

THE PHARMA INNOVATION - JOURNAL

Investigation of relationship between the level of cytokines and clinical parameters of patients with generalized periodontitis under the influence of zinaxin.

S.S. Romanyshyn

1. SIHE “Ivano-Frankivsk National Medical University”, Ivano-Frankivsk, Ukraine

[Email: robogdan@mail.ru]

We have examined 39 persons, including 27 patients with generalized periodontitis of the Ist- IInd degree of severity and 12 patients with clinically healthy periodontium. As a result of a comprehensive clinical and laboratory research the pathogenetic role of cytokine imbalance in the pathological process formation in the periodontal tissues was established. In patients with generalized periodontitis of the Ist - IInd degree the growth of the contents of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) and the reduction of the level of anti-inflammatory cytokines (IL-4) were revealed, in comparison with healthy people. Correlation analysis of cytokines' content in gingival and oral fluid of patients with generalized periodontitis and data of clinical indicators of periodontal tissues has showed that there is a strong direct connection between pro-inflammatory cytokines and clinical parameters.

Keyword: Generalized periodontitis, cytokines, gingival fluid, oral liquid, Zinaxin.

1. Introduction

A lot of studies in the field of periodontology highlight the role of processes of inflammation and immune systems in the complex of mechanisms underlying the pathogenesis of dystrophic-inflammatory periodontal diseases [1, 3, 8, 9]. However, despite this, the given problem cannot be considered as solved one by the end. Recently cytokine model of generalized periodontitis development has become common [4, 5, 6], in the basis of which there is modern understanding of the role of cytokines in the formation, and self-maintenance and self-development of the chronic inflammation in periodontal tissues [7, 12, 13].

Pro-inflammatory cytokines (IL-1 β , IL-2, IL-6, TNF- α , etc.) and hydrolytic enzymes in addition to positive effects on leukocyte chemotaxis, formation of prostaglandins, induce bone resorption [2, 10, 11, 15]. A number of studies have demonstrated the existence of a direct correlation between the activity of cytokines, severity of the

course and intensity of the resorptive processes of alveolar bone tissue. It is proved that some cytokines (IL-1 β , TNF- α) can stimulate the formation of giant polynuclear cells, osteoclasts and also inhibit bone-plastic function of osteoblasts [14, 16, 17]. Taking into account the role of cytokines in the pathogenesis of generalized periodontitis, the research of the contents and activity of pro-inflammatory (IL-1 β , IL-6, TNF- α) and anti-inflammatory (IL-4) mediators in the oral and gingival fluid of patients with generalized periodontitis and searching methods of their regulation by pathogenetically-based therapy is important. In this connection, our attention was attracted by the medicine of multi-factor action – Zinaxin, which affects the production and activity of some cytokines (IL-1 β , IL-6, TNF- α).

The aim of the study is to determine the dynamics of the content of pro- and anti-inflammatory cytokines in patients with generalized periodontitis under the influence of Zinaxin.

2. Materials and methods of study

We have observed 27 patients with generalized periodontitis of the Ist - IInd degree aged 34-55 years. The main group consisted of 13 patients, on the background of basic therapy they were performed imposition of curative periodontal dressings using Zinaxin under the insulating bandage "Septo-pack" (Septodont). In addition to it, patients of this group have taken Zinaxin orally (one capsule 2 times a day). Complex treatment with schemes of local and general treatment, based on generally accepted methods of periodontal pathology treatment was used in 14 patients with generalized periodontitis, who were included in the control group. For comparative evaluation of indicators a group of people (12 persons) with clinically healthy periodontium was taken.

The level of interleukins (IL-1 β , IL-4, IL-6) and tumor necrosis factor α (TNF- α) was determined in the oral and gingival fluids by the method of immune-enzyme analysis using the analyzer "StatFax 303 Plus" using reagents "ProKon" (Ltd. "Protein contour", Russia). The received results were processed by mathematical statistics on personnel PC using the programme "Statistica 5" considering the reliability of differences ($p < 0.05$) according to the Student's criterion.

3. Results and their discussion

As a result of our study it was determined that in the basic and control groups after performed complex treatment compared with output data the decrease in number of parameters (depth of periodontal pockets, bleeding indices, PMA, hygiene index) see (Table 1).

Table 1: Periodontal tissues' condition in patients with generalized periodontitis of the Ist-IInd degree.

Indicators	Basic group (n =13)		Control group (n =14)	
	Before treatment	After treatment	Before treatment	After treatment
Bleeding of gums, balls	2,15 \pm 0,03	*0,19 \pm 0,02**	2,30 \pm 0,04	0,54 \pm 0,06**
Periodontal pocket, mm	2,90 \pm 0,05	2,03 \pm 0,03**	2,70 \pm 0,03	2,10 \pm 0,04**
Index of PMA, %	48,60 \pm 0,87	*5,65 \pm 0,74**	52,40 \pm 0,89	10,50 \pm 1,18**
Index of Green-Vermillion, balls	2,10 \pm 0,04	0,52 \pm 0,03**	2,30 \pm 0,03	0,60 \pm 0,05**

Note: ** - statistically credible difference in comparison with the output data,

* - statistically credible difference between the basic and control groups.

Table 2: The level of cytokines in gingival fluid (pkg/ml) of the patients with generalized periodontitis

Indicators	Basic group (n =13)		Control group (n =14)		Clinically healthy periodontium (n=12)
	Before treatment	Before treatment	Before treatment	Before treatment	
IL-1 β	*194,4 \pm 10,0	52,1 \pm 6,1* p<0,05	*202,4 \pm 15,1	74,2 \pm 5,5*	54,4 \pm 24,5
IL-4	*41,8 \pm 7,3	123,4 \pm 9,1* p<0,05	*39,4 \pm 9,0	95,0 \pm 6,5*	143,2 \pm 21,4
IL-6	*270,1 \pm 24,1	80,7 \pm 10,3*	*280,7 \pm 29,5	101,0 \pm 14,0*	36,8 \pm 22,5
TNF- α	*286,6 \pm 16,1	112,4 \pm 6,1* p<0,05	*295,0 \pm 18,5	151,0 \pm 7,3*	89,7 \pm 43,4

Note: * left - statistically credible difference compared with clinically healthy periodontium,

* Right - statistically credible difference compared to the output data, p-statistically credible difference between basic and control groups.

Taking into account the obtained clinical results, it can be argued that the intended treatment regimen of patients with generalized periodontitis of both groups provide a positive clinical effect. However, a comparative evaluation of clinical parameters (gingival bleeding, index PMA) of the basic and control groups of patients, reveals statistically credible difference, indicating the therapeutic efficacy and expediency of Zinaxin administration in generalized periodontitis.

Analysis of cytokines' content was performed in gingival and oral fluid of patients with generalized periodontitis. During the study of cytokine status it was established the following pattern: the superiority of the content of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) over the anti-inflammatory ones (IL-4) in basic and control groups of patients with periodontal pathology compared with patients with clinically healthy periodontium (see Table. 2, 3).

Table 2 shows, that in comparison with the data obtained in the study of gingival fluid of patients with clinically healthy periodontium, the increase of IL-1 β , IL-6, TNF- α and the decrease of IL-4 has appeared in patients with generalized periodontitis. Evaluating the results after the performed treatment, compared with the output

data, it can be argued that the chosen treatment schemes which were used in both groups, give a positive therapeutic effect, as it's evidenced by the significant decrease in IL-1 β , IL-6, TNF- α and increase of the IL-4 contents. However, when comparing results of treatment between basic and control groups the statistically credible difference of IL-1 β , TNF- α , IL-4 level takes place, indicating a pronounced therapeutic effect of Zinaxin.

During the study of oral fluid in patients with generalized periodontitis in both groups there is a significant increase in IL-1 β , IL-6, and in the basic group - the reduction of IL-4 (see Table 3). When comparing results of treatment between basic and control groups there is a statistically credible difference in the reduction of IL- β and IL-6, which shows a pronounced therapeutic effect of the chosen treatment regimen.

Thus, in patients with generalized periodontitis of the Ist-IInd degree in both groups a statistically credible increase in the contents of pro-inflammatory cytokines (in gingival fluid, IL-1 β , IL-6, TNF- α , in oral fluid - IL-1 β , IL-6) and the decrease in the concentration of anti-inflammatory cytokines (IL-4 - in gingival fluid, oral fluid (basic group)) were observed.

Table 3: The level of cytokines in oral fluid (pkg/ml) of patients with generalized periodontitis

Indicators	Basic group (n =13)		Control group (n=14)		Clinically healthy periodontium (n =12)
	Before treatment	After treatment	Before treatment	After treatment	
IL-1 β	*71,4 \pm 8,3	30,6 \pm 4,1* p<0,05	*72,0 \pm 5,2	45,1 \pm 4,3*	25,1 \pm 12,4
IL-4	*23,2 \pm 9,1	60,5 \pm 16,7	27,2 \pm 12,2	45,0 \pm 11,2	54,7 \pm 9,4
IL-6	*110,6 \pm 13,0	16,5 \pm 5,1* p<0,05	*80,0 \pm 11,3	31,0 \pm 5,4*	25,4 \pm 18,2
TNF- α	80,0 \pm 17,3	45,0 \pm 11,6	83,0 \pm 20,1	61,0 \pm 7,5	61,2 \pm 7,2

Note: *left - a statistically credible difference compared with clinically healthy periodontium, *right - statistically credible difference compared with the output data, p - statistically credible difference between basic and control groups.

After performed treatment, both in basic and control groups, there is a statistically credible reduction of pro-inflammatory cytokines (in gingival fluid - IL-1 β , IL-6, TNF- α , in oral fluid -

IL-1 β , IL-6) and increase of anti-inflammatory cytokines concentration (IL-4 - in gingival fluid). When comparing data received after treatment in basic and control groups, there is a statistically

credible difference of pro-inflammatory cytokines (in gingival fluid - IL-1 β , TNF- α , in oral fluid - IL-1 β , IL-6) and increase of inflammatory cytokines concentration (IL-4 - in gingival fluid). In determination of the index of correlation (r) it was found that between the increase of pro-inflammatory cytokines contents and growth of studied clinical parameters there is a strong direct relationship (see Table. 4). Instead, between the anti-inflammatory cytokines contents and certain clinical parameters there is a strong feedback. Correlation analysis of cytokines' contents in gingival and oral fluid of patients with generalized periodontitis and data of clinical indicators of periodontal tissues has shown that a strong direct relationship exists between pro-inflammatory cytokines and the PMA index and

bleeding, and to a lesser extent, depends on the depth of periodontal pocket (weak direct link). A strong inverse correlation was found between the contents of IL-4 and PMA indicators performance index and bleeding gums.

Performed studies have confirmed the existence of links between cytokine system imbalance and clinical condition of periodontal tissues in patients with dystrophic-inflammatory changes in periodontal tissues. The studies have revealed displacement of biological regulators towards pro-inflammatory links. Elevated level of pro-inflammatory cytokines in gingival and oral fluid of patients with generalized periodontitis confirms the role of its influence on periodontal tissues.

Table 4: Indicators of correlation index (r) of clinical parameters and cytokine status of patients with generalized periodontitis

Indicators		IL-1 β	IL-6	IL-4	TNF- α
Gingival fluid	PMA	0,75***	0,72***	-0,64***	0,66***
	IB	0,72***	0,74***	-0,59***	0,65***
	DPP	0,36*	0,51***	-0,34*	0,39*
Oral fluid	PMA	0,64***	0,52***	-0,47**	0,54***
	IB	0,52***	0,56***	-0,45**	0,52***
	DPP	0,47**	0,47**	-0,25*	0,42**

Note: *** - strong link, ** - average link, * - weak link, «-» - feedback.

Growing production of pro-inflammatory cytokines (IL-1 β and TNF- α), which are regulators of the inflammatory response at all levels, is the response to destructive periodontal changes that occur as a result of prolonged inflammation. In chronic generalized periodontitis cytokines contents is directly dependent on the degree of inflammation in periodontal tissues. The observed imbalance in the system of pro- and anti-inflammatory cytokines and therapeutic efficacy of the applied treatment in patients with generalized periodontitis of the basic group indicates the legality of Zinaxin use in complex treatment of patients with periodontal pathology.

4. Conclusions

1. Patients with generalized periodontitis have an increase in the contents of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α - in gingival fluid, IL-1 β , IL-6 - in oral fluid) and a decrease in anti-inflammatory cytokines (IL-4 - in gingival fluid, oral fluid (basic group)).
2. After the performed treatment in patients of basic group a significant reduction of pro-inflammatory cytokines (in gingival fluid - IL-1 β , TNF- α , in oral fluid - IL-1 β , IL-6) and the increase of anti-inflammatory cytokines level (IL-4 - in gingival fluid) were observed, indicating a pronounced therapeutic effect of Zinaxin in the treatment of patients with generalized periodontitis.

3. The positive dynamics of cytokine contents indicators is most clearly seen in the gingival fluid, allowing us to recommend wider use of this study for the diagnosis of periodontal status and determination of the effectiveness of the performed treatment.

5. References

1. Grigor'yan AS, Grudianov AI, Rabuhina NA, Frolova OA. Boliezni parodonta (Periodontal diseases). - M: MIA, 2004, 289.
2. Ivaniushko TP, Gankovskaia LV, Kovalchuk LV, Ter-Asaturov GP, Kassin VU, Budanova EV. Kompleksnoie izucheniie mehanizmov razvitiia hronicheskogo vospaleniia pri parodontite (Complex study of developmental mechanisms of chronic inflammation in periodontitis). Stomatologiiia (Dentistry) 2000; 4:13-16.
3. Kovalchuk LV, Gankovskaya LV, Rogova MA, Ivaniushko TP, Budanova EV, Shabanova NA. Rol tsitokinov v mehanizmah razvitiia hronicheskogo vospaleniia v tkani parodonta (The role of cytokines in the mechanisms of development of chronic inflammation in periodontal tissue). Immunologiiia (Immunology) 2000; 6:24-26.
4. Mashchenko IS. Obmen tsitokinov u bolnyh generalizovannym parodontitom (Metabolism of cytokines in patients with generalized periodontitis). Sovremennaia stomatologiiia (Modern Stomatology) 2004; 1:73-75.
5. Hans-Peter M. Parodontologiiia (Periodontology). Lviv: Hal Dent 2004; 256.
6. Obidnyak VZ, Fedorov SV, Ostrovskyy MM. Rol tsytokiniv u prohresuvanni zahvoryuvan parodonta (The role of cytokines in the progression of periodontal diseases). Halytskyi likarskyy visnyk (Galician Medical Herald) 2001; 2:162-164.
7. Piuryk VP, Nychyporchuk HP, Hrekulyak VV, Mulyk IB. Tsytokiny: diahnostychni mozhlyvosti i perspektyvy vykorystannya v hirurhichnyi stomatolohiyi (Cytokines: diagnostic possibilities and perspectives of the use in dental surgery). Halytskyi likarskyy visnyk (Galician Medical Herald). 2002; 1:144-150.
8. Samojlenko AV, Makarevych AY. Lokalna patohenetychna terapiya heneralizovanoho parodontytu (Local pathogenetic treatment of generalized periodontitis). Novyny stomatolohiyi (News of Dentistry) 2002; 1:27-28.
9. Chumakova UG. Rol tsitokinov v reguliatsii vospaleniia tkanej parodonta u bolnyh generalizovannym parodontitom (The role of cytokines in regulation of periodontal tissue inflammation in patients with generalized periodontitis). Sovremennaia stomatologiiia (Modern Stomatology). 2004; 4:60-62.
10. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. Journal of Periodontal Research 1993; 28:500-510.
11. Bragger U, Lang NP. The significance of bone in periodontal disease. Semin Orthod 1996; 2(1):31-38.
12. Brynskov J, Nielson O, Annfelt-Ronn J *et al.* Cytokines in inflammatory bowel disease. Scand J Gastroent 1992; 11(27):897-906.
13. Graves DT. The potential role of chemokines and inflammatory cytokines in periodontal disease progression. Clin Infect Dis 1999; 28(3):482-490.
14. Jandinski JJ. Osteoclast activating is now interleukin-1 beta: Historical perspective and biological implications. Journal of Oral Pathology 1988; 17:145-152.
15. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1 α and -1 β in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease.// Journal of Periodontal Research 1990; 25:156-163.
16. Mundy GR. Inflammatory mediators in the destruction of bone. Journal of Periodontal search 1991; 26:213-217.

17. Tatakis DN. Interleukin-1 and bone metabolism: a review. J Periodontal 1993; 64(5):416-431.