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Effect of gingival inflammation on the inflammatory response in patients with idiopathic uveitis

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Abstract

Objectives: This study aimed to investigate the levels of eleven oral species in plaque samples and cytokine levels in biofluid samples of patients with idiopathic uveitis (IU) and systemically healthy individuals (H) with or without gingival inflammation.

Materials & Methods: Twenty-one patients with IU (n=21), and twenty-two systemically healthy individuals (n=22) were enrolled in the study. Clinical periodontal measurements were recorded. Cytokine levels in the biofluid samples were determined by ELISA. Bacteria gene copy numbers were determined by qPCR on plaque microbial DNA preparations.

Results: According to two-step cluster analysis, ANOVA and t-test: GCF, serum and salivary TNF-α, IL-17A, IL-17A/E; GCF and serum IL-6; salivary IL-17F and salivary, serum IL-17A/F levels were higher in the IU group than the H group (p<0.05). However, serum IL-10 and IL-17E levels were higher in the H group than the IU group (p<0.05). A. actinomycetemcomitans, F. nucleatum, S. oralis, A. naeslundi and V. dispar counts were higher in IU group compared to H group (p<0.05).

Conclusion: Altered local and systemic cytokine profiles are associated with differences in the microbial plaque composition in IU. Anti-inflammatory cytokines; IL-10, IL-17E are reduced in patients with IU and Th-1 and Th-17 driven inflammatory responses in biofluids are altered.

MeSH Keywords: Cytokine(s); Dental plaque; Gingivitis; Interleukin(s); Uveitis.

Scientific rationale for study: Importance of Th17 cells and IL-17 in the pathogenesis of the idiopathic uveitis have been reported. Sustained pro-inflammatory cytokines (i.e presence of gingival inflammation) might have some potential to impair the balance of the ocular immune system by stimulating the autoreactive T cells.

Principal findings: Production and regulation of signature cytokines of Th cells especially IL-17A and TNF-α seem to be increased in the presence of IU and microbial; composition of subgingival plaque altered.

Practical implications: Periodontal screening of IU patients, improved oral hygiene, the control of gingival inflammation to influence circulating cytokine levels.

Conflict of Interest and Source of Funding: The authors have no conflict of interests and the study has been funded solely by the institutions of the authors.
Introduction

Uveitis is an inflammatory disease involving the pigmented vascular coat of the eyeball (iris, ciliary body and choroid) and is a major cause of severe visual impairment with the 10-15% of total blindness and its incidence in the USA was reported as 52.4/100.000 people per year (Gritz and Wong, 2004). Idiopathic uveitis (IU) is an isolated type of autoimmune uveal inflammation that may occur without any accompanying autoimmune mediated diseases. It has recently been shown that Th17 cells and interleukin-17 (IL-17) may have an important role in the pathogenesis of the IU (Sun et al. 2015) and the inflammatory process is mainly driven by Th17 cells and is sustained by pro-inflammatory cytokines such as, tumour necrosis factor-alpha (TNF-α), IL-10, IL-1β and IL-6 (Selmi, 2014). IL-17 is potentially an important mediator in periodontal immunopathology due to its pro-inflammatory and bone resorptive activities and furthermore, it has been demonstrated that, IL-17 producing cells are related to severity of gingival inflammation (Cheng et al., 2014). Gingivitis is a very common chronic inflammatory status of the periodontium, in which oral microbiota is the primary etiological factor (Colombo et al., 2009). High levels of IL-17A were reported in gingival crevicular fluid (GCF) of periodontitis patients (Vernal et al., 2005). Consequently, in patients with periodontitis, the association of the increased GCF levels of IL-17 and the presence of Porphyromonas gingivalis in the subgingival biofilm has been reported (Zenobia and Hajishengallis, 2014). IL-10 shows a critical protective role by balancing IL-17 and Th17 pathway in order to prevent immunopathology i.e. caused by oral microbiota in periodontal disease (Moretti et al., 2015). Pro-inflammatory cytokines TNF-α and IL-6 are involved in Th1 and Th2 driven responses, respectively and both are related to gingival inflammation (Garlet, 2010).

Uveitis is more frequently diagnosed in young adults and so is gingivitis (Prete et al., 2014). Therefore, increased IL-17 receptor signalling and elevated production of mediators may shift the acute inflammatory state to chronic persisting inflammatory status, which then may potentially cause inflammation mediated tissue destruction. To date, the relationship between oral microbiota, gingival inflammation in the presence of IU has not been investigated. The hypothesis of this study is that the inflammatory cytokine levels in biofluids and the levels of certain bacteria in dental plaque are elevated in the presence of IU particularly when there is accompanying gingival inflammation. Hence, the aim of the present study was to investigate the levels of eleven oral species in plaque samples, IL-17, IL-6, IL-10 and TNF-α levels in GCF, saliva and serum samples of patients with IU and also systemically healthy individuals.
Materials and Methods

Study population
Twenty-one patients with IU and twenty-two systemically healthy individuals followed by the outpatient clinic of the Department of Ophthalmology, School of Medicine, Ege University, Turkey were enrolled in this study between September 2013 and April 2014. The study was conducted in full accordance with ethical principles, including the World Medical Association’s Declaration of Helsinki, as revised in 2008 and was approved by the Ethics Committee of the Ege University with the protocol number 13-3.2/10. The study protocol was explained and written informed consent was received from each individual prior to enrolment. The study conforms to STROBE guidelines for observational studies (von Elm et al., 2007). Complete medical and dental histories were obtained from each individual. The inclusion criteria of IU group were; age ≥ 18 years, diagnosis of the IU based on the SUN (Standardization of Uveitis Nomenclature) criteria (Jabs et al., 2005). Exclusion criteria were; presence of immunologic diseases and infectious pathologies diagnosed by physical inspection and serologic tests, autoimmune diseases including Behçet disease, ankylosing spondylitis, multiple sclerosis, rheumatoid arthritis, sarcoidosis, pregnancy, lactation, usage of antibiotics or anti-inflammatory, immunosuppressive drugs including biologic agents within the last 6 months, current smoking and smoking within the past 10 years. The comparison group consisted of systemically healthy individuals. Upon confirmation of eligibility for enrolment in the study, all individuals returned to the clinic for clinical periodontal measurements including probing depth (PD), clinical attachment level (CAL), plaque index (PI) (Silness and Löe, 1964) and gingival index (GI) (Löe, 1967). Clinical periodontal measurements were performed at 6 sites on each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations), except for third molars, using a manual periodontal probe (Williams, Hu-Friedy, Chicago, IL, USA) by a single calibrated examiner (AA).
Patient selection was based on clinical and radiographic criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (Armitage, 1999). Diagnosis of gingivitis was assigned when BOP scores exceeded 50 % of all sites, PD < 3 mm at 90% of the measured sites and no more than one site had a PD > 4 mm or CAL ≤ 1 mm, with no clinical and/or radiographic sign of periodontitis.
Biofluid sampling

GCF samples were obtained by filter paper strips (PerioPaper, ProFlow, Amityville, NY) from the buccal aspects of two interproximal sites (PD < 3 mm, CAL ≤ 1 mm) with obvious plaque accumulation and visible signs of inflammation (except for the controls) at single-rooted teeth and at least one multi-rooted tooth from each individual. First, supragingival plaque was removed carefully by sterile curettes and the surfaces were gently air-dried and isolated from saliva by cotton rolls. Then filter paper strips were placed into the gingival sulcus for 30 seconds. Care was taken to avoid mechanical trauma, and strips visually contaminated with blood were discarded. The absorbed GCF volume was estimated by a calibrated instrument (Periotron 8000, Oraflow, Plainview, NY). The two strips from each patient were placed into one polypropylene tube and frozen immediately.

All salivary samples were collected in the morning between 8:00 am and 9:00 am. Participants were asked to avoid oral hygiene measures (flossing, brushing and mouthrinses), eating, and drinking 2 h prior to saliva sampling. All individuals were asked first to rinse their mouth with distilled water for 2 min, wait for 10 min and then expectorate into sterile 50 ml tubes for 5 min.

A total of 5 ml of venous blood were taken into a silicone-coated tube (BD Diagnostics, Franklin Lakes, NJ) by a standard venipuncture method. Once collected, the samples were left at room temperature to allow blood clotting, and then centrifuged for 15 min at 1500 x g at +4°C to remove the fibrin clot and cellular elements.

All biofluid samples were taken before clinical periodontal measurements and immediately frozen and stored at -80°C.

Microbiologic Plaque Sampling and Processing

Approximately 15 minutes after GCF collection, subgingival plaque samples were collected from the interproximal sites of the same sampling teeth by using standardized #30 sterile paper points. A sterile paperpoint was inserted to the base of the sulcus, left there for 10s and then removed gently. Maximum care was taken not to provoke any bleeding in the adjacent tissues. The two plaque samples from each individual were pooled in a dry Eppendorf tube, and kept at -80°C.

For determining microbiological composition the DNA was extracted from the plaque and real-time quantitative polymerase chain reaction (real-time qPCR) was employed as described recently (Nizam et al., 2015) in order to define the quantity (Equivalent colony forming units) of *P. gingivalis*, *T. denticola*, *T. forsythia*, *A. actinomycetemcomitans*, *P.
intermedia, F. nucleaturn, P. micra, A. naeslundi, V. dispar, S. oralis, S. mutans. Briefly, the DNA was isolated from a paperpoint sample using a Masterpure DNA isolation kit (tebu-bio Peterbourgh UK) and the amount of DNA obtained varied between 3 and 9 micrograms as determined by measuring the wavelength at 260 nm. A standard curve DNA was prepared in the same manner from five serial 1/10 dilutions of $1 \times 10^8$ CFU determined by counting visually their growth on Agar (containing approximately 1 - 2 µg bacteria ~400 - 800 ng of DNA) of a laboratory isolates of each assay target bacterium. DNA standards were suspended to a to a final DNA concentration of 200 µg/ml with salmon testes DNA (Sigma Poole UK).

PCR reactions contained 200 ng of microbial DNA and 200 nM - 500 nM of each 16S rRNA gene oligonucleotide primer (concentration dictated by the efficiency $E=10^{-1/\text{slope}}$ determined from Ct v Log$_{10}$ bacterial DNA concentration of validation assays). Standard PCR conditions were run in triplicate as follows: 95°C for 10 min - then 40 cycles of 95°C, 30s; 60°C, 1 min; 72°C, 1min; then 72°C for 5 min - followed by a melt cure analysis gradient (95°C-25°C over 35 min) in a Stratagene MX3000 Real-Time PCR machine (Thermo Fisher Paisley UK). The amount of product was measured using SYBR green with ROS as the reference dye. The Ct values obtained in the real time PCR of each test were compared to the assay standards and the 16S rRNA gene copy number derived by adjusting for the assay dilution and sample volume.

Measurement of TNF-α, IL-6, IL-10, IL-17A, IL-17F, IL-17E, IL-17A/F levels in biofluid samples

Commercial ELISA kits were purchased for the measurement of TNF-α, IL-6, IL-10, IL-17A, IL-17F, IL-17E (Peprotech London UK), IL-17A/F (R & D systems Abingdon UK). The ELISA assays were carried out according to the manufacturers’ recommendations. The optical densities of each well were read at 450 nm with a background subtraction at 570 nm and the samples were compared with the standards. The minimum detection limits for the assays were; TNF-α 0.4 pg/mL, IL-6, IL-10, IL-17A, IL-17F, IL-17E, 0.79 pg/mL, IL-17A/F 1.59 pg/mL. The GCF data were expressed as total amounts per sampling time (pg/30s) and also as concentrations (pg/µL). The findings in saliva and serum samples were expressed as concentrations (pg/mL).

Statistical analysis
The statistical analyses were conducted using commercially available statistical software (SPSS Inc. version 21 IBM, Chicago, USA) and statistical significance was considered at p=0.05.

A statistical power calculation was based on the assumption of log normally distributed data. With an alpha = 0.5 a predicted mean difference equal to the pooled standard deviation the minimum n=17 in each group was required to achieve 80% statistical power. Following Q-Q plot analysis the data was found to approximate a log normal distribution.

The t-test was used to evaluate the differences between group means of the log transformed data. Weighted least squares regression analysis on Univariate tests (t-tests) was performed in a general linear model to correct for potential confounding factors (PI and GI). After randomisation of the data, a two-step cluster analysis was performed. Parameters such as age, and clinical, biochemical, and bacterial data were added to the analysis as continuous variables and the gender and diagnosis as categorical variables. This procedure was followed by ANOVA and Bonferroni or Tamhane post-test depending on the result of a Levenes' test comparing group variances.

The differences in bacteria detected in the study groups were investigated by the analysis described above, represented graphically as a summation plot of the percentage of each bacterial species of the total microbes detected in the assays and by a principal component analysis (PC analysis). A PC analysis is a non-parametric statistical procedure that was used to convert the log copy numbers of each microbe (a set of possibly correlated variables) into orthogonal linearly uncorrelated variables, for each microbe data set by subtracting the mean, dividing by the standard deviation rotating in space and plotting the two greatest variance levels (PC1 & PC2) on a Cartesian diagram.

Correlation between the clinical parameters and the microbial analysis and between GCF, salivary and serum biochemical data were evaluated with Spearman rho rank correlation test.

**Results**

**Periodontal clinical findings**

Table 1 reports demographic variables and periodontal parameters of the study groups. There were no significant differences between the groups for gender, age and clinical periodontal parameters apart from the plaque and gingival index. Differences in gingival inflammation levels were compensated for in a weighted analysis of the clinical and biochemical data.
Cytokine measurements

The number of samples with cytokine levels below the detection limits were as follows; for TNF-α; 5 samples (4 healthy, 1 IU; 13%) in GCF and saliva, 2 samples (1 healthy, 1 IU; 5%) in the serum, for IL-6; 2 samples (1 healthy, 1 IU; 5%) in GCF, for IL-17A/F; 4 samples (3 healthy, 1 IU; 10%) in GCF.

GCF total amounts, serum and salivary TNF-α, IL-17A levels, and IL-17A/IL-17E, IL-17A+F+A/F/IL-17E ratios were higher in the IU group than the systemically healthy controls (p<0.05) (Figure 1-3). Similarly, GCF concentrations of IL-17A, IL-17F, IL-17E, IL-10 and TNF-α were higher in the IU group than the systemically healthy controls (p<0.05) (Figure 1-3).

GCF total amounts and concentrations and serum IL-6 levels, salivary IL-17F levels and salivary, serum IL-17A/F levels were higher in the IU group than the systemically healthy individuals (p<0.05) (Figure 1-3). However, serum IL-10 and IL-17E levels and GCF concentrations of IL-17E were significantly higher in the systemically healthy individuals than the uveitis group (p<0.05).

The two-step cluster analysis resulted in separation into 3 clusters. Cluster 1 consisted mainly of healthy participants with gingivitis, cluster 2 were all healthy participants and cluster 3, the largest grouping, all had uveitis, but in the presence and absence of gingivitis: cluster 1 (n=12); gingivitis (n=10) + IU (n=1) + IU with gingivitis (n=1); Cluster 2 (n=8): all healthy (n=8); Cluster 3 (n=20); uveitis (n=9) + uveitis with gingivitis (n=11). There were 3 outliers in 4 data sets with missing values. Predictors of importance > 0.50 in descending order were: Diagnostic criteria, GCF IL-17A:IL-17E ratio, GCF IL-17F, serum IL-17A/F, A. naeslundi, serum IL-17A, V. dispar, GCF IL-17A+F+A/F, IL-10 and GCF IL-6. ANOVA and Bonferroni or Tamhane post-test analysis indicted all these parameters to be higher in cluster 3 (all IU) and in cluster 1 (mostly gingivitis) than in cluster 2 (all healthy) Figure 4.

Plaque and gingival index scores correlated positively with salivary and serum levels and GCF amounts of TNF-α (rho = 0.311, 0.362, 0.434, 0.462, 0.349 and 0.384, respectively; p < 0.05). Significant correlations were found between plaque index scores and salivary IL-17A and IL-17E levels and serum IL-17E levels as well as salivary ratios of (the sum of IL-17A, IL-17F and IL-17A/F):IL-17E (IL-17A+F+A/F:IL-17E ratios) (rho = 0.326, 0.311, -0.404 and -0.370, respectively; p < 0.05). For the majority of cytokines the measure of each particular analyte in biofluids showed moderate to high correlations (rho = 0.382 to -0.779, p < 0.01) with measurements in the other biofluids (data not shown). Generally, the pro-
inflammatory cytokines correlated positively with levels in the same biofluid (and often levels in other biofluids) of the other pro-inflammatory molecules (rho = 0.312 to 0.576, p < 0.05) and negatively with IL-17E and IL-10 (rho = -0.424 to -0.779; p<0.01). The exceptions to this generalization were salivary IL-17F, serum IL-17A/F and GCF levels of TNF-α, which did not appear to correlate with the other cytokines (data not shown).

**Microbial analyses**

Figure 5a shows the respective relative proportions in the healthy plaque samples compared to the IU plaque samples of *A. actinomycetemcomitans* (1.9 % and 8.4 %), *A. naeslundi* (15 % and 33 %), *F. nucleatum* (2.3 % and 16 %), *P. gingivalis* (9 % and 0.1 %), *P. intermedia* (5.4 % and 0.1 %), *P. micro* (25 % and 6 %), *S. oralis* (0.9 % and 1.7 %), *S. mutans* (19 % and 12 %), *T. denticola* (3.8 % and 0.3 %), *T. forsythia* (0.4 % and 2.5 %), and *V. dispar* (15 % and 18 %) in the subgingival plaque samples collected from patients with IU and systemically healthy controls, respectively. The 16S rRNA gene numbers of *A. actinomycetemcomitans, F. nucleatum, S. oralis, A. naeslundi* and *V. dispar* were significantly higher in IU group plaque samples than in the plaque samples collected from the systemically healthy individuals (p<0.05). The clusters two-step cluster analysis indicated that the same microbes were significantly higher in of IU plaque samples than in the healthy samples supported the original t-test results for the organisms that the healthy plaque had lower copy numbers of (p<0.001).

PC analysis of the selected set of oral microorganisms present in the samples of subgingival plaque in health compared to subgingival plaque in IU indicated clear difference in the variance (mainly on PC1 axis) of the microbial data of the two study groups. The variance for Principal component 1 =44.0% and variance for Principal component 2 was 14.8% (Figure 5b).

The only significant correlation between the presence of particular microbes and the clinical indices was found between the presence of *T. denticola* and the PD, CAL (rho = 0.346 and rho=0.329, respectively; p < 0.05). There were positive correlations between the presence of certain microorganisms and the levels of pro-inflammatory cytokines. In particular, *A. actinomycetemcomitans, A. naeslundi, F. nucleatum, and V. dispar* which correlated with GCF- and salivary TNF-α; GCF- and serum IL-6; GCF-, saliva- and serum IL-17A (rho = 0.314 to 0.617 p < 0.01); GCF-, saliva- and serum IL-17A/F (rho = 0.332 to 0.532 p < 0.01). Negative correlations were observed between the presence of *A. actinomycetemcomitans, A.
naeslundi, F. nucleatum, and V. dispar and serum IL-10 (rho = -0.340 to -0.448; p < 0.01) and (with the exception of A. naeslundi) with serum and salivary IL-17E (rho = -0.370 to -0.420 ; p < 0.01).

**Discussion**

It is suspected that environmental factors (microbial) and/or sustained pro-inflammatory cytokines might impair the balance of the ocular immune system by stimulating the auto reactive T cells and further causing ocular immunopathology. To the best of the authors’ knowledge, this is the first study to examine the possible association between IU and oral microbiota in plaque and biomarkers in biofluids in regards with gingival inflammation. Uveitis is more frequently diagnosed in young adults and so is gingivitis (Prete et al., 2014). An important limitation of the present study is the lack of participants enabling subgroup analysis based on the clinical periodontal status (periodontally healthy vs gingivitis) in the analysis. The inclusion of such subgroups in the analysis was avoided to prevent an underpowering of the analyses. However, a two-step cluster analysis of the data defined 3 clusters. The cluster analysis indicated that the participant diagnosis was the most important predictor for cluster formation. This was followed by certain biofluid cytokine levels (such as the GCF IL-17A:IL-17E ratios) and the presence of certain microbes such as A. naeslundi. Cluster 1 consisted mainly of healthy participants with gingivitis, cluster 2 - healthy participants and cluster 3, all had uveitis, but with or without gingivitis. The presence of inflammation, as indicated by the increased levels of several pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines, may have had a pronounced influence on the formation of clusters 1 and 3. Whether this shows that the presence of uveitis was the major driving factor in the inflammatory process and formation of cluster 3 is moot. These clusters were tested by ANOVA and provided further support for the major findings of the IU group versus healthy group comparisons (t-test).

Nevertheless, the present study indicates elevated cytokine profiles in biofluids of the IU patients compared to those of the systemically healthy controls. TNF-α plays a key role in the pathogenesis of many inflammatory diseases and has been found at high concentrations both in the aqueous humour and in the serum of patients with uveitis (Perez-Guijo et al., 2004). Similarly, in the present study TNF-α were higher in serum as well as GCF and saliva of IU patients compared to systemically healthy controls.
Cytokine levels in the systemic circulation mirror impaired balance in cytokine levels of the inflamed gingival tissue. IL-17 has a substantial role as it is involved in the first-line bacterial infection defence (as bacterial immunity) especially at mucosal and epithelial barriers (Ivanov et al., 2009, Jin and Dong, 2013) and in fact, IL-17 is a key component of a neutrophil feedback mechanism that maintains steady-state neutrophil counts. Although, there is no data on the correlation of the salivary and ocular tissue content, the present study demonstrated elevated salivary levels of IL-17A, IL-17F, IL-17A/F and TNF-α. Saliva is an important diagnostic tool to evaluate mediators originated from serum and may well have a diagnostic value for ocular tissue. Evaluation of the salivary levels of these mediators may contribute the possible diagnostic algorithm or outcome measures of this unique immune-mediated disease.

It was reported that both IL-17A and IL-17F has additional biologic properties on stimulation of pro-inflammatory cytokine IL-6, TNF-α and IL-1β expression (Iwakura et al., 2011). Furthermore, TNF-α stimulates IL-17A expression by cooperation with IL-23 (Liu et al., 2011). Positive correlations were found between IL-17 and IL-6, TNF-α levels in biofluids of the study population (data not shown). Furthermore, GCF and serum levels of IL-6 and TNF-α were elevated in patients with IU supporting such an association. Moreover, it has been reported that TNF-α initiates an immunosuppressive effect via a synergistic association with IL-17 (Han et al., 2014). However, the current study design does not facilitate comment on the bidirectional relationship between these cytokines.

The limiting effect of IL-10 on the Th1 and Th17 inflammatory response in periodontal disease is important in regards not reducing immunopathology (Moretti et al., 2015). IL-17E acts as a suppressor mediator among Th17 cytokines (Lee et al., 2001). While IL-10 and IL-17E play important roles in modulation of chronic inflammation in the mouth, the results of the present study suggest that IL-10 and IL-17E may play protective roles in acute oral inflammatory conditions also, particularly in the presence of other inflammatory conditions. In this study, the participants were free of periodontitis, it might be suggested that regulation of the immune response is impaired because of reduced circulating levels of IL-10 and IL-17E and this might pose a risk for future periodontal disease progression. However, when GCF levels of IL-10 were calculated local production of this molecule was greater in IU. Whether this suggests that local protective levels to the oral microbiome was not impaired in patients with IU is yet to be elucidated.

Oral microbiota and its bacterial components such as lipopolysaccharides and peptidoglycans may serve as a link between innate and cell-mediated response (Pratap et al., 2011), which is
further important to the pro-inflammatory cytokine production and related Th1, and Th17 driven response. Within the limits of the present study we are able to detect all of the selected bacteria in both groups, but not in all patients. There was a distinct change in the composition of the microbial plaque in systemically healthy individuals compared to IU group. Despite the role of oral microorganisms in periodontal health or disease, the oral biofilm may be a source for systemic circulation and the development of systemic infections via the bacterial components (Vieira Colombo et al., 2015). Present findings support this assumption by reporting several significant positive correlations between analysed bacterial species and biochemical parameters in biofluids. However, we did not see a firm association between the microbial species present and the clinical measures of periodontal inflammation with the exception of _T. denticola_. How IU exerts an influence on the gingival microbiota remains unclear. It would appear reasonable to assume that there may be a bidirectional association between the microbial composition of gingival plaque and local and systemic levels of certain cytokines, which regulate local inflammation and host responses to infection. However, it is realised that the selection of a small number of microbes in this investigation is possibly a further limitation of this current study.

It has been hypothesized that several inflammatory processes may induce an abnormal T cell-mediated immune response and their cytokine pathways may impair the blood-retinal barrier (Lee et al., 2012, Horai and Caspi 2011). Therefore, existing gingival inflammation may contribute increased T cell response. Accordingly, the present findings revealed several positive correlations between PI and GI scores and the cytokine levels.

Cytokine levels in blood and ocular fluids; intracellular cytokines in serum have been used to predict disease severity. The present findings possibly suggest a new assessment method using saliva and GCF in order to measure cytokine profile for the patients with IU.

**Conclusions**

Altered local and systemic cytokine profiles are associated with differences in the microbial plaque composition in IU. Regulatory function of anti-inflammatory cytokines IL-10 and IL-17E are reduced in patients with IU and Th-1 and Th-17 driven inflammatory responses in biofluids are altered. Plaque composition and the cytokine profiles investigated show different patterns in the two groups of individuals. However, since correlation is not necessarily causation, the results could well be describing a consequence of an immunological malfunction in IU patients, which allows differential proliferation of certain species.
Better understanding of IU and gingivitis may help to develop new therapeutic strategies aiming at the control of inflammation-mediated responses. Therefore, periodontal screening of these patients and the control of gingival inflammation may influence circulating cytokine levels. Future clinical intervention studies are needed to investigate the possible effects of periodontal treatment on clinical manifestations of IU.
REFERENCES


Tables and figure legends

Table 1: Demographic variables and periodontal parameters.

Figure 1: Serum levels of biochemical data
Concentrations (pg/mL) of TNF-α, IL-6, IL-10, IL-17A, IL-17F, IL-17A/F, IL-17E, IL-17A:IL-17E and IL-17A-A/F:IL-17E in serum. The horizontal lines in the boxplots represent the median values. Each dot represents an individual in the groups. Significant differences between groups are shown on the graphs.

Figure 2: Salivary levels of biochemical data
Concentrations (pg/mL) of TNF-α, IL-6, IL-10, IL-17A, IL-17F, IL-17A/F, IL-17E, IL-17A:IL-17E and IL-17A-A/F:IL-17E in saliva. The horizontal lines in the boxplots represent the median values. Each dot represents an individual in the groups. Significant differences between groups are shown on the graphs.

Figure 3: GCF total amounts and concentrations of biochemical data
Figure 3a: Amounts (pg/30s) of TNF-α, IL-6, IL-10, IL-17A, IL-17F, IL-17A/F, IL-17E, IL-17A:IL-17E and IL-17A-A/F:IL-17E in GCF. The horizontal lines in the boxplots represent the median values. Each dot represents an individual in the groups. Significant differences between groups are shown on the graphs.

Figure 3b: Concentrations (pg/µL) of of TNF-α, IL-6, IL-10, IL-17A, IL-17F, IL-17A/F, IL-17E, IL-17A:IL-17E and IL-17A-A/F:IL-17E (I)) in GCF. The horizontal lines in the boxplots represent the median values. Each dot represents an individual in the groups. Significant differences between groups are shown on the graphs.

Figure 4: Predictor importance in the cluster analyses. Predictors greater than 0.5 are shown.

Figure 5a: Detection and principal component (PC) analysis of selected microorganisms in subgingival plaque
Stacked bar graphs indicate the relative percentages of the sum of the copy numbers of A. actinomycetemcomitans, A. naeslundi, F. nucleatum, P. gingivalis, P. intermedia, P. micra, S. oralis, S. mutans, T. denticola, T. forsythus, and V. dispar in sulcal and subgingival plaque of patients with IU and systemically healthy controls.
Figure 5b: Principal component (PC) analysis of *A. actinomycetemcomitans, A. naeslundi, F. nucleatum, P. gingivalis, P. intermedia, P. micra, S. oralis, S. mutans, T. denticola, T. forsythia,* and *V. dispar* in sulcal and subgingival plaque of patients with IU (red dots) and systemically healthy controls (black dots). A non-parametric statistical procedure; PC analysis was used to convert a set of possibly correlated variables into orthogonal linearly uncorrelated variables, for each data set by subtracting the mean, dividing by the standard deviation rotating in space then plotting on a Cartesian diagram the two greatest variance levels (principal components) obtained for each data set.
Table 1. Demographics and clinical periodontal measurements

<table>
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<th>Idiopathic uveitis (n=21)</th>
<th>Systemically Healthy (n=22)</th>
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<td>Age (years)</td>
<td>39.6 ± 16.8</td>
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<td>1.9 ± 0.9</td>
<td>1.7 ± 0.8</td>
<td>0.59</td>
</tr>
<tr>
<td>PI (Score 0-3)</td>
<td>0.7 ± 0.6*</td>
<td>0.4 ± 0.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>GI (Score 0-3)</td>
<td>0.7 ± 0.7*</td>
<td>0.4 ± 0.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Periodontal status (Healthy/gingivitis) (n)</td>
<td>10 / 11</td>
<td>10 / 12</td>
<td>0.95</td>
</tr>
</tbody>
</table>

**PD** = Probing depth, **CAL** = Clinical attachment level, **PI** = Plaque Index, **GI** = Gingival index. Values are shown as mean ± standard deviation. p-value indicates results of t-test of differences in group means. * IU group mean is significantly greater than healthy group mean.
Figure 3

**GCF IL-17A:IL-17E**

- Healthy: 0
- Uveitis: 3
- Ratio: 5
- p-value: 0.003

**Saliva IL-17A:IL-17E**

- Healthy: 0
- Uveitis: 1
- Ratio: 5
- p-value: 0.025

**Serum IL-17A:IL-17E**

- Healthy: 0
- Uveitis: 5
- Ratio: 15
- p-value: 0.004

**GCF IL-17A-A/F:IL-17E**

- Healthy: 0
- Uveitis: 2
- Ratio: 8
- p-value: 0.017

**Saliva IL-17A-A/F:IL-17E**

- Healthy: 0
- Uveitis: 1
- Ratio: 5
- p-value: 0.001

**Serum IL-17A-A/F:IL-17E**

- Healthy: 0
- Uveitis: 5
- Ratio: 20
- p-value: 0.005
Figure 4

Predictor importance

- Diagnosis
- GCF. IL-17A:IL-17E
- GCF. IL-17F
- Serum IL-17A/F
- A. naeslundii
- Serum IL-17A, A/F & F
- V. dispar
- GCF IL-17A
- Serum IL-17A
- GCF IL-17A, A/F & F