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Tissue plasminogen activator (t-PA) and placental plasminogen activator inhibitor (PAI-2) in gingival crevicular fluid from patients with Papillon–Lefèvre syndrome

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Abstract

Objectives: Numerous patients with Papillon–Lefèvre syndrome (PLS) express a severe periodontal inflammation that results in premature loss of deciduous and permanent teeth. The plasminogen activating (PA) system is involved in physiological and pathological processes including epithelial healing, extracellular proteolysis and local inflammatory reactions. The aim of the study was to explore a possible role of the PA system in patients with PLS.

Material and Methods: Samples of gingival crevicular fluid (GCF) were collected from areas with gingival infection in 20 patients with PLS and in 20 healthy controls. The concentration of tissue plasminogen activator (t-PA) and inhibitor (PAI-2) was measured with ELISA.

Results: The median level of PAI-2 was significantly higher ($p < 0.01$) in PLS patients than in the controls, while the median value of t-PA did not differ between the groups. No difference in t-PA or PAI-2 levels was found regarding age, gender or presence of active periodontal disease.

Conclusion: The findings indicate an atypical activity of the PA system with a disturbed epithelial function in PLS patients, suggesting that the periodontal destruction seen in patients with PLS is secondary to a hereditary defect in the defense system.

Key words: gingival crevicular fluid; gingival inflammation; Papillon–Lefèvre syndrome; plasminogen activating (PA) system

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Papillon–Lefèvre syndrome (PLS) is a rare condition with an autosomal recessive transmission, characterized by erythematous palmoplantar hyperkeratosis and rapid destruction of the periodontal structures (Hart & Shapira 1994). The periodontal involvement is often severe and results in premature loss of deciduous and permanent teeth. Many patients with PLS end up with a complete loss of permanent teeth already in adolescence. Additional findings are intracranial calcifications, retardation of somatic development, onychogryphosis and increased susceptibility to infection.

The dermal component of PLS implies an involvement of the periodontal epithelia and it has been suggested that dyskeratosis, analogous to the dermal changes, may be responsible for a less effective gingival epithelial barrier in PLS (Lyberg 1982). Such a defect might lead to a reduced defense against periodontal bacteria (Preus 1988). Together, this would indicate that the periodontal involvement is secondary to the epithelial disturbance.

The syndrome is reported to be linked to mutations in the cathepsin C gene (Hart et al. 1999, Toomes et al. 1999).

Although the genetic basis of the syndrome has been characterized its correlation to the periodontal involvement in patients with PLS is unclear. Disturbed function of lymphocytes (Haneke 1975, Celenligil et al. 1992, Gongora et al. 1994) and neutrophils (van Dyke et al. 1984, Preus & Gjermo 1987, Firatli et al. 1996, Ghaffer et al. 1999, Liu et al. 2000) have been reported as well as imbalance of the collagenolytic activity (Shoshan et al. 1970). However, other studies have found the defense mechanisms to be normal or similar to those described for other periodontal diseases (Lyberg 1982, Schroeder et al. 1983, Celenligil et al. 1992, Tinanoff 1995). The periodontal pathogen *Actinobacillus actinomycetemcomitans* has been detected in periodontal lesions in PLS patients (Preus 1988, Bimstein et al. 1990, Stabholz et al. 1995, Kleinfelder et al. 1996, De Vree et al. 2000, Wiebe et al. 2001, Pacheco et al. 2002), although other studies have revealed a bacterial flora resembling adult periodontitis or a flora without any particular periodontal pathogen (Lundgren et al. 1998, Robertson et al. 2001). Cathepsin C, a lysosomal cysteine protease has also been shown to activate serine proteases extracellularly, thereby possibly affecting the plasminogen activating (PA) system (Turk et al. 2001).

The PA system is involved in physiological as well as pathological processes, including epithelial healing, extracellular proteolysis, local inflammatory reactions and neoplastic growth and invasion (Rømer et al. 1996, Reuning et al. 1998, Collen 1999, Kinnby 2002). Through the action of the specific plasminogen activators, tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA), the inactive precursor plasminogen is converted into the aggressive broad-spectrum proteinase plasmin. The activators are balanced by the specific inhibitors, plasminogen activator inhibitor 1 (PAI-1) and plasminogen activator inhibitor 2 (PAI-2). A strict control of the PA system is essential for maintaining the integrity of the tissues. Plasminogen is present in high concentrations in blood and other body fluids. The specific activators and inhibitors are produced locally, and in the gingival area t-PA and PAI-2 have been found to be the dominating components of the PA system (Kinnby et al. 1994, Kinnby et al. 1999). The concentrations of t-PA and PAI-2 in gingival crevicular fluid increase during inflammation (Kinnby et al. 1994) and the expression of t-PA as well as PAI-2 in inflamed gingival tissues are enhanced (Lindberg et al. 2001). In the gingiva, the by far strongest expression of t-PA and PAI-2 has been found in the epithelial tissues (Kinnby et al. 1999, Lindberg et al. 2001).

The significance of the PA system for the initiation and development of periodontal disease is not established. Hypothetically, an imbalance between t-PA and PAI-2 might influence the proteolytic activity in the region, which in turn affects the gingival epithelial barrier. To our knowledge, there are no studies on the function of the PA system in gingival or other epithelial tissues of PLS patients. The aim of the present study was to explore a possible role of the PA system for the development of periodontal destruction in patients with PLS. The present investigation was designed to compare the levels of t-PA and PAI-2 in gingival crevicular fluid from patients with PLS and from healthy individuals.

Material and Methods

Study population

A total of 40 subjects were included in the study, 20 diagnosed with PLS and 20 being medically healthy controls. All participants were of Saudi nationality. The controls consisted of medically healthy subjects from a pool of recall dental patients at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. The age of the participants ranged from 4 to 22 years, with an average age of 11 years. Each group consisted of 13 females and seven males. The study was approved by the Research Advisory Council of King Faisal Specialist Hospital and Research Centre. All participants signed an informed consent.

The sampling of gingival crevicular fluid (GCF) from the PLS patients was performed at one of their periodic check-up dental appointments. All PLS patients had a history of destructive periodontal disease in the deciduous and/or the permanent dentition. Eleven of the patients were considered to have areas with active periodontitis and areas with gingivitis at the time of GCF sampling, while nine had gingivitis only. Active periodontitis was defined as one or more sites with a periodontal pocket depth >6 mm combined with bleeding on probing, and in some cases with pus and granulation tissue emerging from the periodontal pockets. All patients with active periodontitis at the time of sampling received periodontal treatment and/or extractions during the period following the sampling of the GCF.

The medically healthy controls were age- and gender-matched to each of the PLS patients and included in the order of appearance at the clinic for regular dental check-up. None of the controls had any signs or any history of destructive periodontal disease (pocket depth $\leqslant 3$ mm), but all showed areas with clear signs of gingivitis.

Sites with visual signs of inflammation but free from bleeding on probing (GI score 1 according to Löe & Silness 1963) and with a pocket depth $\leqslant 3$ mm were accepted for sampling of GCF. The stated condition for the sampling site in relation to level of inflammation apply to control subjects as well as to PLS patients. The sampling sites in both groups were chosen to match each other in respect to level of gingival inflammation. Due to easy access the sampling was restricted to upper cuspids or incisors (buccal aspect). One site fulfilling the criteria above constituted test site. In cases where sampling was unsuitable due to presence of bleeding or pathological pocket depth, another site within the area was selected.

Collection of GCF

GCF was collected by means of small paper discs, 3 mm in diameter, made of Millipore® filter (Durapore GVWP 0.22 μm, Millipore, Sundbyberg, Sweden). Four discs were collected from each test site. The sampling area was sealed off with cotton rolls in order to avoid salivary contamination. The discs were sequentially, and gently, inserted in the gingival crevice until resistance was felt and thereafter left until saturated, as indicated by a clearly visible change in color. The amount of fluid of a saturated disc corresponds to 0.4 μl of serum (Olofsson et al. 2003). If four filled disks could not be obtained from the selected test site, a neighboring site was chosen. After sampling of GCF, the discs were immediately transferred to a plastic tube containing 25 μl of a buffer solution, PBS with EDTA and Tween, thus yielding a 16.6-fold dilution. The samples were then frozen at -70°C until analyzed.

Immunochemical analyses

Before analysis, the samples were thawed at room temperature and shaken for 5 s on a vortex shaker (Vortex-Genie, Scientific Industries, Bohemia, NY, USA) and then centrifuged for 3 min. All samples from each patient were then pooled and again quickly centrifuged, and thus one sample representing each patient was created. PAI-2 antigen was measured with an enzyme-linked immunosorbent assay (ELISA) using monoclonal and polyclonal antibodies (Lecander & Åstedt 1987). This measures complex-bound PAI-2 to 60%. t-PA antigen was measured with an ELISA (Imulyse™ t-PA, Biopool, Umeå, Sweden) that also shows t-PA in complex with PAI-1, PAI-2 and α_2-antiplasmin. For each type of analysis, all samples were analyzed on the same occasion. The intra-assay c.v. of the analyses was 5 and 8% (Kinnby 1995).

Statistical analyses

t-PA and PAI-2 values below the detection limit were set to 0. Values above the upper limit of the standard curve were set to its maximum 10 mg/l. Due to these upper and lower limit adjustments, median values were used in the presentation of data (Table 1). For paired comparisons of the PLS patients with controls, Wilcoxon's signed rank sum test was used. For tests of gender differences and differences between PLS patients with or without signs of active periodontitis, the Wilcoxon–Mann-Whitney rank sum test was applied. Speerman's test of correlation was used for studies of influence of age. p-Values $\leqslant 0.05$ were considered statistically significant.

Results

The individual levels and the median values of t-PA and PAI-2 are presented in Table 1. The median value of PAI-2 showed a statistically significant difference ($p < 0.01$) between the PLS patients (1.38 mg/l) and the controls (0.33 mg/l). t-PA was somewhat lower in the group of PLS patients (0.24 mg/l) than in the control group (0.37 mg/l). However, the difference was not statistically significant. No significant differences in levels of t-PA and PAI-2 were found between genders in the group of PLS patients or in the control group, or between age and levels of t-PA and PAI-2. A tendency toward lower PAI-2 levels with increasing age in the group of PLS patients ($p = 0.12$), as well as in the controls ($p = 0.07$), was noted.

Table 1. Individual values and median values (mg/L) of t-PA and PAI-2 in GCF from patients with Papillon-Lefèvre syndrome and from age- and sex-matched control patients

Subject pair	t-PA		PAI-2	
	PLS-patients	Controls	PLS-patients	Controls
1	0	0.37	1.07	0
2	0.43*	0.31	1.02*	0.51
3	0.25	0.16	5.23	0
4	0.23	0.88	1.55	0
5	0.39	0.80	2.10	0
6	0.14	0.75	0.55	0
7	0.42*	0.36	0*	0.40
8	0.15	0.80	1.21	0
9	0*	0.10	0.90*	0.25
10	0.36	0.44	3.64	0
11	0.26*	0	4.00*	0.41
12	0*	0.08	1.14*	0.47
13	0*	0	0.82*	0.83
14	0*	0.87	1.71*	0
15	0.31*	0	0*	0.82
16	1.10*	0.40	4.62*	0.65
17	0	0.24	1.14	9.86
18	0.22	0.53	6.19	2.28
19	0.54*	0.09	2.13*	0
20	1.06*	1.32	>10*	>10
Median	0.24	0.37	1.38	0.33
Percentil 25	0	0.09	0.93	0
Percentil 75	0.41	0.79	3.91	0.78
Sign. Level	$p > 0.05$		$p < 0.01$	

* = PLS patient with active periodontitis

The patients with PLS were subgrouped according to presence or absence of active periodontitis. Median values of t-PA and PAI-2 were 0.31 versus 0.22, and 1.14 versus 1.55 for the active and non-active group, respectively, thus displaying no significant differences.

Discussion

Previous studies have shown that the PA system is highly active in the dentogingival region (Kinnby 2002). The activators u-PA and t-PA and the inhibitors PAI-1 and PAI-2 are all expressed in the area. When assessed in GCF with ELISA (Kinnby et al. 1994), and in the gingival tissues with in situ hybridization and immunohistochemistry (Kinnby et al. 1999, Lindberg et al. 2001), t-PA and PAI-2 seem to be the dominating components. t-PA and PAI-2 are predominantly produced in the gingival epithelia (Kinnby et al. 1999), and a strong expression in the sulcular and junctional epithelia indicates an important role of the PA-system in these barrier tissues. The PA-system represents a highly regulated enzymatic cascade for extracellular proteolysis, and a disturbed balance of the system may have an effect on the turnover of the gingival epithelia and possibly also on their protective barrier function.

The present study showed the novel finding of significantly higher concentrations of PAI-2 in GCF from the patients with PLS compared to the controls. At the same time somewhat lower concentrations of the activator t-PA were noted in the GCF samples. The GCF levels of the components of the PA system are likely to reflect the expression of these components primarily in the gingival epithelia, and thus, the threefold higher level of PAI-2 in the PLS patients, in combination with a similar or even lower level of the t-PA, indicates a disturbed gingival epithelial function in the PLS patients.

Lyberg (1982) and Preus (1988) suggested that the destructive periodontal disease in patients with PLS might be related to a defective gingival epithelial barrier. According to Lyberg (1982), the protective barrier may be less effective than normal due to dyskeratosis, analogous to that found in the epithelium of the palms and soles. Hart et al (1997) discussed possible

similarities between the palmoplantar epithelium and the junctional epithelium, and speculated that both tissues are affected by the genetic anomaly in PLS. Our findings support the hypothesis of a disturbed homeostasis of the gingival epithelia in patients with PLS. A higher expression of PAI-2, and thus a possible down-balanced extracellular proteolytic activity, might lead to an altered epithelial turnover. How this in turn would affect the barrier function and the protective capacity of the epithelia in the dento-gingival area needs further study, preferably on a biopsy material.

A disturbed epithelial barrier function may lead to a reduced defense against periodontal bacteria in patients with PLS. However, there may be other factors involved in the pathogenesis of destructive periodontal disease in these patients. Toomes et al. (1999) have emphasized that mutations within the cathepsin C gene lead to a loss-of-enzyme activity in patients with periodontal disease and in subjects with PLS. Biochemical analysis has demonstrated almost no detectable cathepsin C activity in leukocytes in three families with PLS (Zhang et al. 2002). Furthermore, Soell et al. (2002) showed that cathepsin C activity in GCF samples from medically healthy, periodontitis-affected patients was reduced with 41% of that in healthy controls. Cathepsin C activity is crucial to the activation of the neutrophils (Adkison et al. 2002) and the loss-of-function mutations in PLS might instigate a defective neutrophil defense contributing to the periodontal breakdown. The above-mentioned connection between cathepsin C and the PA system (Turk et al. 2001) indicates that lack of cathepsin C activity is likely to cause disturbances in the proteolytic cascade system, further emphasizes the likelihood of more than one mechanism to be involved in the pathogenesis of PLS.

No significant differences in level of t-PA or PAI-2 were seen in the PLS patients with or without active periodontitis. This is in contrast to previous studies where inflammation have been a factor influencing the expression of the components of the PA system (Kinnby 2002). Our findings indicate an atypical activity of the PA system in the PLS patients. This supports the hypothesis formulated by Preus (1988) that the periodontal destruction in patients with PLS is a result of the establishment of a virulent bacterial flora in areas with a hereditary epithelial defect.

The PA system has implications on various pathological and physiological events, including proliferation and differentiation of normal and abnormal epidermis. An altered activity of the PA system has been reported in epidermal disorders like psoriasis, pemphigus, bullous pemphigoid and lupus erythematosis (Baird et al. 1990, Jensen et al. 1990, Gissler et al. 1993, Spiers et al. 1994, Lyons-Giordano et al. 1994, Bechtel et al. 1996). Elevated levels of t-PA were found in lesions from patients with psoriasis, pemphigus and bullous pemphigoid, in contrast to normal epidermis where no t-PA was detected at all. In studies of lesions of lupus erythematosis, an increase in epidermal PAI-2 was observed, but u-PA or t-PA was not detected (Bechtel et al. 1996). The present finding of an enhanced level of PAI-2 in GCF from patients with PLS indicates an altered epithelial activity of the PA system also in this group of patients, as for lupus erythematosis where PAI-2 seems to be upregulated without any effect on t-PA. This may be due to an altered reaction pattern of the epithelium or simply a sign of an increased amount of epithelium. However, before PLS can be added to the list of skin disorders connected with altered activity of the PA system, studies of epidermal lesions with hyperkeratosis using in situ hybridization techniques and immunohistochemistry are needed.

In summary, the present study showed significantly higher concentrations of the inhibitor PAI-2 in GCF from patients with PLS compared to controls. As no significant difference was noted for t-PA, the findings point to an atypical activity of the PA system, indicating a disturbed epithelial function in this group of patients. No difference in the PA system was seen between PLS patients with or without active periodontitis, which supports the theory that the periodontal destruction seen in patients with PLS is secondary to a hereditary defect of the epithelial barrier and consequently in the defense system. Thus, the periodontal affection may be an entity of its own and not part of the syndrome.

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