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RESEARCH ARTICLE

CLINICAL RELEVANCE OF IL-1B/IL-10 AND TNF-A/IL-10 RATIO IN CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

Background: Chronic periodontitis is a multifactorial polymicrobial infection characterized by an inflammatory process that leads to destruction of teeth supporting tissues. In periodontal tissue destruction, cytokines appear to have a central role, and there is a complex network of pro-and anti-inflammatory cytokines acting in the inflamed periodontal tissues.
Aims of the study: This study was designed to evaluate the ratio between pro-and anti-inflammatory cytokines (IL-1β\IL-10 and TNF-α\IL-10) in chronic periodontitis patients.
Subjects and Methods: A total of 50 patients with chronic periodontitis and 25 healthy volunteers were studied, who were considered as control. Periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, clinical attachment level and bleeding on probing. Serum concentrations of IL-1β, TNF-α and IL-10 were assessed by means of enzyme-linked immune-sorbent assay.
Results: The current results revealed that median serum levels of pro-inflammatory cytokines (IL-1β and TNF-α) were significantly higher in patients than in healthy controls ($p<0.001$), whereas the serum levels of anti-inflammatory cytokine (IL-10) was significantly low in patients when compared to control ($p<0.001$). On the other hand, the ratios of IL-1β\IL-0 and TNF-α\IL-0 were significantly higher in patients when compared with the ratios in control group.
Conclusion: The present results may provide direct evidence for the systemic activation of immune cells in periodontitis, and suggests that cytokines may play an important role in pro-inflammatory response in serum of patients with chronic periodontitis. Moreover imbalance between pro and anti-inflammatory cytokines could be involved in the initiation and progression of chronic periodontitis and is indicative of a stronger systemic pro-inflammatory state in disease.

INTRODUCTION

Periodontitis is a multifactorial infection characterized by a destructive inflammatory process affecting tooth supporting tissues and resulting in periodontal pocket formation and alveolar bone resorption, which might eventually lead to tooth loss (1). The chronic form of periodontitis, termed chronic periodontitis (CP), is the most prevalent disease type (2). Although, periodontal bacteria are the main causative agents inducing the initiation of periodntitis, subsequent progression and disease severity are also determined by the host immune response. The continuous challenge of host immune and resident cells by periodontopathogens and their virulence factors results in a complex network of pro- and anti-inflammatory cytokines acting in the inflamed periodontal tissues. These host mediators directly or indirectly participate in periodontal tissue destruction and particularly in bone resorption (3). In periodontal disease, the balance between pro- and anti-inflammation is directed towards pro-inflammatory activity (4). In periodontal tissues IL-1β is known to stimulate the proliferation of keratinocytes, fibroblasts, and endothelial cells, and to enhance fibroblast synthesis of collagenase, hyaluronate, fibronectin, and PGE2. IL-1β upregulates matrix metalloproteinases (MMP) and downregulates tissue inhibitors of metalloproteinase production and it are also a potent stimulator of bone resorption (5). TNF-α fuels tissue pathology towards periodontal connective tissue destruction and bone loss (4). TNF-α is a multi-potential pro-inflammatory cytokine produced as a result of bacterial stimulation by monocytes/ macrophages, polymorphonuclear, leucocytes,fibroblasts, epithelial cells, endothelial cells and osteoblasts. TNF-α enhances connective tissue and bone destruction via enhanced osteoclast formation and activity, induction of MMP expression and stimulation of PGE2 production. In addition, apoptosis of fibroblasts is stimulated by TNF-α, resulting in

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limited repair of the periodontal tissues (6). In a complex network of pro- and anti-inflammatory cytokines acting in the inflamed periodontal tissues, IL-10 is an example of a cytokine with anti-inflammatory effects. IL-10 is a regulatory cytokine, which on the one hand limits inflammatory responses by inhibiting the expression of pro-inflammatory cytokines (7).

It was assessed using P (Mann-Whitney-test) and P (Bonferroni-test). Correlation between the different parameters was calculated by the spearman test and P values of P<0.01 and P<0.05 were considered significant.

Table 1 Demographic and clinical periodontal parameters in CP patients and healthy control groups

	CP Patients (n=50)	Healthy Control (n=25)	P -Value
Demographic Parameters			
Age Range	(23-60)	(21-50)	
Age Mean ± SD	40.1±7.6	33.4±9.1	P=0.07[NS]
Male	40(80%)	18(72%)	P=0.15[NS]
Female	10(20%)	7(28%)	P=0.88[NS]
Clinical periodontal Parameters			
Plaque Index	1.4±0.4	0.6± 0.1	<0.001**
Gingival Index	1.6±0.3	0.5±0.1	<0.001**
Proping Pocket Depth (mm)	2.1±0.4	1.2±0.6	<0.001**
Clinical Attachment Loss (mm)	1.4±0.4	0	<0.001**
Bleeding on Probing (BOP) "percentage of bleeding surfaces"	21.8±29.2	2.6±1.5	=0.003**

Table 2 CP patients-healthy control difference in serum median levels of IL-1β, IL-10, TNF-α, IL-1β/IL-10 and TNF-α/IL-10 (pg/ml)

	Range		Median		Inter-quartile range		Mean Rank		P (Mann-Whitney)
	CP N=50	Healthy N=25	CP N=50	Healthy N=25	CP N=25	Healthy N=50	CP N=50	Healthy N=25	
IL-1β	(0.106 - 368.9)	(0.106 - 117.8)	34.57	27.21	(13.046 - 86.817)	(9.691 - 42.161)	40.1	32.4	0.003**
IL-10	(0.010 - 0.82)	(0.013 - 0.839)	0.039	0.095	(0.027 - 0.051)	(0.034 - 0.165)	33.19	47.62	0.007**
TNF-α	(0.1- 1.90)	(0.09 - 0.81)	1.51	0.52	(0.35 - 0.542)	(0.406 - 0.577)	45.2	36.8	0.012**
IL-1β/IL-10	(3.12- 4044.4)	(0.41 - 2661.3)	663.7	290.1	(279.6 - 1804.25)	(19.88- 853.7)	42	28.68	0.012*
TNF-α/IL-10	(0.35 - 53.9)	(0.55 - 43.46)	11.94	5.87	(7.69 - 16.16)	(1.87 - 11.69)	42.72	28.56	0.008**

Table 3 Correlation coefficient among cytokines levels in CP patients

Cytokines	Serum IL-1β	Serum IL-10	Serum TNF-α	Serum IL-1β \IL10 ratio	Serum TNF-α \IL10 ratio
Serum IL-1β	1	-0.60**	0.78**	0.745**	0.303*
Serum IL-10	-0.60**	1	-0.54**	0.475*	0.712**
Serum TNF-α	0.78**	-0.54*	1	0.336*	0.157
Serum IL-1β \IL10 ratio	0.745**	0.475*	0.336*	1	0.188
Serum TNF-α \IL10 ratio	0.303*	0.712**	0.157	0.188	1

The current study was carried out to evaluate the ratio between pro-and anti-inflammatory cytokines (IL-1β\IL-10 and TNF-α\IL-10) in chronic periodontitis patients.

SUBJECTS AND METHODS

A total of 50 patients with chronic periodontitis were studied, their ages range from 23-60 years with a mean age of 40.1±7.6 years. Apparently healthy volunteers consisted of 25 individuals who were their age range (21-50) years with a mean age of 33.4±9.1 years considered as control. Periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, clinical attachment level and bleeding on probing. Blood samples were collected from CP patients and healthy control groups to assess serum concentrations of IL-1β, TNF-α and IL-10 by means of enzyme-linked immune-sorbent assay.

Statistical analysis

RESULTS

In the present study the age of CP patients ranged between 23-60 years with a mean age of 40.1±7.6 years. Furthermore, there was male's predominance among patients Regarding the differences in clinical periodontal parameters in patients and healthy controls, the differences in clinical periodontal parameters in patients and healthy controls are summarized in table (1). Table (2) revealed a significant elevation in median serum IL-1β level among CP patients (34.57pg /ml) in comparison to that of healthy control (27.21 pg /ml) (p<0. 01, on the other hand, there are significant decrease of median serum of IL-10 level in CP patients (0.039pg /ml) as compared to healthy control (0.095pg /ml), (p<0.01), while a significant elevation in median serum level of TNF-α was observed among CP patients (1.51 pg/ml) in comparison to that of healthy control (0.52 pg/ml), (p<0.05). The present study revealed significant strong positive linear correlation between IL-1β and TNF-α, p<0.01.While strong negative correlation

was noticed between IL-10 and each of IL-1β and TNF-α, $p<0.0$, as clearly shown in table (3).

DISCUSSION

To clarify possible deviation in the systemic inflammatory status, the present study investigate the role of pro-inflammatory to anti-inflammatory cytokine ratios in patients with CP, and presented as the ratios of serum, IL-1β/IL-10 and TNF-α/IL-10, from protective to destructive (8).

The results of the present study showed that the levels of IL-1β and TNF-α were significantly higher in patients with CP as compared with control group, these findings are consistent with other studies reported by (9, 10) who observed similar increase of these cytokines. In contrast other reports mentioned that there were no differences in the concentrations of IL-1β and TNF-α between CP patients and control (11, 12). In 2005 Keles and colleagues reported that IL-1β level is a sensitive and reliable marker of chronic inflammatory disease activity, and IL-1β elevation may demonstrate tissue destruction (13). A number of studies indicate that peripheral blood monocytes challenged by bacterial LPS produce inflammatory mediators like IL-1β and TNF- α (14, 15). On the other hand, low level of IL-10 in periodontitis subjects was observed when compared with healthy control in the current study, this result was consistent with (16, 17), but at variance with Bodet and colleagues, who reported that there was no significant differences in the level of serum IL-10 between CP patients and healthy group (18). Because of the interactions between cytokines, the ratios were studied in this work, and the findings was found out that the ratios was significantly higher in the periodontitis group when compared with the ratios in the control, indicating a stronger systemic pro-inflammatory state in chronic periodontitis, this result is consist with Poassaja and associates who mentioned that the elevation levels of pro-inflammatory cytokines in serum, may contribute to increased tissue pathology locally in the periodontal and final result accumulation of pro-inflammatory and inhibition the activity of IL-10 (17). As mentioned above that inflammatory mediators produced by distant immune cells, also these mediators can leak from inflamed periodontal pocket areas into the circulation, both of these pathways contributed to the down-regulation of anti-inflammatory mediators. In conclusion these findings may provide direct evidence for the systemic activation of immune cells in periodontitis, and suggests that cytokines may play an important role in pro-inflammatory response in serum of patients with chronic periodontitis. Moreover imbalance between pro and anti-inflammatory cytokines could be involved in the initiation and progression of chronic periodontitis and is indicative of a stronger systemic pro-inflammatory state in disease.

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الأهمية السريرية للنسبة بين المدور الخلوي بيتا 1 \ المدور الخلوي-10

و العامل المنخر للخلايا السرطانية-ألفا\ المدور الخلوي-10 في المرضى المصابين بمرض النساغ المزمن

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الخلاصة

الخلفية العلمية: مرض النساغ المزمن هو مرض متعدد العوامل, وكذلك متعدد المكروبات يتميز بعملية التهابية تؤدي إلى تدمير الأنسجة الداعمة للأسنان.كما أن هناك شبكة معقدة من المدورات الخلوية الحاثة والمضادة للالتهابات تؤثر في أنسجة ما حول الأسنان الملتهبة. **أهداف الدراسة:** تم تصميم هذه الدراسة لتقييم النسبة بين المدورات الخلوية الحاثة للألتهابات (بيتا-1 و و العامل المنخر للخلايا السرطانية-ألفا) و المدور الخلوي المضاد للألتهاب(10).

المرضى و طرائق العمل: تمت دراسة ما مجموعه 50 مريضا مصاب بمرض النساغ المزمن وتألفت مجموعة السيطرة من 25 من المتطوعين الاصحاء. وكانت معلمات ما حول الأسنان المستخدمة في هذه الدراسة هي مؤشر الصفيحة الجرثومية و مؤشر ألتهابات اللثة وعمق جيوب اللثة وفقدان الأنسجة الرابطة والنزيف أثناء الفحص. التراكيز المصلية للمدورات الخلوية الحاثة للألتهابات (بيتا-1 و العامل المنخر للخلايا السرطانية-ألفا) و المدور الخلوي المضاد للألتهاب(10). تم قياسها بأستخدام تقنية مقايسة الأنظيم المرتبط الممتز المناعية. **النتائج:** النتائج الحالية كشفت عن أن متوسط المستويات المصلية للمدورات الخلوية الحاثة للالتهابات (1 بيتا و العامل المنخر للخلايا السرطانية-ألفا) كانت أعلى في المرضى مقارنة بمجموعة السيطرة من ناحية أخرى، كانت مستويات المصل للمدور الخلوي المضاد للألتهاب (10) في المرضى $p<0.001$ منخفضة بالمقارنة مع السيطرة .

$p<0.001$

في حين لوحظ علاقة سالبة عكسية قوية بين المدور من المثير للاهتمام تم العثور على علاقة موجبة قوية بين كل من (بيتا-1 و العامل المنخر للخلايا السرطانية-ألفا)، المدور الخلوي 10 ، و نسبة المدور \ وكانت نسبة العامل المنخر للخلايا السرطانية-ألفا.الخلوي 10 وكل من المدور الخلوي 1 بيتا و العامل المنخر للخلايا السرطانية-ألفا .الخلوي بيتا-1\ المدور الخلوي 10أعلى بكثير في المرضى بالمقارنة مع النسب في مجموعة السيطرة

الأستنتاج: النتائج الحالية قد توفر دليلا مباشرا على التنشيط الجهازي للخلايا المناعية في ألتهاب ما حول الأسنان, وتشير إلى أن المستويات المصلية للمدور الخلوي 1بيتا و العامل المنخر للخلايا السرطانية-ألفا قد تلعب دورا مهما في هذا المرض وعلاوة على ذلك عدم الموازنة بين مستويات المدورات الخلوية الحاثة و المضادة للألتهابات قد تشارك في بدء وتطور ألتهاب ما حول الأسنان المزمن و كذلك يدل على وجود حالة ألتهابية جهازية قوية في هذا المرض.

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