Cytokines, matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 in gingival crevicular fluid from patients with Papillon-Lefèvre syndrome

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Ullbro C, Crossner C-G, Nederfors T, Parhar R, Al Mohanna F, Meikle MC, Reynolds JJ, Twetman S. Cytokines, matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 in gingival crevicular fluid from patients with Papillon-Lefèvre syndrome. Acta Odontol 2004;62:70–74. Oslo. ISSN 0001-6357.

The aim of the present study was to compare concentrations of cytokines, matrix metalloproteinases (MMPs) and a metalloproteinase inhibitor (TIMP-1) in gingival crevicular fluids (GCF) from sites with gingival inflammation in 28 young patients with Papillon-Lefèvre syndrome (PLS), and in age- and gender-matched controls. Each group consisted of 17 females and 11 males with a mean age of 11.0 years (range 4–22 years). In both groups, anterior upper sites with a clinical diagnosis of gingival inflammation and with pockets $\leq$3 mm were selected for sampling of GCF, which was carried out with filter disks inserted into the gingival crevice until saturated. The concentrations of cytokines (IL-1α, IL-1β, TNF-α, and IL-8), matrix metalloproteinases (MMP-1, MMP-3, MMP-8, and MMP-9), and their tissue inhibitor (TIMP-1) were analysed using commercial ELISA kits. Significantly higher levels of IL-1β ($P < 0.001$) and MMP-8 ($P < 0.05$) were disclosed among the PLS patients compared with their controls, while the opposite was found for IL-8 ($P < 0.05$) and MMP-1 ($P < 0.001$). The individual variations were considerable in both groups. When comparing the expression of cytokines, MMPs, and TIMP-1 in PLS patients with clinically active and non-active periodontitis, the non-active PLS patients showed significantly higher values of IL-1β than the patients with active periodontal disease (ANOVA, $P < 0.01$). In conclusion, this study was unable to demonstrate a clear-cut pathognomonic expression of cytokines or MMPs in patients with PLS, but further studies on cytokine and MMP output are warranted. □ *Cytokines; matrix metalloproteinases; Papillon-Lefèvre syndrome; periodontitis; tissue inhibitor of matrix metalloproteinases-1*

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Papillon-Lefèvre syndrome (PLS) is an autosomal recessive disorder with palmoplantar hyperkeratosis and aggressive periodontitis as its main features (1). The disorder has been linked to a deficiency of cathepsin C activity secondary to mutations in the cathepsin C gene (2, 3). Although the genetic defects have been localized it has not been possible to explain why patients with PLS develop an aggressive periodontal infection. Earlier reports suggested an immune dysfunction with impaired function of leukocytes (4–6) as well as anatomical defects of the epithelial barrier (7, 8) and/or presence of aggressive periopathogens (9, 10). The loss-of-function mutations of cathepsin C may be associated with a reduced response towards bacteria in dental plaque, since cathepsin C has an essential role in the activation of the granule serine proteases granzyme A and B, expressed in bone-marrow-derived effector cells of both myeloid and lymphoid series (11). These proteases are implicated in immune and inflammatory processes, including phagocytic destruction of bacteria and local activation of cytokines and other inflammatory mediators (3).

Bacteria are essential for the induction of the infection in periodontal tissues, but the progress and the severity of the disease are dependent on the host's immune and inflammatory response. Endotoxins and other antigens from the cell walls of periodontal pathogens stimulate circulating mononuclear phagocytes and resident cells to produce cytokines that drive the inflammatory response (12). A wide spectrum of enzymes are released by gingival cells, resident macrophages, and infiltrating mononuclear cells initiating destruction of periodontal tissue by the formation, secretion, and activation of matrix metalloproteinases (MMPs) (13, 14). The MMPs are key enzymes in tissue degradation and each of the major cell types in periodontal tissues is capable of expressing MMPs when stimulated (15). MMPs can synergistically digest all the macromolecules of connective tissue matrices at physiological pH and in health, a balance of MMP activity with naturally occurring tissue inhibitors of metalloproteinases (TIMPs) controls normal tissue turnover. In disease states, an imbalance between MMPs and TIMPs occurs, leading to a breakdown of the extracellular matrix (16).

A study of the composition of the gingival crevicular fluid (GCF) can provide useful information on the host response to exogenous antigens. Enzymes and cytokines released by fibroblastic, epithelial, or inflammatory cells

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DOI 10.1080/00016350310008571 © 2004 Taylor & Francis

may lead to an explanation of how the mutations of the cathepsin C gene could be involved in the development of periodontitis in patients with PLS. Our hypothesis was that this premature and rapidly progressive periodontal condition may be caused by either an increased production of cytokines and/or MMPs or a decrease in the production of the balancing TIMPs. Consequently, the aim of this study was to investigate whether the presence of the cytokines, interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), and the matrix metalloproteinases, collagenase-1 (MMP-1), stromelysin-1 (MMP-3), neutrophil collagenase (MMP-8), gelatinase B (MMP-9), and tissue inhibitor of metalloproteinases-1 (TIMP-1), as presented within the GCF, differs between young patients diagnosed with PLS and medically healthy, age-matched and gender-matched controls.

Materials and methods

Study population

A total of 56 patients were included in the study, 28 diagnosed with PLS; 28 subjects were medically healthy controls. All participants were of Saudi nationality and enrolled as patients at the King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, KSA. The controls consisted of a convenience sample of subjects selected from a pool of restorative recall patients. These medically healthy subjects were age-matched and gender-matched to each of the PLS patients. All participants signed an informed consent and the study protocol was approved by the Research Advisory Council at KFSH&RC. The age of the participants ranged from 4 to 22 years, with an average age of 11.0 years, and each group consisted of 17 females and 11 males.

All patients and controls in the study exhibited at least two areas with gingival inflammation diagnosed as redness of the gingival margin and bleeding on probing, with no pocket depths exceeding 3 mm. Every PLS patient had a history or signs of periodontal infection in the deciduous and/or the permanent dentition. Thirteen of them (46%) were considered to have areas with active periodontitis at the time of the sampling of GCF, while the rest of the PLS patients had gingival infection only. The diagnosis of active periodontitis was used in patients with periodontal pockets depths reaching or exceeding 6 mm, and gingival bleeding on probing, and in some cases pus and granulation tissue emerging from the periodontal pockets. All patients with the diagnosis of active periodontitis received conservative periodontal treatment and/or extractions during the period following sampling of the GCF. None of the controls had any signs or any history of periodontal infection.

Collection of GCF

The sampling of GCF from the control subjects was performed during a routine dental visit, while the sampling of the PLS patients was performed during one of their periodic check-up appointments. The GCF was collected by means of small disks, 3 mm in diameter, made of Millipore© filter (Durapore GVWP 0.22 µm, Millipore, Sundbyberg, Sweden). In both groups, sites with gingival inflammation in the upper incisor or cuspid areas were chosen as primary test sites. These areas were chosen since they were not affected by periodontal disease and also in order to diminish the risk of saliva contamination. In cases where sampling was unsuitable at any of these sites, an adjacent site was selected as close as possible. In case of bleeding from the gingival tissue during sampling the disk was discarded and a new test site, close to the original, was chosen. The sampling area was sealed off with cotton rolls in order to avoid salivary contamination. The disks were sequentially, and gently, inserted in the gingival crevice until resistance was felt and thereafter left in the crevice until saturated as indicated by a clearly visible change in color, corresponding to 0.39 µL of serum (17). After sampling of GCF, the disks were immediately transferred to a plastic tube containing 25 µL of a buffer solution (PBS with EDTA and Tween) and kept frozen at -70°C until analyzed.

Analysis of GCF

Before analysis, the samples were thawed at room temperature. The content of cytokines (IL-1α, IL-1β, TNF-α, and IL-8), matrix metalloproteinases (MMP-1, MMP-3, MMP-8, and MMP-9), and their tissue inhibitor (TIMP-1) were analysed using commercial ELISA kits (Amersham Pharmacia Biotech, Freiburg, GDR) according to the manufacturer's recommendations, and results were expressed as pg/mL or ng/mL.

Statistical analysis

Statistical analysis was performed using SPSS 10.0 for the microcomputer (SPSS Inc., Chicago, Ill., USA). Differences between the matched pairs were analysed by means of Student's paired two-sided *t* test or Wilcoxon paired sign test when indicated. Data on active versus non-active periodontitis were subjected to analysis of variance. A P value <0.05 was considered as statistically significant.

Results

The results of cytokine assays are presented in Table 1, and for the matrix metalloproteinases and TIMP-1 in Table 2. The statistical analysis showed significantly higher concentrations of IL-1β and MMP-8 ($P < 0.001$ and $P < 0.05$, respectively) in PLS patients as compared to their controls. For IL-8 and MMP-1 the situation was the opposite, with significantly lower levels being observed among the PLS patients ($P < 0.05$ and $P < 0.001$, respectively). There was a similar expression of IL-1α, TNF-α,

Table 1. Concentration of IL-1α, IL-1β, TNF-α (*p*g/mL), and IL-8 (*n*g/mL) in gingival crevicular fluid from anterior upper sites with gingival inflammation in patients with Papillon-Lefèvre syndrome (PLS) and in matched controls (*n* = number of subjects). The *P* values denote differences between the groups

	PLS patients			Healthy controls			
	n	Mean ± SD	Range	*n*	Mean ± SD	Range	*P* value
IL-1α	28	56.5 ± 23.3	8.3–99.1	25	64.6 ± 28.6	12.5–100.2	ns
IL-1β	27	34.7 ± 9.5	13.2–46.5	27	22.6 ± 16.2	1.2–48.8	$P < 0.001$
TNF-α	28	2.2 ± 3.9	0.4–21.5	28	1.4 ± 1.5	0.2–7.2	ns
IL-8	28	2.36 ± 1.45	0.29–4.83	28	3.24 ± 1.47	0.52–5.36	$P < 0.05$

Statistical analysis performed by means of Student's paired two-tailed *t* test. ns = not significant.

MMP-3, MMP-9, and TIMP-1 in both groups and no difference between genders for any of the variables was displayed. When comparing the expression of cytokines, MMPs, and TIMP-1 in PLS patients with clinically active and non-active periodontitis, the non-active PLS patients showed significantly higher concentrations of IL-1β than the patients with active periodontal disease ($P < 0.01$). When relating the concentrations of cytokines and MMPs to age, there was a tendency to higher IL-1β and MMP-3 levels with increasing age in both groups.

Discussion

Evidence for the role of cytokines and MMPs is well established in the pathogenesis of periodontal destruction (16). Considering the aggressiveness of the periodontal infection in patients with PLS it was tempting to hypothesize that these patients would present with an exaggerated inflammatory and immune reaction leading to an increased release of pro-inflammatory cytokines and tissue proteolytic enzymes. In this study, the contents of IL-1β and MMP-8 were significantly higher in subjects with PLS compared to controls, while, surprisingly, the levels of IL-8 and MMP-1 were significantly lower. For this type of cross-sectional data the selection of sample sites in both the diseased and non-diseased subjects is crucial. To be included, the subjects had to exhibit anterior upper areas with mild to moderate gingival inflammation according to a clinical judgement but, naturally, the severity of gingival affection on a histological level might have differed among the groups and the individuals. Furthermore, the widespread age distribution with various stages or phases of the inflammation could also be a confounding factor. Although GCF has proven to be a reliable source when analyzing the presence of cytokines, proteases, and other factors of inflammation (19), the quantity of exudates increases with the severity of the periodontal infection (20). It might be suspected that an increased amount of GCF fluid could dilute the enzymes and proteases, but studies have suggested that the concentration of total enzyme is positively associated with the volume of the exudates (21). Therefore, it would certainly have been of interest to measure the total amount of cytokine and MMP production over time, although the present experimental set-up did not allow us to estimate the flow rate of the GCF.

The finding of an increased level of IL-1β in the PLS patients in this study, as well as previous findings in patients with both early on-set periodontitis (EOP) and adult periodontitis (22), supports the hypothesis of an increased release of cytokines in patients with periodontal disease. When comparing the levels of IL-1β within the PLS group it was surprising to find higher levels of IL-1β in PLS patients without any active periodontal disease compared to those assessed with active disease. The clinical experience that the activity of the periodontal disease seems to cease with increasing age in PLS patients might partly offer an explanation for this finding. Most of the PLS patients with non-active disease belonged to the older age interval and statistical analyses disclosed a tendency to increasing levels of Il-1β with age in both

Table 2. Concentrations of MMP-1, MMP-3, MMP-8, MMP-9, and TIMP-1 (*n*g/mL) in gingival crevicular fluid from anterior upper sites with gingival inflammation in patients with Papillon-Lefèvre syndrome (PLS) and in matched controls (*n* = number of subjects). The *P* values denote differences between the groups

	PLS patients			Healthy controls			
	n	Mean ± SD	Range	*n*	Mean ± SD	Range	*P* value
MMP-1	28	14.11 ± 5.67	4.74–25.95	28	23.42 ± 9.97	7.35–47.81	$P < 0.001$
MMP-3	28	50.40 ± 7.58	43.13–68.74	28	52.17 ± 5.18	41.63–68.24	ns
MMP-8	10	2.98 ± 3.03	0.51–9.38	10	0.73 ± 0.49	0.48–2.04	$P < 0.05$§
MMP-9	28	202.92 ± 161.90	48.92–670.75	28	151.82 ± 97.60	50.97–482.56	ns
TIMP-1	28	36.32 ± 13.51	18.07–71.22	28	40.24 ± 24.93	14.03–130.59	ns

Statistical analysis performed by means of Student's paired two-tailed *t* test. Ns = not significant. §Wilcoxon paired sign test.

groups of patients. Consequently, the increased cytokine levels seemed to be related more to age than to the disease itself.

IL-8 is an important chemo-attractant for polymorphonuclear leukocytes (PMNs), affecting migration and chemotaxis as well as activation of the PMN cells (23). We found lower concentrations of IL-8 in GCF in PLS patients compared with controls, although no difference in PLS patients with active and non-active periodontal disease was evident. Defective phagocytic and lytic PMN activities have earlier been shown in PLS patients (4) and it would be reasonable to assume that low tissue levels of IL-8 would increase the susceptibility to periodontal infection through poor recruitment and activation of the PMN cells. Our IL-8 findings are in agreement with previous data from patients with localized juvenile periodontitis (LJP) and chronic periodontitis (24, 25), but in contrast to an earlier report on PLS patients (5). Strong protein and mRNA expression of IL-8 have been found in both epithelial cells and gingival connective tissue cells in patients with generalized aggressive and adult periodontitis (26). It could thus be speculated that the level of IL-8 measured in GCF does not correspond to the level measured within the gingival tissue.

The ability of cytokines to induce MMP expression in gingival fibroblasts is well accepted (27) and the correlation between MMP expression and the severity of periodontal disease has been established in many studies (14, 16, 18). For the MMP-8 comparison, significantly higher levels were displayed for the PLS patients than for the controls. Since tetracycline has shown ability to inhibit production and activation of matrix metalloproteinases and reduce the collagenase activity in GCF (28), only PLS patients without a history of tetracycline medication and their controls were included for the MMP-8 assay. Elevated levels of MMP-1 in GCF have earlier been described in patients with LJP compared to cases with adult periodontitis and control patients (18). Therefore, our finding of slightly lower levels of MMP-1 in patients with PLS than in controls was somewhat unexpected. However, in an in vitro study fibroblasts from patients with LJP and slowly progressive periodontitis have shown to be lower in collagen-degrading activity than fibroblasts from healthy control patients (29). Whether this represents a specific fibroblast phenotype or is dependent on the fact that fibroblasts in periodontal disease are less active or less efficient in healing and remodelling of the tissue is questioned. It is suggested that periodontal disease may be linked to inadequate—as well as to excess—MMP expression. This study supports the former theory. It should be noted, however, that the analyses of the MMPs in the present study reflected the total content of the investigated proteases found in GCF, and with the method used it was not possible to reveal the activated fraction of the MMPs. Such an assessment would be of great interest when evaluating the possible effect that the mutations of the cathepsin C gene may have on activation of proteolytic enzymes in patients with PLS.

In conclusion, it was not possible in this study to demonstrate a clear-cut pathognomonic expression of cytokines or MMPs in patients with PLS. Periodontal disease is a multifactorial condition and further investigations will have to be performed prior to attaining an explanation of the development of periodontal disease in patients with PLS.

Acknowledgements.—We thank Dr Alison Brown for skilful assisting during the GCF sampling. Grants for this study were received from the Research Fund, Department of Dentistry, KFSH&RC and Colgate Research Fund.

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Received for publication 18 August 2003
Accepted 3 December 2003