VARIATION OF THE CYTOKINE PROFILES IN GINGIVAL CREVICULAR FLUID BETWEEN DIFFERENT GROUPS OF PERIODONTALLY HEALTHY TEETH

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VARIJACIJA PROFILA CITOKINA U GINGIVALNOJ ZGLOBNOJ TEČNOSTI IZMEĐU RAZLIČITIH GRUPA PARODONTALNO ZDRAVIH ZUBA

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ABSTRACT

Profiling of biomarkers of physiological process represents an integrative part in optimisation of diagnostic markers in order to adjust the diagnostic ranges to the potential effects of the local factors such occlusal forces in case of periodontal tissues. The objective of this study was estimation of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-17, IL-22, TNFα and IFNγ concentrations in gingival crevicular fluid samples (GCF) between different groups of teeth. Two hundred fifty-nine systemically healthy non-smokers having at least one vital tooth without restorations, with healthy periodontal tissues, were clinically examined and the GCF sample was retrieved. The cytokine levels were estimated using flow cytometry and compared between central incisors (CI), lateral incisors, canines, first premolars, second premolars, first molars and second molars. Cytokine profiles varied between different groups of teeth with tendency of increase in pro-inflammatory cytokines from anterior teeth toward molars. Molars might be considered teeth with natural predisposition for faster bone resorption while the adjustment of diagnostic range of periodontal biomarkers for anterior or posterior teeth should be considered within diagnostic context. Cytokine profiles varied between different groups of teeth with tendency of increase in pro-inflammatory cytokines from anterior teeth toward molars. Molars might be considered teeth with natural predisposition for faster bone resorption while the adjustment of diagnostic range of periodontal biomarkers for anterior or posterior teeth should be considered within diagnostic context.

Keywords: biomarkers, gingival crevicular fluid, cytokines, periodontal ligament, occlusal forces, periodontal disease

SAŽETAK

Profilisanje biomarkera fiziološkog procesa predstavlja integrativni deo optimalizacije dijagnostičkih markera, kako bi se dijagnostički rasponi prilagodili potencijalnim uticajima lokalnih faktora poput okluzijskih sila u slučaju parodontalnih tkiva. Cilj ove studije bila je procena koncentracija IL-1b, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-17, IL- 22, TNFα i IFNγ u uzorcima gingivalne tečnosti (GT) kod različitih grupa zuba. Klinički je pregledano dvesta pedeset devet sistemski zdravih nepušača sa najmanje jednim vitalnim zubom bez restauracija, sa zdravim parodontalnim tkivima, i uzet je GT uzorak. Nivoi citokina procenjeni su protočnom citometrijom i upoređeni između centralnih sekutića (CS), bočnih sekutića, očnjaka, prvih i drugih premolara, kao i prvih i drugih kutnjaka. Profil citokina varirao je između različitih grupa zuba sa tendencijom povećanja pro-upalnih citokina od prednjih zuba do kutnjaka. Molari se mogu smatrati zubima sa prirodnom predispozicijom za bržu resorpciju kosti, dok bi podešavanje dijagnostičkog raspona parodontalnih biomarkera za prednje ili zadnje zube trebalo razmotriti unutar dijagnostičkog konteksta.

Ključne reči: biomarkeri, gingivalna tečnost, citokini, periodontalni ligament, okluzijske sile, parodontalna bolest

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INTRODUCTION

The cytokines were widely investigated in periodontology as host response markers of periodontal diseases (1-3) The strong tendency in periodontology toward personalized medicine approach. This approach refers to the clinical decision-making supported by molecular findings for the selection of optimal treatment tailored to the individual patient. The reason for that is clinical periodontal parameters which are not sensitive enough to provide refined diagnostic information on nature of pathological process, disease activity, its magnitude and responsiveness on the performed treatment. On the other hand, cytokines being the objectively measurable regulators of periodontal inflammation are able to provide the real-time information on ongoing processes in the tissue. Additionally, the gingival crevicular fluid (GCF) is a diagnostic specimen that is easily accessible without additional invasive procedures qualitatively corresponding to the liquid biopsy (4). Briefly, GCF evolves by the serum transudation through the vessels of gingival plexus therewith skimming all biological markers on its flow from the local vessels' endothelium, junction epithelium, gingival crevices and entire gingival sulcus/ pockets. Therefore, cytokines in GCF reliably reflect ongoing processes in the periodontal tissues and provide the exact information on their nature and magnitude. However, when optimizing biochemical markers around metabolically active tissues such as periodontal ligament (PDL) and bone tissue it is of substantial importance the adjustment of the diagnostic ranges to the local physiological factors (5). In relation to that, biomarkers of normal biological processes represent the independent field in the biomarkers research (6). One of the ultimate specificities of teeth and supporting periodontal tissues is exposure to the strong occlusal forces counting about 160-240N at incisors and 490-840N in molars. The crucial role in amortization of these forces plays PDL interposed between root cement and alveolar bone while its structural integrity represents the key determinant of function. The main structural units of PDL are periodontal fibers and intercellular matrix with reach cellular content including fibroblasts, mesenchymal stem cells, osteoblasts, osteoclasts, cementoblasts and cementoclasts responsible for formation and remodeling of PDL, cement and alveolar bone (7). The activity of these cells is regulated by proprioceptors stimulated by occlusal forces via cytokine networks and corresponding autocrine/paracrine mechanisms (8, 9). In brief, stimulation of the mechanoreceptors leads to the local release of balanced concentrations of neuropeptides, growth factors and cytokines with subsequent physiological remodeling of the periodontal tissues (8, 10). Subsequently, in the case of inappropriate mechanical loading, the balance between pro-resorptive and pro-formative mediators remains disrupted leading to the structural changes in PDL and inflammatory osteoclastogenesis via receptor-activator nuclear factor kappa-B ligand (RANKL) (11). Moreover, different groups of teeth are exposed to the different intensity of occlusal forces depending of their anatomical position and primary function. However, the studies reporting the profile of cytokines around periodontally healthy teeth at different anatomical positions are very scarce.

Thus, we hypothesized that cytokine profiles in GCF are different between periodontally healthy teeth at different anatomical positions.

The objective of this study was estimation of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-17, IL-22, TNFα and IFNγ concentrations in GCF samples of central incisors, lateral incisors, canines, first premolars, second premolars, first molars and second molars from periodontally healthy teeth.

MATERIAL AND METHODS

Study population

Two hundred fifty-nine (259) adults aged 30-60 visiting the Clinic for Stomatology, Military Medical Academy, Belgrade, Serbia were recruited from November 2013 to August 2015. Patients were selected on the basis of having at least one vital tooth without restorations, with healthy periodontal tissues including no bleeding on probing (BOP), probing depth (PD) ≤ 3mm, clinical attachment level (CAL) =0, absence of mucogingival defects and absence of traumatic occlusal contacts.

Participants were included in the study if they:
- were systemically healthy non-smokers,
- had at least 24 natural teeth,
- did not have active periodontal inflammation measured as full-mouth bleeding scores (FMBOP) < 15% and
- had good oral hygiene measured as full-mouth plaque scores (FMPS) < 25%
- and if they lacked the following exclusion criteria:
- periodontal treatment in the preceding year,
- intake of antibiotics and/or anti-inflammatory agents in the preceding 3 months
- pregnancy and/or lactation in female patients
- orthodontic treatment
- bruxism and oral parafunctions
- fresh postextractional or traumatic wounds in the area of investigated teeth.

Participants were informed about study characteristics and agreed to participate by signing the informed consent form while the study was approved by the institutional ethics committee (permission reference: VMA/10-12/A.1).

Experimental Design

This study was designed as a cross-sectional study comparing the profile of 13 cytokines in the GCF of periodontally healthy teeth at different anatomical positions in adults. The participants aged between 30 and 60 were selected in order to match the age of population affected by chronic periodontitis seeking for biomarkers for disease monitoring. In the first visit, the clinical and anamnestic parameters were recorded to verify the eligibility of the participants, while the second visit for collection of GCF specimen was scheduled

second visit for collection of GCF specimen was scheduled 24-72 hours following a clinical examination in order to avoid the contamination of the sample. In relation to the representative tooth affiliation the following experimental groups were created: 1) central incisors (CI); 2) lateral incisors (LI); 3) canines (CA); 4) first premolars (PM1); 5) second premolars (PM2); 6) first molars (M1); 7) second molars (M2).

Clinical Outcome Variables

The full-mouth periodontal measurements in six sites per tooth were performed using periodontal probe graded in mm to record the following clinical parameters:

- PD as a distance between gingival margin and the bottom of the sulcus/pocket (expressed in mm).
- CAL as a distance between the cement-enamel junction and the depth at which the probe met resistance (expressed in mm).
- Bleeding on Probing (BOP) - measured 15 s after probing and recorded as presence (1) or absence (0).
- Visible Plaque Accumulation (PI) - measured along the mucosal margin and recorded as presence (1) or absence (0).

The clinical measurements were performed by two trained and calibrated examiners (E.T., V.S. and Z.T.). Intra-examiner calibration was performed twice, before and during the study, by assessing PD and CAL with a degree of agreement within ±1mm of 95.7%. All teeth were evaluated with the exception of third molars and teeth where the cemento-enamel junction could not be accurately distinguished.

GCF sampling and storing

The GCF sample was retrieved from the bucco-mesial aspect of one representative tooth in each patient being the participant unit of analysis. The representative tooth was selected randomly using computer software, while in the case when computer selected the tooth that was not present/eligible, the process was repeated until the selection of appropriate tooth. The protocol of one representative tooth per patient was selected for the purpose of initial population screening since there is no available evidence in the published literature on cytokines profiles between different groups of teeth. Additionally, it was difficult to recruit the adults with all present teeth in the mouth; hence, to ensure the homogeneity in the protocol one tooth per patient was selected.

The samples were retrieved using previously described filter paper technique (12).

Quantification of cytokines using multiplexed bead immunoassay

Cytokines were quantified in the GCF samples with commercial flow cytometric assay (Ebioscience Human Th1/Th2/Th9/Th17/Th22 13 plex kit, Bender MedSystems GMBH, Campus Vienna Biocentar, Austria, EU) on Beckman Coulter FC500 cytometer (Brea, California, USA). The concentrations of measured markers were expressed as total amount (picograms) of each cytokine per site in 30 seconds (pg/site).

Data analysis

The primary outcome variables were GCF levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-17, IL-22, TNFα and IFNγ expressed as total pg/site/30s.

Distribution of age, sex and number of the remaining teeth between the groups were compared using Fisher exact test. Distribution of biochemical data was tested for normality using Shapiro–Wilk test. Following that, the intergroup comparisons of the biochemical markers were tested with the Kruskal-Wallis test, whereas the differences were evaluated using the Mann-Whitney U test. Thereafter, the p-values were adjusted using the Bonferroni correction. Comparison of cytokines levels between maxilla and mandible was performed using Mann-Whitney test. Furthermore, the correlation between cytokine levels in the different groups of teeth were estimated using Spearman's rank correlation test. The power analysis was performed for the IL-1β levels and resulted in sample size of 28 participants per group for α=0.05 and power of 0.95. The statistical analysis was performed using commercial software (Prism 5.0, GraphPad Software, Inc., La Jolla, CA, USA) with the significance level set at 5 % (p <0.05).

RESULTS

The study sample included 112 women and 147 men, so the distribution according to the sex was similar and that was confirmed statistically. 259 investigated teeth included 32 central incisors, 34 lateral incisors, 31 canines, 36 first premolars, 47 second premolars, 38 first molars and 41 second molars, hence the quantitative composition of the groups was similar according to the statistical analysis as well. Regarding qualitative homogeneity of the groups, distribution of the age, sex, remaining teeth and periodontal status between the groups were similar as well based on the absence of any statistical significance (Table 1).

The detectability rate for evaluated cytokines between investigated teeth varied and this is outlined in Table 2. In general, the highest detectability rate for evaluated cytokines was observed in canines (10/13 cytokines) and second molars (9/13 cytokines), while in the lateral incisor no cytokine demonstrated detectability in more than 80% of samples.

Furthermore, the greatest number of cytokines with highest concentrations was observed in canines where four pro-resorptive bone cytokines including IFNγ, IL2, IL12 and IL17 demonstrated the highest levels. Further, the first premolars were the next group of teeth by the number of cytokines detected in the highest concentration including three pro-osteogenic cytokines IL-22, IL-13 and IL-4. In molars,

and IL-10 and IL1$_\beta$ in the second molars. Central incisors and second premolars showed the highest levels for one cytokine including IL-9 and IL-5, respectively while in lateral incisors no evaluated cytokines represented the highest concentration.

Table 1. Demographic and Clinical Characteristics of the Groups

Group of teeth / Parameter	Central Incisor n=32	Lateral Incisor n=34	Canine n=31	First Premolar n=36	Second Premolar n=47	First Molar n=38	Second Molar n=41
Mean age (interval)	45.2 (35-58)	46.1 (34-54)	47.8 (33-60)	46.6 (33-59)	43.9 (30-53)	45.7 (31-58)	44.2 (31-52)
Gender							
Female	18	15	15	17	21	17	17
Male	14	19	16	19	26	21	24
FMPD (mm)	2.74±2.11	2.52±1.21	2.27±0.75	2.51±1.82	2.36±1.54	2.23±0.93	2.56±1.51
FMCAL (mm)	1.98±0.77	1.65±0.89	1.56±0.75	1.65±1.15	1.89±1.25	1.75±1.45	0.98±1.01

Table 2. Detectability rate and the highest GCF levels of cytokines per different group of teeth

| Tooth	The highest average concentration	Detectability rate > 80%
Central incisor	IL9	IL12, IL10, IL9
Lateral incisor	-	-
Canine	IL2, IL12, IL17, IFNγ	IL12, IL2, IL10, IL9, IL22, IL13, IL4, IL5, IL1β, TNFα
First premolar	IL13, IL4, IL22	IL13, IL1β
Second premolar	IL5	IL12, IL2, IL1β
First molar	IL6, TNFα	IL12, IL6, IL4, IL1β
Second molar	IL1β, IL10	IL12, IL2, IL10, IL9, IL22, IL6, IL5, IL1β, TNFα

Comparison of cytokine levels in GCF samples between different groups of teeth is depicted in Fig.1. Generally, the most distinct cytokine profile was observed in central incisors that demonstrated significantly increased levels of IL-9 when compared to all other groups of teeth and significantly lower levels of 10/13 cytokines when compared to canines, premolars and molars. Briefly, in the CI the following cytokines were significantly lower when compared to the corresponding groups of teeth: IL1β < PM2, M1 and M2; IL-2 and IL-12 < CA, PM2 and M2; IL-4 and IL-5 <CA, PM2, M1 and M2, IL-6 < M1; IL-13 <CA, PM1, M1 and M2; IL-17 < CA, PM1, M1 and M2; TNFα< CA and M2 and IFNγ< CA. In the CA group the following cytokines showed significantly higher concentration compared to the corresponding groups: IL-2 > M1; IL-9 > LI and PM1; IL-17 > M1 and IFNγ > CI and PM2. In the M1 group the following cytokines showed significantly higher concentration compared to the

corresponding groups: IL-6 > CI, LI, PM1 and PM2 and IL-4 > LI as well as significantly lower level of IL-13 when compared to PM1. In the M2 group, the following cytokines showed significantly higher concentration when compared to the corresponding groups: IL-1β > CI, LI, CA, PM1 and PM2; IL-10 > PM2 and M1; IL-12 > CI and M1; TNFα > CI, LI and PM2 and IFNγ > PM2.

Comparison of cytokine levels between GCF samples from maxillary and mandibular teeth showed significantly higher levels of IL-4, IL-9 and TNFα in maxilla and significantly higher levels of IL-1β and IL-12 in mandible (Fig.2). IL-22 concentration was visibly higher in the GCF of maxillary teeth, but this was not statistically significant.

**Fig.1.** Cytokine levels in GCF samples between different groups of teeth
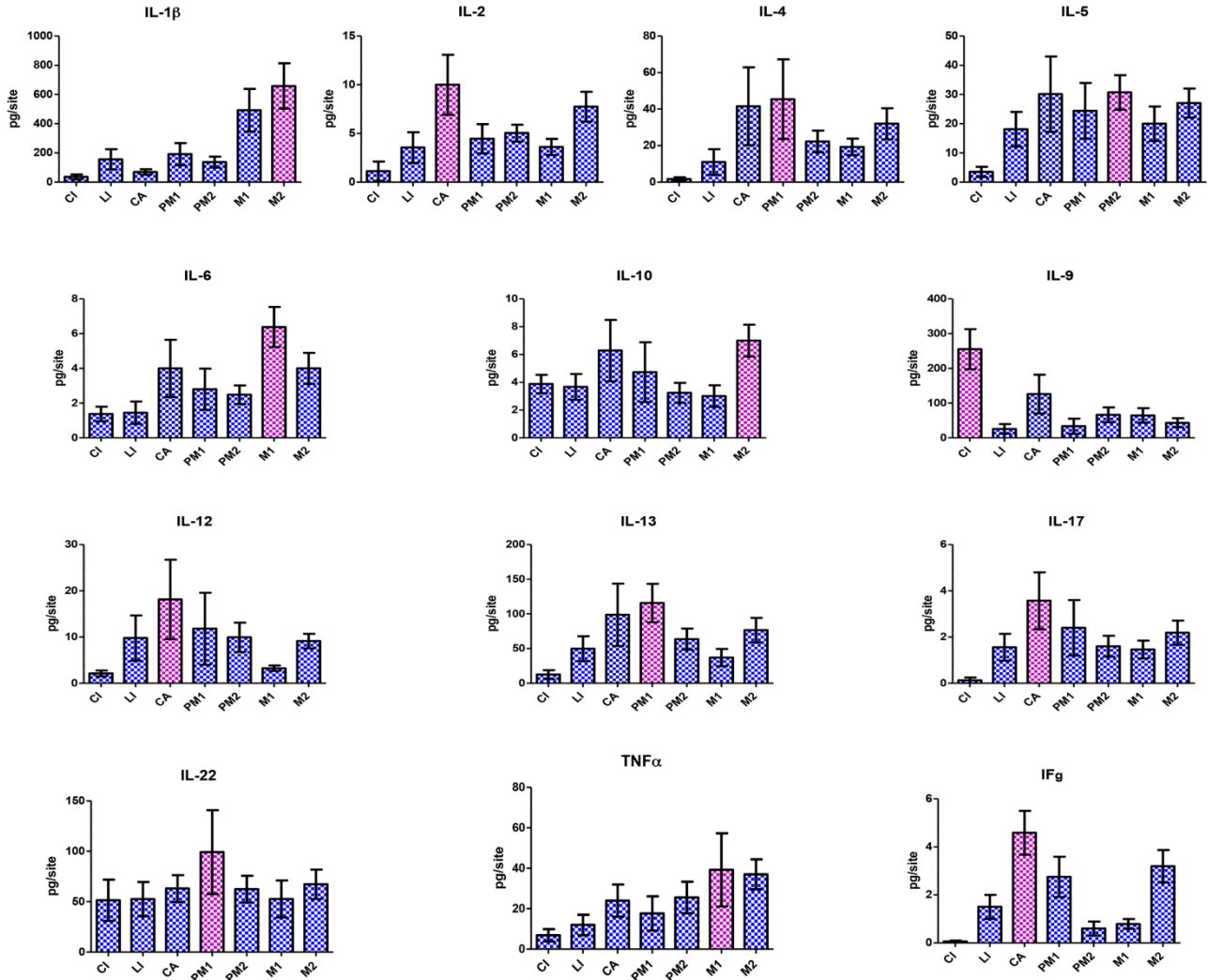
IL1b	IL2	IL4	IL5	IL6	IL9	IL10	IL12	IL13	IL17	IL22	TNFa	IFNg
PM2>CI*	CA>CI*	CA>CI*	CA>CI*	M1>CI**	CI>LI*	M2>PM2*	CA>CI*	CA>CI*	CA>CI*		CA>CI*	CA>CI*
M1>CI*	PM2>CI*	PM2>CI*	PM2>CI*	M1>LI*	CI>PM1*	M2>M1*	PM2>CI*	PM1>CI*	PM1>CI*		M2>CI*	CA>PM2*
M2>1*	M2>CI *	M1>CI**	M1>CI*	M1>PM1*	CI>PM2*		M2>CI*	PM2>CI *	M1>CI*		M2>LI*	M2>PM2*
M2>LI*	CA>CI *	M2>CI*	M2>CI*	M1>PM2*	CI>M1*		CA>M1*	M2>CI*	M2>CI*		M2>PM2*	
M2>CA*	CA>M1*	M1>LI*			CI>M2*		M2>M1*	PM1>M1*		-		
M2>PM1*					CA>LI*							
M2>PM2*					CA>PM1*							

The schema bellow the charts indicates statistically significant differences between different groups of teeth according to the Man Whitney tests following Bonferroni adjustment (*). The box plots are plotted on mean value while the read lines indicate standard error. CI-central incisor; LI- lateral incisor; CA-canine; PM1- first premolar; PM2- second premolar; M1- first molar; M2- second molar; the violet pattern indicates the group of teeth with highest level of the corresponding cytokine, **- p<0.001.

Fig.2. Comparison of cytokine levels between maxillary and mandibular teeth.
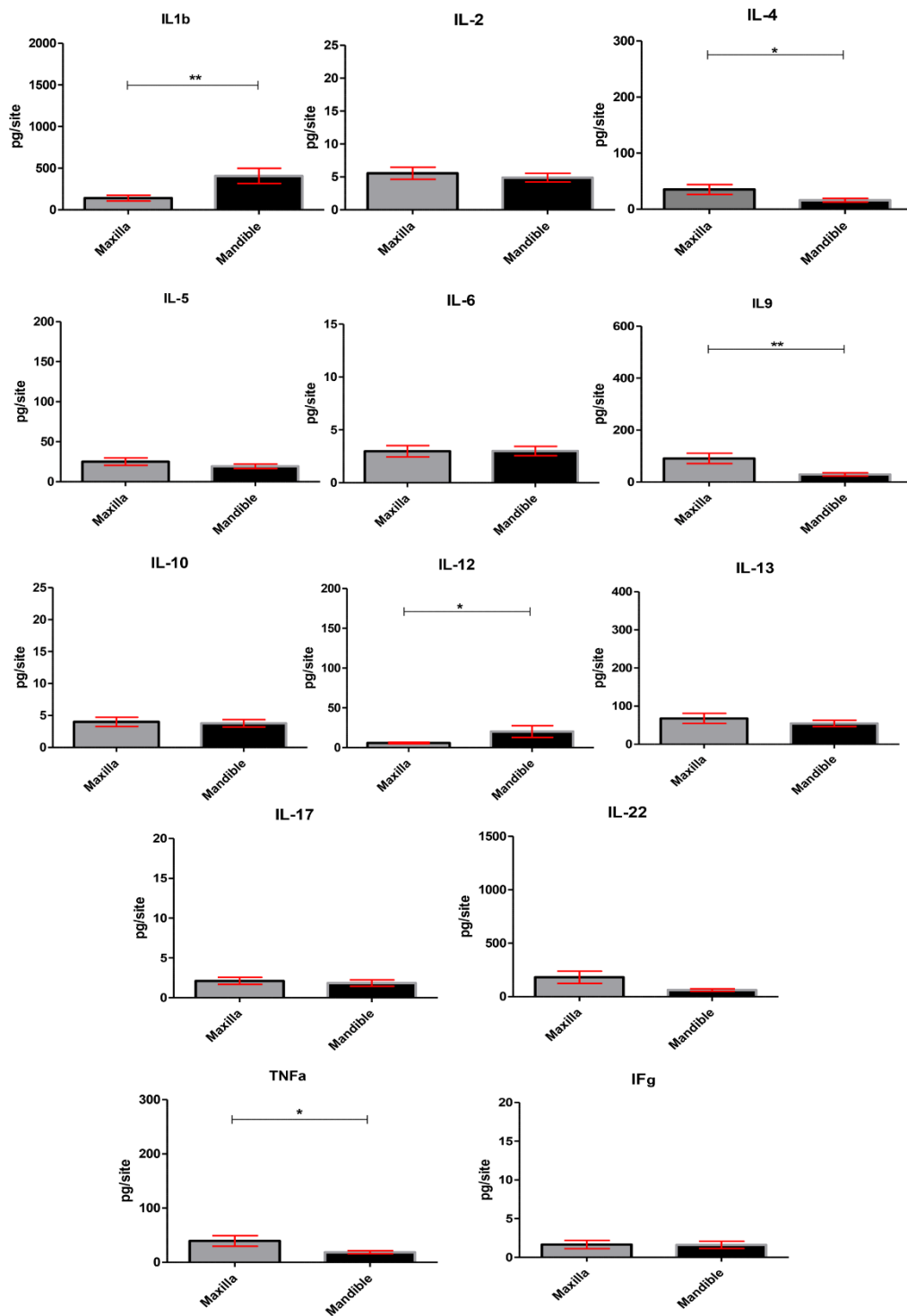
The chart depicts significantly higher levels of IL-4, IL-9 and TNFα in
maxilla and significantly higher levels of IL-1$_{\beta}$ and IL-12 in mandible; [*]- p<0.05; [**]- p<0.001.

DISCUSSION

Results of this study showed that cytokine profiles in GCF varied between different groups of teeth while the teeth demonstrating the most distinctive profile were central incisors with low levels of evaluated cytokines with exception of anti-inflammatory IL-9, canines with increased levels of both pro and anti-inflammatory cytokines and first and second molars with generally increased pro-inflammatory cytokines. Hence, the active metabolic activity around canines and tendency of increase in pro-inflammatory cytokines toward posterior teeth was observed. Moreover, the comparison of cytokine concentrations between maxilla and mandible demonstrated significantly higher levels of IL-9, IL-4 and TNFα in maxilla and significantly higher concentrations of pro-inflammatory IL-1β and IL-12 in mandible.

Cytokines in GCF have been widely investigated in periodontology for better understanding of the physiology and pathology of periodontal tissues as well as for the purpose of identification of biochemical markers of periodontal disease (1,13,14). Surprisingly enough, although cytokine networks in the periodontal tissues orchestrate the perception and entire regulation of mechanical loading, the studies reporting the cytokine profile between different groups of teeth exposed to different intensity of occlusal forces are very scarce. Generally, the studies in orthodontics estimated cytokine levels in patients undergoing orthodontic treatment and demonstrated increase in pro-resorptive bone cytokines immediately following the application of orthodontic forces while the cytokine levels decreased gradually with time (15-19). There is only one study in orthodontics that evaluated longitudinally the MMP9, TIMP1, TIMP2, RANKL and OPG GCF levels between different groups of teeth in patients undergoing orthodontic treatment and showed clear difference in concentration of all investigated mediators between tooth with different anatomical position, canines and second molars (20). Hence, this is the first study to report cytokine levels in GCF samples within different groups of teeth.

Flow cytometry method used in this study represents the method for proteomic analysis of secreted proteins considered as a powerful platform for both the research and clinical settings concerning biomarkers and patient-stratification (21). This method allows one-shot analysis of the wide selection of biomarkers thus providing a comprehensive overview of the local cytokine profile. For the purpose of this study, the set of 13 pro and anti-inflammatory cytokines was selected from five different T-helper (Th) sub-sets including Th1/Th2/Th9/Th17/Th22. This approach allowed the functional profiling by providing information of the exact groups of Th responsible for different functions as well as by providing the overview of inter-relations between secreted cytokines. Regarding the selected cytokines, majority of estimated cytokines were already investigated in periodontology including Th1 pro-inflammatory cytokines that participate in bone resorption: IL-1β, IL-12, TNFα and IFNγ; pro (IL-2) and so called anti-inflammatory Th2 cytokines act in both soft and bone tissue related processes: IL-4, IL-5, IL-6, IL-

10 and IL-17 reported (22) as an important osteoclastogenic factor. However, some recent cytokines considered of interest for periodontal physiology were also investigated in this study including IL-9, IL-13 and IL-22. IL-22 is an anti-inflammatory cytokine, member of IL10 superfamily, produced by activated DC and T cells, with the main role of regulating local antimicrobial defense (23). In addition to that, it was reported that IL-22 could have protective role in local inflammation and even regenerative function by inducing mineralization via periodontal ligament cells (24, 25). IL9 is nowadays recognized as cytokine with potent antitumor effects. IL9 induces indirectly potent antitumor response, initiating CCL20 production that mediates recruitment of dendritic cells and CD8 cytotoxic T lymphocytes expressing CCR6 (26). IL-13 secreted predominantly by Th2 cells, regulates numerous biological functions like resistance to Leishmania and Listeria species, but also processes of tissue remodeling and fibrosis (27), and could be associated with colonization of specific microbiota (28).

In this study, the parameter of cytokine detectability was followed since it is considered that, in healthy conditions, the levels of cytokines and growth factors are extremely low and frequently undetectable (due to concentrations bellow detectability threshold of the diagnostic assays). Hence, in our study we used the detectability rate >80% as an indicator of active tissue metabolism. In relation to that, canines showed the highest number of cytokines with detectability above 80% including 10/13 estimated markers. Since both pro and anti-inflammatory cytokines were in this group such an active metabolism can be explained by the role of canines as natural stress breakers (29, 30) of masticatory forces indicating their permanent exposure to the strong biomechanical stimulation (31).Moreover, it was recently indicated that proprioceptives of canine teeth are more responsive due to their role in anterior guidance, hence it seems that such hyper-responsiveness consequentially increase the levels of locally released neurotransmitters and cytokines as well (30). The next group of teeth by the number of cytokines with high detectability rate were second molars with the rate of 9/13 markers but with more expressed impact of pro-inflammatory cytokines than in canines. Such an increased detectability of pro-inflammatory cytokines in second molars as well as in first molars and second premolars who demonstrated increased detectability of three pro-inflammatory cytokines can be explained by the exposure of these teeth to the stronger masticatory forces due to their physiological function. From the biochemical aspect, stimulation of periodontal proprioceptors by mechanical loading leads to activation of transcription factors such as nuclear factor-kB, c-Fos and c-jun responsible for biosynthesis of pro-inflammatory cytokines (32, 33). Hence, such an increase in pro-inflammatory cytokines in the lateral teeth can be explained by the exposure to stronger masticatory forces that subsequently affect cytokine levels in the course of its increase. Therefore, significantly higher levels of IL-1β, IL-6 ,IL-12, TNFα and IFNγ in molars with clear tendency of their increase from anterior toward posterior teeth in this study support this physiological rule. In relation to that, the optimal occlusal loading is a key

determinant of the periodontal homeostasis (34) since under physiological loading the amounts of pro-inflammatory cytokines are competitively balanced by anti-inflammatory cytokines. This fact explains the high detectability rate and increased levels of both pro and anti-inflammatory cytokines in canines opposite to the molars where the pro-inflammatory cytokines dominated in the profile. In fact, canines are natural stress breakers of occlusale forces but are exposed to the approximately four times lower forces than molars due to different masticatory function. In addition to that, significantly lower levels of pro-inflammatory $IL1_\beta$, IL-2 , IL-5 ,IL-6 , I IL-12 L-13 , IL-17 , TNFα, IFNγ as well as anti-inflammatory IL-4 and IL-13 in central incisors indicate the balanced periodontal metabolism in the group of teeth exposed to the lowest occlusal forces.

On the other hand, in the case of excessive biomechanical loading, the amount of secreted pro-inflammatory cytokines exceeds adaptive capacity of anti-inflammatory cytokines causing the osteoclastogenesis and the structural changes in PDL associated with occlusal traumatism. Significantly higher levels of the main pro-osteoclastogenic cytokines ($IL1_\beta$, IL-6 , I IL-12 and TNFα) in molars, compared to almost all anterior teeth, can be considered as an important pathophysiological feature of molars. In fact, such an initial physiological increase in these pro-inflammatory cytokines might facilitate achievement of their critical concentrations being the trigger of inflammatory osteoclastogenesis in periodontitis and therewith provide the natural predisposition of molars for periodontitis.

Furthermore, in this study the levels of cytokines between maxilla and mandible were compared since it was reported that the metabolic activity of cancellous bone in maxilla is about 20% more expressed when compared to mandible.

This analysis revealed variation in profiles of IL-1$_\beta$, IL-4, IL-9, IL-12 and TNFα between different jaws, but further investigation in the studies with the sample size harvested in relation to the number of maxillary and mandibular teeth should be performed. In this study, the jaw appurtenance of the teeth was not considered since it is considered that there is no difference in acting of occlusal forces between maxillary and mandibular teeth of the same group of teeth.

Moreover, since this was the first study to investigate the cytokine profiles between different groups of teeth in adults, the protocol of one tooth per patient was selected for initial screening of population and this can be considered as a relative limitation of the study. Although it is considered that the inter-individual variations in cytokine networks are expressed in disease rather than in healthy condition, for the future studies the analyses of cytokine profiles around different groups of teeth in the same mouth of healthy participants should be considered. Moreover, the same analyses should be conducted in patients with periodontitis in order to establish whether different groups of teeth exhibit substantially different cytokine profile that should be considered in setting of diagnostic range of potential biochemical markers.

CONCLUSION

Results of this study indicated that cytokine profiles in GCF within different groups of teeth vary with clear tendency of increased pro-inflammatory cytokines concentration from anterior teeth toward molars. Therefore, molars might be considered as teeth with natural predisposition for faster bone resorption, while the adjustment of diagnostic range of periodontal biomarkers for anterior or posterior groups of teeth should be possibly considered.

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