

Glycemic Control and Interleukin-1 β in Periodontal Patients with Type 1 and 2 Diabetes in a Group of Subjects in South-West Romania

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Interleukin-1 β is an important mediator of the inflammatory response and is involved in the pathophysiology of periodontitis. The purpose of the study was to investigate the influence of periodontal disease and glycemic control on biomarker levels in crevicular fluid. Patients with aggressive periodontitis were characterized by a higher ratio of IL-1 β compared to patients with chronic periodontitis.

Keywords: periodontitis, diabetes mellitus, interleukin-1beta, crevicular fluid.

Periodontal disease result from interactions of specific subgingival microbial species and sensitive host, leading to the release of inflammatory products that mediate tissue destruction[1]. Periodontal infections are specific, gram-negative, bacterial anaerobic infections leading primarily to the destruction of connective tissue and then to the alveolar bone, the substrate supporting the teeth[2].

Periodontal diagnosis generally requires appreciation of periodontal tissue destruction (gingival trench depth and clinical attachment level) and gingival inflammation (bleeding at the sounding and gingival index). Although the techniques used are simple and noninvasive[3], these parameters are static and reflect the history of the disease without showing the activity level of the disease[4]. Therefore, it is necessary to perform diagnostic tests that can identify periodontal active sites with the evaluation of disease progression as well as the response to periodontal treatment[5].

Parodontopathogenic bacteria increase the risk of periodontitis by modifying immune responses against bacterial products and subsequent secretion of proinflammatory cytokines with crucial effects in the destruction of periodontal tissue [6,7].

The levels of biomarkers in crevicular fluid are potentially important as predictors of disease progression [44]. These molecular messengers are the determinants of effective innate and adaptive responses and allow for fine-tuning the interaction between the various components of the immune system[8]. Changes in cytokine levels appear to be guided by the microbiota present at each stage of periodontitis; however, there are very limited data on changes in cytokine levels relative to the total mass and composition of subgingival biofilms. It is also recognized that subgingival biofilms exhibit great variability in their microbial composition [9].

Sites next to different teeth or even on the same tooth may differ considerably in the levels and proportions of colonization of bacterial species[1]. Clarification of the key immunological mechanisms involved in the response to polydimorphic biofilms associated with periodontal disease could lead to the development of new therapies for periodontal infections. Previous trials of interaction investigation between subgingival and host species have focused on examining the effects of single bacterial species

or models in-vitro of single mixed biofilms composed of several species on different types of host cells[10].

Interleukin-1 β (IL -1 β) is an important mediator of the inflammatory response and is involved in cellular proliferation, differentiation and apoptosis, and in the pathophysiology of periodontitis[11].

Several studies have reported a correlation between the inflammatory status of periodontal disease and elevated levels of pro-inflammatory cytokine crevicular fluid such as IL-1 β , TNF- α and arachidonic acid metabolites such as prostaglandin E2 (PGE2). PGE2, the cyclooxygenase metabolite, is the most potent mediator in alveolar bone loss in a periodontitis. PGE2 is known to have activity on fibroblasts and osteoclasts, induces matrix metalloproteinase (MMPs), IL-1 β and other cytokine matrix synthesis. An increased level of PGE2 was detected in gingival tissue and crevicular fluid in proportion to the severity of periodontal disease[12].

Diabetes mellitus is a heterogeneous group of metabolic disorders associated with metabolism of carbohydrates, fats and proteins.

The new data support the idea that in the periodontitis associated with diabetes, the modified inflammatory response of the host plays a critical role[13].

According to previous studies, the prevalence of periodontitis in patients with type 1 diabetes aged between 13 and 18 is about 9.8% [14], while in the 20-29 age group the rate increases to 40%[15].

Since type 1 diabetes develops generally at a very early stage of life, there may be a high probability of severe periodontitis, with early onset, leading to tooth loss if not detected quickly enough. Moreover, periodontitis can affect metabolic control and adequate treatment of diabetic patients, and is associated with a reduction in glycosylated hemoglobin levels[16,]. Progression to a severe periodontitis with loss of support structures and periodontal remodeling during orthodontic movements are mediated by other factors, including the selection of a broad spectrum of inflammatory and destructive mediators such as cytokines (TNF α , IL-1 β and IL-6), chemokine (IL -8), acute phase proteins and MMPs[17,18], and prostaglandin E2 (PGE2)[19,20]. Furthermore, increases in IL-1 α and serum TNF- α have been shown to cause changes in lipid metabolism leading to hyperlipidemia[21]. TNF- α is a 157-

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amino acid cytokine that is known to play a central role in lipid and glucose metabolism and is produced by a wide variety of cell types[22].

Type 2 diabetes is characterized by elevated blood glucose levels due to increased glucose production in the liver and increased peripheral insulin resistance, which could lead to a reduction in insulin secretion[23]. Monocytes from diabetic patients produce significantly higher amounts of IL-1 β and PGE2 in vitro than non-diabetic controls. It is assumed that type 2 diabetes produces changes in crevicular fluid in inflammatory mediators, this being part of the mechanisms by which diabetes affects periodontal health.

The excessive production of some mediators such as interleukin-1- β , tumor necrosis factor and prostaglandins generates a persistent inflammatory process that is the cause of tissue destruction[24].

The purpose of the study was to investigate the influence of periodontal disease and glycemic control on biomarker levels in crevicular fluid by comparing the levels in subjects with aggressive periodontitis and type 1 diabetes with the levels in subjects with chronic periodontitis and type 2 diabetes in a group of patients from south-west Romania.

Experimental part

A group of 19 patients from South-West Romania with periodontal disease was divided in two subgroups. The first subgroup (G1) – 8 patients with type 1 diabetes and aggressive periodontitis ages 21-29 years, the second (G2) – 11 patients with chronic periodontitis and type 2 diabetes between the ages of 47-54 years. The study was approved by the Ethics Committee of University of Medicine and Pharmacy of Craiova, Romania. All patients came from the urban environment, none of whom do not have anti-inflammatory treatments that could affect their periodontal status at least six months before the study. Subjects were excluded if they had other systemic condition that would influence the course of the disease or periodontal treatment or medical conditions that would require antibiotic prophylaxis for routine dental procedures. According to the HbA1c, the groups were divided in 2 subgroups: in G1 group – G1G - 4 patients with HbA1c < 6.2 (good glycemic control) and G1P - 4 patients with HbA1c > 6.2 (poor glycemic control), in G2 group G2G - 4 patients with HbA1c < 6.2 (good glycemic control) and G2P - 7 patients with HbA1c > 6.2 (poor glycemic control).

The crevicular fluid sampling

After the isolation of each tooth with a cotton roll, the supragingival plaque was carefully removed without touching the marginal gingiva. The gum was then gently dried with an air syringe. The crevicular fluid was collected with a Periopaper strip at 1 mm subgingival for 30 s and then measured with Periotron 8000. The strips were immediately placed in microcentrifuge tubes and stored at -20 ° C until assays. The levels of IL1beta were assessed with ELISA test in gingival crevicular fluid, expressed in ng/ml and statistical analysis t-test was performed for statistical significance differences between the values in G1 and G2 groups and between subgroups (p < 0.05).

Results and discussions

IL1beta levels in the G1 group were 1.6 times higher than in the G2 group with the statistically significant difference (p < 0.05) (fig.1).

Statistical differences were found within the two groups G1 and G2 among the subgroups G1G/G1P and G2G/G2P as well as between G1P/G2P with p < 0.05 (fig.1).

Values in ng/ml in the four subgroups

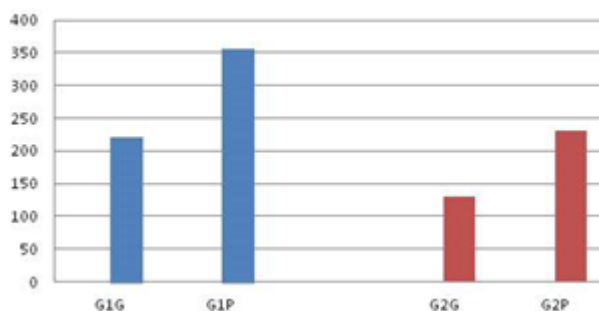


Fig. 1. Levels of IL1beta with statistical significant differences between G1G, G1P, G2G, G2P

Literature suggests that IL-1 β can be an important mediator of the loss of attachment in a periodontitis and indicates that its presence may be useful for localizing the activity sites of periodontal disease. There is some evidence that IL-1 β is produced by periodontal cells can be detected in gingival crevicular fluid. Levels of IL-1 β in crevicular fluid were quantified by ELISA. The mean IL-1 β concentrations in the type 2 diabetes, periodontal disease and control groups were 200.1 ± 65.34 pg/ μ L, 131.35 ± 67.66 pg/ μ L and 80.0 ± 36.08 μ g/ μ L. Levels in diabetic patients were significantly higher than in subjects with periodontal disease and control. There were no significant correlations between the IL-1 β level and any of the clinical data parameters for each group [25].

In a study conducted in 2014, the IL-1 β level was the highest in the chronic diabetic-periodontium group, with no statistical significance. A recent meta-analysis has shown that patients with type 2 diabetes with chronic periodontitis have been found to have significantly higher IL-1 β values in crevicular fluid than their systemic healthy counterparts[26].

The severity and extent of periodontitis were significantly higher in patients with diabetes than in non-diabetic patients. No significant differences in IL-1 α -899, -1 β + 3954 genotypes were found between diabetic patients and non-diabetic patients. The IL-1 α -889 TT genotype (1.20) genotype, the IL-1 β + 3954TT genotype (1.25) were significantly associated with periodontitis. The presence of a positive genotype for IL-1 was significantly associated with periodontitis (1.04 to 2.49) [27].

The level of IL-1 β and TNF- α gingival fluid in subjects with type 1 diabetes was significantly higher than in subjects with type 2 diabetes. In subjects with type 1 diabetes, significant negative correlations were found between the duration diabetes mellitus and IL-1 β and between diabetes mellitus and TNF- α [28].

A study of group of patients with type 1 diabetes indicated that IL-1 β levels were increased compared to healthy individuals and showed differences in groups at 7-21 days, while healthy patients showed increases in IL-1 β to 14-21 days (p < 0.05). Differences were seen in MMP-9 among patients with and without diabetes at 7-14 days (p < 0.05). The presence of orange complex species and plaque index measurements showed a superior correlation with biomarker levels compared to other clinical complexes or measurements during experimental gingivitis [29].

IL-1 and IL-18 concentrations were higher in gingival fluid in patients with periodontitis than in patients with gingivitis. IL-18 concentrations were greater than those of IL-1. In serum, very low levels of cytokines were found [30].

A study of a group of 60 patients divided into three groups (I-without periodontal disease, II-with gingivitis, III-with

periodontitis) in which samples were collected showed IL-1 β concentrations in gingival crevicular fluid at patients in group III statistically higher ($P < 0.0001$) than those in group II and the IL-1 β concentration in groups II and III is statistically much higher ($P < 0.0001$) than in group I subjects [31].

IL-1 β showed a significant positive correlation with loss of attachment ($r = 0.33$, $p = 0.035$). A dose-response relationship was observed between the severity of periodontitis and TNF- α ($p = 0.012$) [32].

The group of a study of diabetic patients showed lower levels of IL-1 β than the group of patients with periodontal disease ($p < 0.01$). The levels of PGE2, plasminogen activator (t-PA) and plasminogen 2 activator inhibitor (PAI-2) were similar for the groups with diabetes mellitus and periodontal disease ($p > 0.05$). PGE2, t-PA levels were higher in the diabetes and periodontal disease groups than the healthy patient group ($p < 0.05$). PAI-2 was higher in the diabetes group than the healthy patient group ($p < 0.05$) [33].

Higher levels of IL-1 β and IL-8 in clinically sound situations suggest that inflammatory mechanisms occur in gingival tissues before being clinically detected. In line with these findings, Zhang et al. (2002) [34] demonstrated in gingivitis that IL-1 β levels in crevicular fluid increased after only 3 days of plaque accumulation to the occurrence of clinical signs of inflammation. Our results are consistent with data from Engebretson et al. (2002) [35], which reported that subjects with severe periodontal disease had higher levels of IL-1 β in crevicular fluid in superficial sites compared to superficial sites in mild/moderate periodontal disease subjects. They concluded that IL-1 β expression in crevicular fluid was in part a host response, possibly explained by genetic mechanisms [36].

Periodontal clinical and glycemic (HbA1c) measurements were significantly correlated with IL-1 β from crevicular fluid. Patients with HbA1c greater than 8% had significant mean IL-1 β levels than patients with HbA1c less than 8% [37].

The results of a study indicate that all clinical indices, with the exception of plaque index, were significantly elevated in poorly controlled and well controlled diabetics compared to systemically healthy patients but only in subjects without periodontal disease ($P < 0.05$). Significant results were the association between periodontal disease and IL-1 β level in crevicular fluid, in non-periodontal patients, there was an insignificant increase [38].

Interleukin-1 β (IL-1 β) concentrations by ELISA in gingival crevicular fluid were 1.75 to 97.13 $\mu\text{g}/\mu\text{L}$ in patients with chronic periodontitis. The crevicular fluid was collected for 5 s on filter paper and a second sample was collected for 30 s 1 min later. There were no statistically significant correlations with plaque index, bleeding index, or depth at probing [39].

In the case of diabetic subjects, people with moderate to severe periodontitis have a significantly higher PGE2 and TNF- α monocyte secretion after challenge with LPS and significantly higher levels in PGE2 and IL-1 β crevicular fluid compared to gingivitis patients or periodontal disease in lighter forms [13]. Cytokines profile was determined also in other pathologies like colorectal cancer [40-42].

In contrast, others failed to confirm an association between IL-1 β levels and diabetes in people with periodontal disease [43]. The subject remain in debate, levels of this interleukin in different groups of population must be determined, with a larger number of patients.

Conclusions

Subjects with aggressive periodontitis and diabetes type 1 with poor glycemic control were characterized by a higher ratio of IL-1 β compared to those with chronic periodontitis and type 2 diabetes.

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