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Local and systemic immune responses in gingivitis and periodontitis

Research Article

Gediminas Zekonis*[1], Ingrida Barzdziukaite[1], Jonas Zekonis[1], Renata Sadzeviciene[1], Sandrita Simonyte[1], Juozas Zilinskas[2]

1 Lithuanian University of Health Sciences, LT-44307 Kaunas, Lithuania

2 Public institution "Odontologijos studija", LT-44296 Kaunas, Lithuania

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Abstract: Objective. The aim of this study was to determine the effect of gingivitis and periodontitis on white blood cell (WBC) count and differential WBC count in gingival microvascular blood (GMB) and in venous blood (VB). Material and methods. 102 systemically healthy adult patients – 32 with gingivitis, 36 with periodontitis, and 34 controls – underwent evaluation of the total WBC count, and the count of different types of WBC in VB and GMB. Results. Inflammation of periodontal tissues was persistently associated with a systemic (in VB) elevation of the WBC count ($p<0.05$ in gingivitis and $p<0.01$ in periodontitis), compared to that in control group subjects, and with elevated systemic and local lymphocyte counts ($p<0.05$), compared to the analogous cell count in the control group. Patients with periodontitis were found to have reduced polymorphonuclear leucocyte (PMN) counts in GMB, compared to patients with gingivitis. Conclusion. Persistent chronic bacterial infection affects the systemic elevation of WBC in subjects with gingivitis and periodontitis. A local decrease in PMN in periodontitis patients and a systemic increase in lymphocytes in gingivitis and periodontitis patients may indicate the inability of these patients' organisms to defend against periodontopathic bacteria - and thus susceptibility to disease.

Keywords: *Gingival microvascular blood • White blood cell count • Periodontitis • Gingivitis*

1. Introduction

Periodontal diseases are among the most common chronic infections in humans [1]. Gingivitis and periodontitis are caused by microbial plaque that accumulates in the region of the gingival crevice and induces an inflammatory response in the supporting tissues of the teeth, characterized by a gradual loss of periodontal attachment and alveolar bone [2]. Although bacteria are essential for the induction of the inflammatory response, they are insufficient to cause the disease [3]. In conjunction with the bacterial challenge, the host's immune response plays an important role in the onset and progression of periodontitis [4].

The innate immune response involves the recognition of pathogen-associated molecular patterns by host cells [5], and this event is mediated by toll-like receptors (TLR_S) expressed by resident cells and leukocytes [6]. *Porphyromonas gingivalis* lipopolysaccharide (LPS) preferentially utilizes toll-like receptor-2 rather than toll-like receptor-4 [7]. The data of Wang et al. (2000) indicated that *P. gingivalis* LPS bound the toll-like receptor-4 in gingival fibroblast [8]. The resident cells involved in the innate host response are many, including epithelial cells, gingival and periodontal ligament

* *E-mail: gediminas.zekonis@gmail.com*

fibroblasts, osteoblasts, and dendritic cells [9]. Once TLR_s on the surface of the resident cells recognize pathogen-associated molecular patterns, they initiate the activation of several transcription factors including activator protein 1 and the receptor activator of nuclear factor kappa β ligand (RANKL) through the mitogen-activated proteinkinase cascade [9,10]. These, in turn, activate different innate immunity pathways – including cytokine and chemokine production – that recruit non-resident leukocytes to the periodontal space.

A variety of immune-associated cell populations are responsible for the pathogenesis of periodontal diseases. Activated monocytes, macrophages, and fibroblasts produce cytokines within periodontal lesions, including tumor necrosis factor-α, interleukin-1β, and interleukin-6 [11-13]. These cytokines orchestrate the cascade of destructive events that occur in the periodontal tissues, and trigger the production of an array of inflammatory enzymes and mediators, including matrix metalloproteinases and prostaglandins, and also osteoclast recruitment and differentiation through RANKL-dependent and independent pathways, thus resulting in irreversible hard and soft tissue damage [5,14].

A white blood cell (WBC) is the principal component of the immune system and the inflammatory response, and WBC count elevation is one of the clinical markers of an inflammatory process [15].

Most authors noticed that venous blood of patients with chronic periodontitis had elevated WBC counts [15-17], which after intensive non-surgical treatment decreased significantly for non-smokers [18,19], yet stayed the same for smoking patients [18].

Polymorphonuclear leukocytes (PMN) comprise approximately 90% of the leucocytes present in the gingival crevice [20], and are the predominant cells in the gingival pocket epithelium and the adjacent connective tissue [21]. They are among the first innate immune effector cells to be recruited to the site of infection, and are a critical component of the local inflammatory response in periodontal disease [22].

A large number of scientific papers have been published on the role of genes and their variants (polymorphisms) in host responses in periodontitis [23-25]. Moreover, there is now a consensus that genetic factors play a role in the susceptibility to and severity of periodontitis [26,27].

It is possible that genetically determined differences in immune regulation or in homeostatic bone remodeling are also important for the outcome of periodontal disease [28]. Studies in infectious diseases other than periodontal diseases provide convincing evidence that host genetic factors are important in determining who will succumb to the pathogen and who will not [29]. Susceptibility or resistance to many infectious diseases is dependent on genetically controlled differences in inflammatory responses [30,31].

The literature, however, is scarce about WBC count and differential WBC count in venous blood and in areas proximate to the inflammatory process (in gingival microvascular blood) of patients with gingivitis and periodontitis.

The aim of the present study was to investigate how variables such as WBC and differential blood cell count are affected by gingivitis and periodontitis locally (in gingival microvascular blood (GMB)) and systemically (in venous blood (VB)) .

2. Material and methods

2.1. Patient selection

Patients (n=102) were selected for the study from a large number of individuals treated at the Faculty of Odontology, Kaunas University of Medicine (currently – Lithuanian University of Health Sciences). They were examined clinically and radiographically. All patients were distributed into 3 groups: patients with gingivitis (n=32), patients with periodontitis (n=36), and the control group (n=34).

Patients were included if they were between 18-50 years of age and had ≥20 teeth. Oral hygiene was assessed using the approximal plaque index (API) according to Lange et al. [32]: the percentage of plaque-positive proximal tooth surfaces was assessed using a dental probe. To assess the severity of gingival inflammation, the gingival index (GI) was evaluated by a scale of 0 to 3 at the buccal and lingual surfaces at each tooth according to Loë&Silness [33]. The mean GI was calculated by dividing the sum of all scores by the total number of examined surfaces. Pocket probing depth (PPD) was recorded using a North Carolina probe (Hu-Friedy, Chicago, IL) at mesio- and distobuccal sites of ≥12 teeth in screening examinations.

For separate patient groups, additional inclusion criteria were used.

Patients without systemic pathology were included into the gingivitis group; additional clinical symptoms that were present were the following: redness, gingival swelling, and bleeding on probing. Probing depth was up to 3 mm. Radiographically, no bone resorption was visible.

Patients who had additional clinical symptoms on top the aforementioned ones (pocket depth ≥6 mm in

at least 3 teeth/quadrant, and radiographic evidence of horizontal and vertical bone loss) were included into the third group – the chronic periodontitis group.

The control group consisted of 34 systemically healthy individuals with healthy periodontal tissues. WBC count in their venous blood and gingival microvascular blood was used as the control measure for WBC and differential WBC counts in the other experimental groups. Inclusion criteria for the controls were the absence of clinical and radiographic manifestations of periodontal disease, no history of periodontal disease, and ≥20 teeth present.

Patient exclusion criteria were the following: smoking, alcohol intake, pregnancy, lactation period, intake of antibiotics or anti-inflammatory drugs during the previous 3 months preceding the study, a history of systemic periodontal therapy, and the presence of any systemic conditions that may affect the periodontal status and WBC count.

All clinical parameters were assessed by two trained periodontitis experts, and a calibration exercise was performed to obtain acceptable inter-examiner reproducibility.

2.2. Venous and gingival microvascular blood collection

Venous blood from each subject was collected from the cubital fossa by venipuncture to the sterile blood collection tubes containing lithium heparin (20 u/mL). GMB of periodontal tissues was collected by a puncture of interdental papillae (in the mandible) by the apex of a sterile scalpel after cleaning the vestibulum with 70% alcohol and light air drying. GMB was collected in a sterile Pasteur pipette containing lithium heparin, and was placed in sterile plastic tubes.

Venous blood and GMB were collected under standardized conditions, between 8.00 to 9.00 a.m., after overnight fasting, and with the patient sitting in a dental chair; the procedure was performed by one investigator.

The laboratory analysis of WBC count and differential blood cell count was performed immediately in a Neubauer chamber, and was morphologically evaluated by May-Grünwald-Giemza staining at the Department of Laboratory Medicine, Lithuanian University of Health Sciences.

All experiments were conducted in accordance with the rules and regulations approved by Kaunas Regional Bioethics Committee (approval received on April, 11, 2009, No. BE-2-21). All subjects in this study signed the informed consent form approved by Kaunas Regional Bioethics Committee.

2.3. Statistics

Statistical analysis of the data was performed by using software packages for data storage and analysis – SPSS 13.0. Every data set was tested for normality with the Kolmogorov-Smirnov Test. Differences between the groups were established by applying the non-parametric one-way analysis of variance (Kruskal-Wallis test). Descriptive parameters were shown as the mean, standard deviation, median, and interquartile range. The difference was considered to be statistically significant when the level of significance p was less than 0.05.

3. Results

All 102 patients (54 males and 48 females; mean age – 33.2 years) completed the study (Table 1). There were no differences in age or sex between the patient groups ($p>0.05$).

The results of the blood sample study are presented in Table 2. The analysis of WBC count in venous blood showed that WBC count in venous blood was rising with an increasing severity of the clinical symptoms of periodontal disease: the WBC count in venous blood of gingivitis patients was markedly higher than the analogous cell count of the control group subjects ($p<0.05$). In periodontitis patients, venous WBC count showed significantly higher values than analogous cell counts in venous blood of gingivitis patients ($p<0.05$), or subjects of the control group ($p<0.01$).

Variables of the WBC count in gingival microvascular blood of patients with periodontal diseases had the opposite pattern comparing to the WBC count in venous blood. WBC counts markedly increased in GMB of gingivitis patients, and were remarkably higher than analogous cell counts in control group subjects ($p<0.01$) and periodontitis patients ($p<0.05$). The WBC count in GMB of gingivitis patients, were also remarkably higher ($p<0.01$), compared to that in VB of these patients. The lowest WBC counts in GMB were observed in periodontitis patients, and they were also lower ($p<0.05$) than the counts in venous blood of the same patients.

It is interesting that advancing clinical symptoms of periodontal lesions did not have any notable influence on absolute neutrophil count (ANC) in venous blood of the analyzed groups ($p>0.05$). It is noteworthy that significantly increased ANC in GMB were found in patients with gingivitis ($p<0.05$), compared to those with periodontitis, or the control group subjects.

Our research data showed that variables of absolute lymphocyte count (ALC) depended on the severity of

Table 1. Patients characteristics, oral hygiene status in approximal plaque index (API), gingival index (GI) and pocket probing depth (PPD) (means±SD, median and interquartile range).

Characteristics		Total	Controls	Gingivitis	Periodontitis
N		102	34	32	36
Gender	Male	65 (52.7%)	16 (47.1%)	17 (53.1%)	20 (55.6%)
	Female	57 (47.3%)	18 (52.9%)	15 (46.9%)	16 (44.4%)
Age (years)		33.2±4.0 33.1 (31-36)	31.0±4.2 31.0 (28-34)	32.1±3.4 32.0 (29-37)	36.4±4.4 36.3 (34-40)
Number of teeth		25.7±1.4 25.6 (24-28)	27.3±1.1 27.3 (26-28)	28.7±1.2 28.6 (25-31)	23.1±1.6 23.0 (22-28)
API (%)		29.5±2.4 29.5 (22.1-43.0)	20.6±1.2 20.5 (16.6-29.3)	30.6±2.3* 30.5 (22.3-44.1)	34.1±2.8* 34.0 (27.1-49.2)
GI (point)		1.6±0.1 1.6 (0-2.6)	0	2.2±0.2* 2.2 (1.6-2.7)	2.6±0.2* 2.6 (1.9-2.9)
PPD (mm)		3.1±0.2 3.0 (1.8-4.9)	1.8±0.1 1.8 (1.2-2.0)	2.7±0.1 2.7 (2.4-2.9)	4.9±0.3** 4.9 (4.1-5.3)

**P-value <0.05 compared to controls;*
***P-value <0.01 compared to subjects with gingivitis.*

the inflammation of the periodontal tissue. ALC in the venous blood of patients with gingivitis and periodontitis was higher ($p<0.05$) than the analogous cell counts of the control group. There was no significant difference between ALC in GMB of patients with gingivitis and periodontitis, yet ALC statistically significantly exceeded analogous cell counts in GMB of the control group subjects. It is noteworthy that ALC in GMB of patients with gingivitis significantly exceeded analogous cell counts in VB of the aforementioned patients.

A significant increase in monocyte counts was found in GMB of patients with gingivitis. This increase significantly exceeded analogous cell counts in the venous blood of the aforementioned patients and in GMB of patients with periodontitis and subjects of the control group. An increase in eosinophil counts was observed in GMB – especially in subjects with periodontitis ($p<0.05$). In VB and GMB, the count of basophils did not differ significantly between the groups.

4. Discussion

The relationship between acute bacterial infectious diseases and WBC count and ANC has been recognized for many years. Increased leukocyte events due to an inflammatory host response to bacteria manifest as a periodontal disease [34]. Our results of WBC count in gingival microvascular blood of subjects with gingivitis confirmed the previously stated facts. WBC count increased significantly in GMB of patients with gingivitis. Lower WBC counts in GMB of patients with periodontitis could be associated with a decrease in PMN chemotaxis [35,36]. Patients with periodontitis had lower WBC counts in GMB than in venous blood. This significant decrease in WBC count could be explained by a theory that when the inflammatory process reaches deeper areas of periodontal tissues, a part of leukocytes are diverted to the deeper inflamed area by the blood vessels that are supplying that area, and thus the surface blood might have lower counts of WBC. High levels of gingipain activity of *P. gingivalis* detected in gingival fluid could implicate a role for gingipains in the destruction of the highly vascular periodontal tissue, and can alter cell adhesion molecules and induce endothelial cell death [37]. This could cause extensive microvascular angiopathy in advanced periodontitis, and could also explain the attenuation of the inflammatory reaction in periodontal tissue [38]. Besides, repeated LPS stimulation or degradation of CD14 receptors (the main receptor for bacterial cell surface components such as LPS) on the surface of human macrophage-like cells by gingipains of *P. gingivalis* result in hyporesponsiveness of macrophages to LPS stimulation, and may contribute to an increased capacity of *P. gingivalis* and other periodontopathogens to evade host immune system mechanisms [39,40], resulting in chronic inflammation [41], which may also entail decreased count of WBC in GMB in periodontitis patients. Decreased counts of WBC in GMB of these patients may be influenced by an increased coagulation in vasculature of periodontal tissue via the degradation and inactivation of endothelial

Table 2. Distribution of white blood cells count, absolute neutrophil, lymphocyte, monocyte, eozinophil, and bazophil count ($\times 10^3/\mu l$) in venous blood (VB) and gingival microvascular blood (GMB) of patients with gingivitis and periodontitis.

		Characteristics of patients			
		1. Controls (n=34)	2. Gingivitis (n=32)	3. Periodontitis (n=36)	P-value
White blood cells	VB	6.02±0.07 6.00 5.97-6.06	6.28±0.16 6.27 6.19-6.39	7.40±0.23 7.39 7.17-7.49	$P_{1,2}<0.05$ $P_{1,3;2,3}<0.01$
	GMB	6.08±0.11 6.08 6.03-6.13	7.59±0.30** 7.60 7.36-7.79	6.82±0.18* 6.89 6.72-6.93	$P_{1,3;2,3}<0.05$ $P_{1,2}<0.01$
Neutrophils	VB	3.71±0.12 3.71 3.59-3.79	3.68±0.17 3.67 3.52-3.83	3.83±0.22 3.83 3.67-3.93	$P_{1,2;1,3;2,3}>0.05$
	GMB	3.76±0.14 3.76 3.68-3.85	4.38±0.17** 4.38 4.19-4.52	3.46±0.13 3.46 3.39-3.53	$P_{1,2;2,3}<0.05$ $P_{1,3}>0.05$
Lymphocytes	VB	1.74±0.09 1.76 1.65-1.83	2.04±0.18 2.02 1.91-2.10	2.96±0.19 2.94 2.81-2.99	$P_{1,2;2,3}<0.05$ $P_{1,3}<0.01$
	GMB	1.75±0.12 1.74 1.66-1.82	2.52±0.15* 2.50 2.40-2.66	2.75±0.13 2.76 2.68-2.84	$P_{1,2;1,3}<0.01$ $P_{2,3}>0.05$
Monocytes	VB	0.42±0.02 0.42 0.39-0.45	0.43±0.01 0.42 0.38-0.46	0.45±0.03 0.45 0.33-0.49	$P_{1,2;1,3;2,3}>0.05$
	GMB	0.43±0.03 0.42 0.38-0.49	0.53±0.04* 0.52 0.46-0.49	0.44±0.05 0.44 0.41-0.50	$P_{1,3;2,3}>0.05$ $P_{1,2}<0.05$
Eozinophils	VB	0.11±0.01 0.11 0.08-0.14	0.11±0.01 0.11 0.09-0.12	0.09±0.01 0.09 0.08-0.13	$P_{1,2;1,3}>0.05$
	GMB	0.11±0.01 0.11 0.08-0.14	0.13±0.01 0.13 0.10-0.16	0.15±0.02* 0.14 0.09-0.18	$P_{1,2;2,3}>0.05$
Bazophils	VB	0.04±0.004 0.04 0.02-0.06	0.04±0.005 0.04 0.03-0.06	0.05±0.01 0.05 0.03-0.06	$P_{1,2;1,3}>0.05$
	GMB	0.04±0.004 0.04 0.02-0.07	0.05±0.01 0.05 0.02-0.07	0.04±0.01 0.04 0.02-0.06	$P_{1,2;1,3}>0.05$

**P-value <0.05; **P-value <0.01 compared to venous blood.*

thrombomodulin by gingipains of *P. gingivalis,* resulting in local consumption of WBC [42]. However, we failed to find any literature data on WBC counts in GMB of patients with periodontitis and gingivitis.

Our data showed that inflammation of periodontal tissues (gingivitis and periodontitis) affects the systemic elevation of WBC count, and that the counts of WBC in venous blood were increasing with disease progression. WBC counts in venous blood of patients with gingivitis were higher than the respective counts in the control group. WBC counts in venous blood of periodontitis patients were significantly higher ($p<0.05$) than those in patients with gingivitis. Hayashi et al. [34] proposed non-exclusive mechanisms by which *P. gingivalis* oral infection could induce and maintain inflammation at sites distant from oral infection. *P. gingivalis* enters immune cells such as monocytes/macrophages or dendritic cells in the diseased/inflamed oral mucosal lesion; these cells then leave the inflamed tissues of the oral cavity, enter the circulation, localize, and – via diapedesis – enter the vascular intima at the sites of activated vascular endothelium (pathogen-activated or activated by inflammation associated with atherosclerosis) [34].

Systemic inflammation characterized by an elevated WBC count in venous blood [43] could be caused by higher endotoxin levels detected in the plasma of periodontitis patients, compared to those in healthy patients

[44]. Circulating endotoxin is the trigger for the systemic inflammatory response [45].

Activation of pro-inflammatory cytokines occurs in part via the TLRs – a family of innate immune recognition receptors that detect conserved microbial patterns and endogenous ligands – and play a key role in innate immune signaling and the initiation of inflammatory responses [46]. It therefore appears that *in vivo, P. gingivalis* can survive in blood and host tissues [47,48]. Our results showing higher WBC counts in venous blood of patients with periodontitis concur with the results of other authors [15,17,18,49].

Only few studies have found no significant differences in WBC count in venous blood between periodontitis patients and healthy controls. Wakai et al. [50] reported on an independent association between WBC count and periodontal disease severity after adjustment for smoking and other periodontal factors. Also, only few studies have analyzed the effect of non-surgical treatment of periodontal tissues on the WBC count. Fredriksson et al. [49] studied the effect of periodontal therapy in smoking periodontitis patients and smoking controls. The difference between smoking patients with periodontitis and smoking controls was not statistically significant. Christan et al. [18] and Bokhari et al. [51] found a significant reduction in WBC counts in venous blood after non-surgical therapy among non-smokers. Current knowledge suggests that smoking is associated with a less favorable response to periodontal therapy [50].

Findings of Al-Gwaiz & Babay [52] indicated that ANC was far more sensitive in predicting inflammation, compared to WBC count. Our data showing that ANC in GMB of patients with gingivitis was higher than the same cell counts in venous blood and in GMB of controls do not contradict the findings of the previous studies. However, ANC in GMB of patients with periodontitis was lower than the ANC in patients with gingivitis. PMNs are the predominant cells responsible for host response against bacterial infection, are the crucial cells in the early stages of infection [53], and are the first leukocytes that migrate to the site of infection [54]. Intracellular adhesion molecule expression by vascular endothelium represents a crucial process for the trans-endothelial migration of leukocytes into the inflamed tissue. The data of Yun et al. (2006) provide evidence that gingipains of *P. gingivalis* can reduce the functional expression of adhesion molecules on endothelial cells by cleaving these molecules [55]. This could have influenced lower ANC levels in GMB of periodontitis patients found in our study. The loss of PMN defense – due either to deficient number or function – strongly predisposes to bacterial infections such as periodontitis [56].

Our results showed a marked ALC increase in the venous blood of patients with gingivitis and periodontitis, and in GMB of gingivitis patients, in comparison to ALC in GMB of the controls and venous blood of these subject groups. This could probably be associated with migrations of these cells to periodontal tissues. Our results indirectly support previous authors' histological research data about extensive plasma cell infiltration in periodontal tissues of patients with periodontal disease [57], and also show that the B cells are responsible for T cell-dependent osteoclastogenesis in periodontitis patients through the involvement of IL-6 and IL-7, and via RANKL and TNF-α over-expression [58,59]. It is noteworthy that the severity of the disease does not correlate with ALC levels in GMB. This seems to be associated with an increased coagulation in the vasculature of periodontal tissue via the degradation and inactivation of endothelial thrombomodulin by gingipains of *P. gingivalis,* resulting in local consumption of WBC and ALC [42]. Extensive plasma cell infiltration in the periodontal tissue may indicate the host's systemic humoral immune response to infection [57]. The predominance of the host humoral response indicates inability to defend against periodontopathic bacteria–and thus a predisposition to periodontitis [57].

In literature, we have found a claim that the levels of circulating monocytes are increased in patients with periodontitis [60]. However, our study does not corroborate with these findings, and confirms the findings of other authors [61] who did not detect any elevation in circulating monocyte levels among patients with periodontitis. Our findings about increased monocyte counts in GMB of patients with gingivitis indirectly confirm immunohistochemical research data presented by Lins et al. (2008) on macrophage deposits in gingival tissues of patients with gingivitis [62]. During inflammation, macrophages colonize tissue in two distinct ways – by recruitment of monocyte precursors, and by proliferation of resident cells [63]. Accumulation of PMN in sites of inflammation is accompanied by the release of a wide range of granule proteins [64]. PMNs granule proteins seeded on the endothelium by adherent PMN allow for a direct activation and subsequent adhesion of monocytes, efficiently supporting the recruitment of inflammatory monocytes at inflamed sites [65] via the activation of formyl-peptide receptors [66]. Thus, neutrophil secretion products pave the way for inflammatory monocytes; this process takes place during the early stages of inflammation, and this corresponds to the findings of our study.

The data presented by Zhou et al. [67] showed that supernatant of *P. gingivalis* increased the migration of monocytes into the local environment for their

maturation into macrophages or osteoclasts, which is crucial in the pathogenesis of periodontal disease.

There are data [68] indicating elevated eosinophil counts in gingival crevicular fluid of patients with periodontitis, compared to eosinophil levels in venous blood. Our findings showing elevated eosinophil counts in GMB of patients with periodontitis indirectly confirm the data presented by the aforementioned authors [68]. Eosinophils are one type of the major pro-inflammatory cells in chronic inflammation. Eosinophils are accompanied by a wide variety of inflammatory responses due to the release of toxic inflammatory mediators, which may result in severe tissue/organ damage at the site of eosinophilic infiltrations due to the degranulation [69]. We failed to find any literature data on the function of eosinophils in gingival crevicular fluid. These cells are thought to play a certain role in the development of periodontitis [68].

5. Conclusion

Persistent chronic bacterial infection affects the systemic elevation of WBC in subjects with gingivitis and periodontitis. A local decrease in PMN in periodontitis patients and a systemic increase in lymphocytes in gingivitis and periodontitis patients may indicate the inability of these patients' organisms to defend against periodontopathic bacteria – and thus susceptibility to disease.

Conflict of interest statement

Authors state no conflict of interest.

References

[1] Pussinen PJ, Paju S, Mäntylä P, Sorsa T. Serum microbial- and host-derived markers of periodontal diseases: a review. Curr Med Chem 2007;14:2402-2412

[2] Kinane DF, Lappin DF. Clinical, pathological and immunological aspects of periodontal disease. Acta Odontol Scand 2001;59:154-160

[3] Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000 1997;14:216-248

[4] Ebersole JL, Taubman MA. The protective nature of host responses in periodontal diseases. Periodontol 2000 1994;5:112-141

[5] Di Benedetto A, Gigante I, Colucci S, Grano M. Periodontal disease: linking the primary inflammation to bone loss. Clin Dev Immunol. 2013;2013:503754

[6] Mahanonda R, Pichyangkul S. Toll-like receptors and their role in periodontal health and disease. Periodontol 2000. 2007;43:41-55

[7] Hirschfeld M, Ma Y, Weis JH, Vogel SN, Weis JJ. Cutting edge: repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. J Immunol. 2000;165(2):618-622

[8] Wang PL, Azuma Y, Shinohara M, Ohura K. Toll-like receptor 4-mediated signal pathway induced by Porphyromonas gingivalis lipopolysaccharide in human gingival fibroblasts. Biochem Biophys Res Commun. 2000 Jul 14;273(3):1161-1167

[9] Hans M, Hans VM. Toll-like receptors and their dual role in periodontitis: a review. J Oral Sci. 2011 Sep;53(3):263-7261

[10] Hayashi C, Gudino CV, Gibson FC 3rd, Genco CA. Review: Pathogen-induced inflammation at sites distant from oral infection: bacterial persistence and induction of cell-specific innate immune inflammatory pathways. Mol Oral Microbiol. 2010;25(5):305-316

[11] Morandini AC, Sipert CR, Gasparoto TH, Greghi SL, Passanezi E, Rezende ML, Sant'ana AP, Campanelli AP, Garlet GP, Santos CF. Differential production of macrophage inflammatory protein-1alpha, stromal-derived factor-1, and IL-6 by human cultured periodontal ligament and gingival fibroblasts challenged with lipopolysaccharide from P. gingivalis. J Periodontol. 2010;81(2):310-317

[12] Scheres N, Laine ML, de Vries TJ, Everts V, van Winkelhoff AJ. Gingival and periodontal ligament fibroblasts differ in their inflammatory response to viable Porphyromonas gingivalis. J Periodontal Res. 2010;45(2):262-270

[13] Jung IH, Lee DE, Yun JH, Cho AR, Kim CS, You YJ, Kim SJ, Choi SH. Anti-inflammatory effect of (-)-epigallocatechin-3-gallate on Porphyromonas gingivalis lipopolysaccharide-stimulated fibroblasts and stem cells derived from human periodontal ligament. J Periodontal Implant Sci. 2012;42(6):185-195

[14] Kirkwood KL, Cirelli JA, Rogers JE, Giannobile WV. Novel host response therapeutic approaches

to treat periodontal diseases. Periodontol 2000. 2007;43:294-315
[15] Inoue K, Kobayashi Y, Hanamura H, Toyokawa S. Association of periodontitis with increased white blood cell count and blood pressure. Blood Press 2005;14:53-58
[16] Hussain Bokhari SA, Khan AA, Tatakis DN, Azhar M, Hanif M, Izhar M. Non-surgical periodontal therapy lowers serum inflammatory markers: a pilot study. J Periodontol 2009;80:1574-1580
[17] Renvert S, Ohlsson O, Pettersson T, Persson GR. Periodontitis: a future risk of acute coronary syndrome? A follow-up study over 3 years. J Periodontol 2010;81:992-1000
[18] Christan C, Dietrich T, Hägewald S, Kage A, Bernimoulin JP. White blood cell count in generalized aggressive periodontitis after non-surgical therapy. J Clin Periodontol 2002;29:201-206
[19] Radafshar G, Shad B, Ariamaid E, Geranmayeh S. Effect of intensive non-surgical treatment of the level of serum inflammatory markers in advanced periodontitis. J. Dent (Tehran) 2010;7(1):24-30
[20] Attström R. Presence of leukocytes in crevices of healthy and chronically inflamed gingivae. J Periodontal Res 1970;5:42-47
[21] Tsukamoto Y, Usui M, Yamamoto G, Takagi Y, Tachikawa T, Yamamoto M, Nakamura M. Role of the junctional epithelium in periodontal innate defense and homeostasis. J Periodontal Res. 2012;47(6):750-757
[22] Van Dyke TE, Hopp GA. Neutrophil function and oral disease. Crit Rev Oral Biol Med 1990;1: 117-133
[23] Laine ML, Crielaard W, Loos BG. Genetic susceptibility to periodontitis. Periodontol 2000. 2012;58(1):37-68
[24] Chai L, Song YQ, Leung WK. Genetic polymorphism studies in periodontitis and Fcγ receptors. J Periodontal Res. 2012;47(3):273-285
[25] Mousavi Jazi M, Solgi G, Asl Roosta H, Noshad S, Moslemi N, Sadrimanesh R, Moradi B, A Amirzargar A. HLA-DRB and HLA-DQA/HLA-DQB allele and haplotype frequencies in Iranian patients with aggressive periodontitis. J Periodontal Res. 2013;48(4):533-539
[26] Loos BG, John RP, Laine ML. Identification of genetic risk factors for periodontitis and possible mechanisms of action. J Clin Periodontol. 2005;32 Suppl 6:159-179
[27] Tonetti MS, Claffey N; European Workshop in Periodontology group C. Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. J Clin Periodontol. 2005;32 Suppl 6:210-213
[28] Baker PJ, Dixon M, Roopenian DC. Genetic control of susceptibility to Porphyromonas gingivalis-induced alveolar bone loss in mice. Infect Immun 2000;68:5864-5868
[29] Lama J, Planelles V. Host factors influencing susceptibility to HIV infection and AIDS progression. Retrovirology 2007;25;4:52
[30] Pretzl B, El Sayed N, Cosgarea R, Kaltschmitt J, Kim TS, Eickholz P, Nickles K, Bäumer A. IL-1-polymorphism and severity of periodontal disease. Acta Odontol Scand 2012;70(1):1-6
[31] Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, Höhler T. Differential regulation of interleukin-10 production by genetic and environmental factors – a twin study. Genes Immun 2002;3:407-413
[32] Lange DE, Plagmann HC, Eenboom A, Promesberger A. Clinical methods for the objective evaluation of oral hygiene. Dtsch Zahnarztl Z 1977;32:44-47
[33] Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-551
[34] Hayashi C, Gudino CV, Gibson FC 3rd, Genco CA. Review. Pathogen-induced inflammation at sites distant from oral infection: bacterial persistence and induction of cell-specific innate immune inflammatory pathways. Mol Oral Microbiol 2010;25:305-316
[35] Gursoy UK, Marakoglu I, Oztop AY. Relationship between neutrophil functions and severity of periodontitis in obese and/or type 2 diabetic chronic periodontitis patients. Quintessence Int 2008;39:485-489
[36] Yagi M, Kantarci A, Iwata T, Omori K, Ayilavarapu S, Ito K, Hasturk H, Van Dyke TE. PDK1 regulates chemotaxis in human neutrophils. J Dent Res 2009;88:1119-1124
[37] Sheets SM, Potempa J, Travis J, Casiano CA, Fletcher HM. Gingipains from Porphyromonas gingivalis W83 induce cell adhesion molecule cleavage and apoptosis in endothelial cells. Infect Immun. 2005;73(3):1543-1552
[38] Pinchback JS, Taylor BA, Gibbins JR, Hunter N. Microvascular angiopathy in advanced periodontal disease. J Pathol 1996;179:204-209
[39] Sun Y, Li H, Yang MF, Shu W, Sun MJ, Xu Y. Effects of aging on endotoxin tolerance induced by lipopolysaccharides derived from Porphyromonas gingivalis and Escherichia coli. PLoS One. 2012;7(6):e39224

[40] Duncan L, Yoshioka M, Chandad F, Grenier D. Loss of lipopolysaccharide receptor CD14 from the surface of human macrophage-like cells mediated by Porphyromonas gingivalis outer membrane vesicles. Microb Pathog. 2004;36(6):319-325
[41] Sugawara S, Nemoto E, Tada H, Miyake K, Imamura T, Takada H. Proteolysis of human monocyte CD14 by cysteine proteinases (gingipains) from Porphyromonas gingivalis leading to lipopolysaccharide hyporesponsiveness. J Immunol. 2000;165(1):411-418
[42] Inomata M, Ishihara Y, Matsuyama T, Imamura T, Maruyama I, Noguchi T, Matsushita K. Degradation of vascular endothelial thrombomodulin by arginine- and lysine-specific cysteine proteases from Porphyromonas gingivalis. J Periodontol. 2009;80(9):1511-1517
[43] Loos BG. Systemic effects of periodontitis. Ann R Australas Coll Dent Surg 2006;18:27-29
[44] Shaddox LM, Wiedey J, Calderon NL, Magnusson I, Bimstein E, Bidwell JA, Zapert EF, Aukhil I, Wallet SM. Local inflammatory markers and systemic endotoxin in aggressive periodontitis. J Dent Res 2011;90(9):1140-1144
[45] Kelly JL, O'Sullivan C, O'Riordain M, O'Riordain D, Lyons A, Doherty J, Mannick JA, Rodrick ML. Is circulating endotoxin the trigger for the systemic inflammatory response syndrome seen after injury? Ann Surg 1997;225(5):530-541
[46] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006;124:783-801
[47] Mydel P, Takahashi Y, Yumoto H, Sztukowska M, Kubica M, Gibson FC 3rd, Kurtz DM Jr, Travis J, Collins LV, Nguyen KA, Genco CA, Potempa J. Roles of the host oxidative immune response and bacterial antioxidant rubrerythrin during Porphyromonas gingivalis infection. PLoS Pathog 2006;2:76
[48] Hajishengallis G, Wang M, Bagby GJ, Nelson S. Importance of TLR2 in early innate immune response to acute pulmonary infection with Porphyromonas gingivalis in mice. J Immunol 2008;181:4141-4149
[49] Fredriksson MI, Figueredo CM, Gustafsson A, Bergström KG, Asman BE. Effect of periodontitis and smoking on blood leukocytes and acute-phase proteins. J Periodontol 1999;70:1355-1360
[50] Wakai K, Kawamura T, Umemura O, Hara Y, Machida J, Anno T, Ichihara Y, Mizuno Y, Tamakoshi A, Lin Y, Nakayama T, Ohno Y. Associations of medical status and physical fitness with periodontal disease. J Clin Periodontol 1999;26:664-672
[51] Bokhari SA, Khan AA, Butt AK, Azhar M, Hanif M, Izhar M, Tatakis DN. Non-surgical periodontal therapy reduces coronary heart disease risk markers: a randomized controlled trial. J Clin Periodontol. 2012;39(11):1065-1074
[52] Al-Gwaiz LA, Babay HH. The diagnostic value of absolute neutrophil count, band count and morphologic changes of neutrophils in predicting bacterial infections. Med Princ Pract 2007;16:344-347
[53] Venuprasad K, Chattopadhyay S, Saha B. CD28 signaling in neutrophil induces T-cell chemotactic factor(s) modulating T-cell response. Hum Immunol 2003;64:38-43
[54] van Zandbergen G, Klinger M, Mueller A, Dannenberg S, Gebert A, Solbach W, Laskay T. Cutting edge: neutrophil granulocyte serves as a vector for Leishmania entry into macrophages. J Immunol 2004;173:6521-6525
[55] Yun PL, Decarlo AA, Hunter N. Gingipains of Porphyromonas gingivalis modulate leukocyte adhesion molecule expression induced in human endothelial cells by ligation of CD99. Infect Immun. 2006;74(3):1661-1672
[56] Nussbaum G, Shapira L. How has neutrophil research improved our understanding of periodontal pathogenesis? J Clin Periodontol 2011;38 Suppl 11:49-59
[57] Lappin DF, McGregor AM, Kinane DF. The systemic immune response is more prominent than the mucosal immune response in the pathogenesis of periodontal disease. J Clin Periodontol 2003 Sep;30(9):778-786
[58] Brunetti G, Colucci S, Pignataro P, Coricciati M, Mori G, Cirulli N, Zallone A, Grassi FR, Grano M. T cells support osteoclastogenesis in an in vitro model derived from human periodontitis patients. J Periodontol. 2005;76(10):1675-1680
[59] Colucci S, Mori G, Brunetti G, Coricciati M, Pignataro P, Oranger A, Cirulli N, Mastrangelo F, Grassi FR, Grano M. Interleukin-7 production by B lymphocytes affects the T cell-dependent osteoclast formation in an in vitro model derived from human periodontitis patients. Int J Immunopathol Pharmacol. 2005;18(3 Suppl):13-19
[60] Buhlin K, Hultin M, Norderyd O, Persson L, Pockley AG, Rabe P, Klinge B, Gustafsson A. Risk factors for atherosclerosis in cases with severe periodontitis. J Clin Periodontol 2009;36(7):541-549
[61] Monteiro AM, Jardini MA, Alves S, Giampaoli V, Aubin EC, Figueiredo Neto AM, Gidlund M. Cardiovascular disease parameters in periodontitis. J Periodontol 2009;80(3):378-388

[62] Lins RD, Figueiredo CR, Queiroz LM, da Silveira EJ, Freitas Rde A. Immunohistochemical evaluation of the inflammatory response in periodontal disease. Braz Dent J 2008;19(1):9-14

[63] Jenkins SJ, Ruckerl D, Thomas GD, Hewitson JP, Duncan S, Brombacher F, Maizels RM, Hume DA, Allen JE. IL-4 directly signals tissue-resident macrophages to proliferate beyond homeostatic levels controlled by CSF-1. J Exp Med. 2013;210(11):2477-2491

[64] Tonetti MS. Molecular factors associated with compartmentalization of gingival immune responses and transepithelial neutrophil migration. J Periodontal Res. 1997;32(1 Pt 2):104-109

[65] Soehnlein O, Zernecke A, Weber C. Neutrophils launch monocyte extravasation by release of granule proteins. Thromb Haemost. 2009;102(2):198-205

[66] Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, Herwald H, Bidzhekov K, Rottenberg ME, Weber C, Lindbom L. Neutrophil secretion products pave the way for inflammatory monocytes. Blood. 2008;112(4):1461-1471

[67] Zhou J, Zhang J, Chao J. Porphyromonas gingivalis promotes monocyte migration by activating MMP-9. J Periodontal Res. 2012;47(2):236-242

[68] Suzuki T, Sugita N, Yoshie H, Hara K. Presence of activated eosinophils, high IgE and sCD23 titers in gingival crevicular fluid of patients with adult periodontitis. J Periodontal Res 1995;30(3):159-166

[69] Xue FM, Zhang HP, Hao HJ, Shi ZY, Zhou C, Feng B, Yang PC. CD98 Positive Eosinophils Contribute to T Helper 1 Pattern Inflammation. PLoS One. 2012;7(12):e51830