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EXPRESSION OF IL6 IN PERIODONTAL AFFECTED SITES WITH DIFFERENT ATTACHMENT LOST.

ЕКСПРЕСИЈА НА IL6 ВО РЕГИИ СО РАЗЛИЧНО ИЗРАЗЕН ГУБИТОК НА ПРИПОЈ

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Abstract

Introduction: Adult periodontal disease is characterized by a chronic inflammatory process that destroys the supporting tissues of the teeth. It has been proposed that periodontal pathogens stimulate the inflammatory cytokine expression. Some cytokines and their role during periodontal disease have been thoroughly investigated, but the expression of IL6 in our population has never been investigated. **Objective:** Our objective was to study the levels of IL6 in gingival tissue from sites with different attachment loss.**Material and methods:** Gingival tissue biopsies were obtained from 30 patients. Patients with different degrees of attachment loss and with no gingival recession were selected. All examined patients were between 20 and 40 years old and they all had regions with healthy periodontal tissue. Gingival tissue biopsies were taken from the examined region and the healthy region of each patient. Obtained samples were frozen on -80 °C. Using ELISA method, the levels of the examined cytokine was determined in frozen tissue sections. **Results:** Analysis of the values of the examined cytokine led to the conclusion that IL6 levels exhibited no statistical difference concerning attachment loss or gingival inflammation. Statistical significances were noted only using ANOVA for the groups. **Conclusion:** Regarding the role of IIL6 in periodontal disease, our results pointed out that for bone destruction, gingival inflammation, attachment loss during periodontal disease are not associated with elevated levels of IL6 in our population. **Key words:** periodontal disease, cytokines, IL6, inflammation, attachment loss.

Апстракт

Вовед: Пародонтопатија се вбројува во инфламаторните процеси, која покрај разрушувањата на ткивата на пародонтот, се карактеризира и со експресија на за неа карактеристични цитокини. И покрај тоа што одредени цитокини се карактеристични за пародонталната афекција, сепак нивното присуство кај нашата популација досега не е испитувано. **Цел:** Целта на нашето испитување беше да се докаже присуството на IL6 во регии со различен губиток на припој. **Материјал и метод:** Испитувањето е спроведено кај 30 пациенти со различен степен на пародонтална деструкција, без гингивална рецесија, на возраст помеѓу 20-40 години. Пациентите покрај здравствената состојба беа селектирани и така да секој има барем една здрава регија. По земената согласност и запазените етички принципи кај пациентите беше земен ткивен исечок од засегнатата и здравата регија. Исечоците беа замрзнати на -80 °C. По хомогенизацијата со помош на ELISA method system Biotrak™ од Amersham Pharmacia Biotech беа одредени количествата на IL6 следејќи ги упатствата на производителот. **Резултати:** По анализата на добиените резултати не добивме статистички сигнификантна разлика на вредностите на IL6 кај региите ниту со различен губиток на припој, ниту со различно изразена гингивална инфламација. **Заклучок:** Резултатите не наведуваат на заклучок дека гингивалната инфламација и различната пародонтална деструкција кај нашата популација не е поврзани со зголемени количества на IL6. **Клучни зборови:** пародонтална деструкција, цитокини, IL6, инфламација.

Introduction

The periodontium is a complex tissue structure comprised of resident cells, including epithelial cells, fibroblasts and bone, as well as various types of inflammatory cells, which emigrate from the microvasculature of the gingiva in response to plaque accumulation. In response to initial stimulation, resident cells in the gingival tissue release various cell communication signals in form of chemical cytokines. Individually or collectively, these molecules participate in the resolution or destruction of periodontal tissues.

Large amounts of evidence indicate that cytokines released during periodontal affection are primarily responsible for the course of the disease[1,2], due to their mediating role during inflammation. Among the various types of cytokines, interleukins play a significant role during chronic inflammatory responses by transmitting messages between different types of leukocytes[3,4,5].

Cytokines released in the earliest stages of disease determine the type of immune response (6)

Since the first cytokines discovered originated from leucocytes, they were referred to as lymphocytes or interleukines. Different types of cytokines belong to this

group, such as, growth factors responsible for anabolic events in the tissue, chemokines responsible for tissue cell migration, interferons responsible for lymphocyte activaton, and tumor necrosis factor with pleotropic effects.

The profile of cytokine secretion depends on numerous circumstances, including local and individual genetic factors.

Numerous cytokines are closely associated with chronic periodontal disease. Elevated levels of IL1 are considered to be a risk factor for disease severity. Il6 is traditionally believed to be associated with gingival tissue inflammation and progressive bone resorption, but resent data contradicts the conventional belief. Elevated levels of IL6 in some studies have been proven to have protective effects on periodontal tissues.

Lack of information regarding IL6 expression in our population during periodontal disease prompted us to conduct the present study, the primary objective of which was to:

Determine the expression of IL6 in gingival tissue in our population at different stages of attachment loss during periodontal disease.

Material and methods

Examinations were conducted on 30 patients with diagnosed periodontal disease at the clinic of Periodontology and oral disease in Skopje. All of the patients met the following criteria:

- Age between 20 and 40 years old
- Absence of any kind of systemic disease
- Clinically diagnosed adult periodontal disease according to the criteria proposed from the American academy of Periodontology (7)
- Presence of regions with inflammation - free tissue
- Gingival margin positioned on or above the cement enamel junction.

According to attachment loss, the patients were divided into 3 groups

- **First group** - patients with attachment loss not greater than 3mm
- **Second group** - patient with attachment loss between 3and 6 mm
- **Third group** - patient with attachment loss equal or greater then 6mm

According to the criteria proposed by Silness-Loe, gingival inflammation and gingival bleeding were also recorded for every patient.

Tissue samples were obtained from the affected region, as well as from the healthy ones for each patient.

Tissue samples were frozen at - 80 °C. After tissue homogenization Il6 was detected by using ELISA method system Biotrak™ from Amersham Pharmacia Biotech. All assays were conducted in accordance with the manufacturer's instructions. All investigations regarding tissue samples homogenization, safe keeping and detection of Il6 were preformed at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, "Ss. Cyril and Methodius" University, Skopje, Republic of North Macedonia. Results were expressed as picograms of cytokine per milligram of tissue.

Statistical evaluation of data was performed with computer program Statistic 6, 0 using Student t Test in order to establish differences between healthy and diseased tissue samples and ANOVA in order to establish differences within the groups.

Results

The results obtained for IL6 in tissue samples were as follows:

- For healthy gingiva –0,286±0, 54 picograms of cytokine per milligram of tissue
- For attachment loss of 3mm-0,163±0,2659 picograms of cytokine per milligram of tissue
- For attachment loss between 3-6mm-0,142±0,14 picograms of cytokine per milligram of tissue
- For attachment loss over 6mm-0,204±0, 21 picograms of cytokine per milligram of tissue

No statistical significance was established between the healthy and diseased tissue samples (Figure 1.)

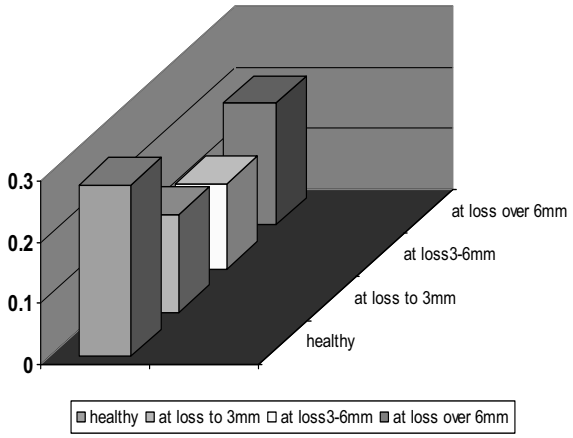

Figure 1. *Average level of IL6 in tissue samples from periodontally affected patients adherent to sites with different attachment loss*

Lack of statistical differences, imposed the need to regroup the obtained data according to the level of tissue inflammation recorded. There were no statistically significant differences between the different degrees of inflammation nor between the healthy and the diseased (Table 1).

Table 1. Averege level of IL6 in tissue samples from periodontally affected patients with different gingival inflammation (according to Sillnes-Loe index(S.L) for gingival inflammation)

	X	SD	df	t	r	F	R
healthy	0.16	0.26					
S.L 1	0.28	0.76	29	0.52	0.37	7.41	0.09
S.L 2	0.49	0.22	27	0.91	0.44	18.1	0.08
S.L 3	0.23	0.11	24	0.77	0.60	1.38	0.54

Table 2. post hoc Scheffe`s test.

	SS	df	MS	F	p
Between groups	0,509791	2	0,254895	12,62974	0,004759
Into the group	0,141275	7	0,020182		
total	0,651066				

Statistical differences was established for ANOVA between the three groups for p=0,004. Results for ANOVA test and post hoc Scheffe`s test are presented in Table 2.

Discussion

Interleukin 6 is a product of lymphocytes, fibroblast and monocytes. IL6 realise can be elicited by LPS, IL1, and TNFalpha. Female hormones such as progesterone and estrogens can diminish IL6 synthesis. Takashaki et al.[8] have reported elevated levels of m RNA IL6 in diseased tissue principally associated with endothelial cells, fibroblasts and macrophages. In vitro, IL6 is a potent inducer of monocytic differentiation into multi-nucleated giant cells which are capable of resorbing bone. Yamazaki et al.[5] have reported an elevation of IL6 secreting cells in periodontitis compared to gingivitis.

Studies by Reinhardt[10] have demonstrated that IL6 is elevated in gingival cervical fluid of refractory patients as compared to stable adult periodontitis. The authors have also shown enhanced levels of IL6, IL1 and IL8 in estrogen-deficient women compared to estrogens supplemented women pointing out the association of these cytokines, estrogens and bone resorption.

IL6 is traditionally associated with periodontal disease. Ellis[10] indicates that elevated levels of IL6 in periodontal-affected tissue contribute to chronic inflammation by protecting the macrophages from apoptotic cell death. Elevated levels of IL6 during periodontal disease have been found in the studies of Yamazaki[5], Reinhardt[9] Balta[11] Shengnan Z[12].

Our results revealed no statistically significant differences between the healthy tissue samples and the samples from inflamed regions with different attachment loss. Lack of statistically significant differences may be due to variety of values of IL6 obtained from our patients and the higher values of standard deviation.

The possible explanation of our results may be found in the studies of Yumoto[13]. He points out that the cytokine profile of periodontal cells is generally the same, but varies based on the type of bacterial colonization, interaction between them and the host immune system.

Different microorganisms of dental plague induce varying levels of IL6 production in fibroblasts. Studies conducted in vitro confirm that a lower concentration of Treponema denticola icreases production of IL6, while a higher concentration inhibits the production of IL6[14]. Micro organisms which have the ability to produce trypsine like enzymes such as Porphiromonas gingivalis are capable of degrading IL6[15]. It is possible that such process had occurred in our material since we had no data regarding the microbial colonization of plague. Furthermore, the detection of various components in tissue samples represents a frozen moment in different stages of a very dynamic process, such as periodontal inflammation with no specific evidence of past or future events.

IL-6 has traditionally been regarded as a proinflammatory mediator, because it is induced by IL-1 and TNF-α, early in the inflammatory cascade, and because it stimulates the expression of acute-phase proteins. However, recent data demonstrate that IL-6 lacks many typical proinflammatory properties and also exerts a number of anti-inflammatory activities. Balto[16] in his study emphases that the anti-inflammatory properties of IL-6 predominate in inflammatory responses. Although the mechanisms of action have yet to be defined, they may involve either the

direct suppression of IL-1 or the induction of endogenous antagonists or inhibitors of IL-1. Individual host response, which some reports consider to be a characteristic of parts or entire populations, remains to be considered. Cytokines production is genetically determined, allowing for a similar response to different types of microbial challenge in chronic disease such as periodontal disease. Studies conducted on large group of our population showed no significant differences for allele, genotype, and haplotype distributions of the IL-6 (174, kt 565) between healthy and periodontaly affected individuals, nor between periodontally affected individuals with different attachment loss[17].

Conclusion

Our investigation revealed no statistically significant difference between tissue levels of IL 6 between samples obtained of periodontaly affected tissue adherent to sites with different attachment loss. No statistical significance was found in tissue samples from different stages of gingival inflammation.

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