative or anaerobic microorganisms (25). Microbiologic and immunologic data from this previous study suggested that *P. intermedia*, *W. recta*

and *P. gingivalis* are important in the etiology of periodontitis in adult patients with NIDDM. In the present study, GCF IL-6 levels were determined in the patients with NIDDM, adult periodontitis and healthy controls at the beginning of the treatment. After the collection of the GCF, periodontal treatment was performed but no GCF sampling was repeated after the treatment. The patients who were participants in this study were not using any hyphoglicemic agents, they were controlling their blood sugar level by diet alone.

We could find no reports about the GCF IL-6 levels of the NIDDM patients with periodontitis in previous studies. However, Reinhardt et al. (4) studied the GCF levels of IL-1 beta and IL-6 in such subjects compared to premenopausal and postmenopausal estrogen treated females with moderate-severe periodontitis. Thirteen premenopausal or postmenopausal females on estrogen therapy (ES) and 13 postmenopausal females not on estrogen supplements (ED) were evaluated. IL-6 was detected more frequently in ED subjects (without estrogen therapy). In the another study, Reinhardt et al. (5) evaluated GCF IL-1 and IL-6 levels in refractory periodontitis. They found that gingival IL-1 and IL-6 production is different in response to local and systemic factors associated with periodontitis and sites from refractory patients produced significantly more IL-6 versus stable periodontitis. Geivelis et al. (3) reported the measurements of IL-6 in GCF from adults with destructive periodontal disease. Their results indicated that the amount of IL-6 in the GCF samples from diseased sites were markedly higher than the tested sites. In our study, IL-6 levels of NIDDM patients with periodontitis were higher than adult periodontitis and healthy controls ($p < 0.05$). Ohno et al. (16) studied the in vitro production of IL-1 , IL-6 and tumor necrosis factor-alpha in insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus patients (NIDDM). They reported that the production of IL-1 and IL-6 by monocytes was significantly lower in IDDM patients than in NIDDM patients.

In the present study, we found no correlation between the clinical parameters and IL-6 levels. This could be related to the limited area of GCF sampling. In order to eliminate the risk of contamination with saliva, GCF was only collected from the vestibular aspects of maxillary incisors.

Although similar clinical parameters were found between the NIDDM and adult periodontitis patients, the higher levels of IL-6 in NIDDM patients can be explained by different and complex host response and the local microbiological challenge. In order to better understand the role of high IL-6 levels in pathogenesis of periodontal breakdown in NIDDM patients, further studies are needed.

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