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High Concentrations of Pyridinoline in Gingival Crevicular Fluid of Patients with Periodontitis and Gingivitis

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Abstract *Serum and urinary concentrations of pyridinoline (PYD) are used as specific markers of bone resorption in various systemic diseases. The purpose of the present study was to determine PYD levels in gingival crevicular fluid (GCF) of patients with periodontitis and gingivitis. GCF was collected from the buccal gingival sulcus of the study tooth from patients with periodontitis (n=11), periodontitis with periodontal abscess (n=9) and gingivitis (n=12) using microcapillary tubes. PYD was measured by the inhibition ELISA method. The mean PYD concentration in patients with periodontitis (4.2 ± 0.6 nmol/L, ± SD) was significantly higher than in those with gingivitis (2.5 ± 0.8 nmol/L, p<0.01). The highest levels were found in GCF of patients with periodontitis associated with abscess formation (6.6 ± 0.9 nmol/L, p<0.01). Our results suggested that PYD levels in GCF could be a potentially useful marker of alveolar bone remodeling in human periodontal diseases.*

Key words : Gingival crevicular fluid, Pyridinoline, Periodontitis, Gingivitis

INTRODUCTION

One of the major consequences of periodontitis and periodontal diseases is loss of alveolar bone through osteoclastic bone resorption [1~3]. Various markers have been developed recently to monitor bone metabolism in systemic bone diseases. Collagen cross-links are probably the most promising markers for bone resorption [4~10]. Serum and urinary concentrations of pyridinoline (PYD) are specific markers for bone resorption used in various bone diseases, such as osteoporosis and Paget's disease [8~10]. In beagle dogs with experimentally-induced periodontitis, we have demonstrated that the levels of PYD in gingival crevicular fluid (GCF) correlated with histochemically-determined osteoclast activity in alveolar bone areas exhibiting bone resorption [11]. We also reported in another study [12] the presence of high PYD serum levels in ligature-induced periimplantitis in dogs. These studies suggested that PYD in GCF seems to be a sensitive marker of alveolar bone resorption in animal models.

The purpose of the present study was to compare PYD levels in GCF of patients with periodontitis and with gingivitis using enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Patients

Twelve patients with gingivitis (2 females and 10 males; age, 23.7 ± 2.8 years, mean ± SD), 11 patients with periodontitis (6 females and 5 males ; age, 53.6 ± 9.1 years) and 9 patients with periodontal abscesses (5 females and 4 males; age, 55.2 ± 6.6 years) were enrolled in the present study. All patients were healthy apart from the aforementioned dental conditions, and agreed to participate in the present study. Patients were examined clinically and their gingival index, probing pocket depth, attachment level and bleeding on probing were determined. A standardized X-ray for abscess formation was also performed. A study tooth was selected in each subject of the above three groups based on typical symptoms.

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Collection of gingival crevicular fluid samples

GCF was collected from the buccal gingival sulcus of the study tooth by placing a microcapillary tube (Microcaps, Doramondo, USA) into the periodontal pocket. The GCF in microcapillary tubes were decanted repeatedly into microtubes until 25 μl of the volume. The collected samples were stored at −50°C until measurement.

ELISA measurement

The PYD was measured by ELISA kit (serum PYD, Quidel, San Diego, CA)[13]. The resolved samples were filtrated by centrifugation. The samples were diluted with standard solution, then 25 μl of the solution containing PYD antibody were added to PYD coated wells and allowed to react overnight at 4°C. After washing, enzyme conjugated solution was added in volume of 150 μl/well. The enzyme substrate was added and color was allowed to develop for 40 min at room temperature. The plates were measured by Multiscan (Toso, Japan) at a wavelength of 405 nm. The values for PYD in GCF were expressed in nM calculated from standard curves.

Statistical analysis

All data are expressed as mean ± standard deviation. Differences between the three groups were examined for statistical significance using the Mann-Whitney test. A P value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

The PYD levels in GCF in our patients were above the detection levels determined in standard serum samples from healthy subjects. The PYD levels in GCF of the three groups were significantly higher than that in serum of healthy adults.

The mean PYD concentration in patients with periodontitis (4.189 ± 0.582 nmol/L) was significantly higher than in those with gingivitis (2.457 ± 0.851 nmol/L, $p<0.01$). The highest levels were detected in GCF of patients with periodontitis associated with

Table 1. GCF concentration of Pyridinoline in periodontal disease

Gingivitis	Periodontitis	Periodontal abscess
	*	
2.257 ± 0.851	4.189 ± 0.582	6.558 ± 0.934
(12)	(11)	(9)
*		*

PYD(n mol/L), Mean ± SD, ( ) : Number of subjects, * : $p<0.01$

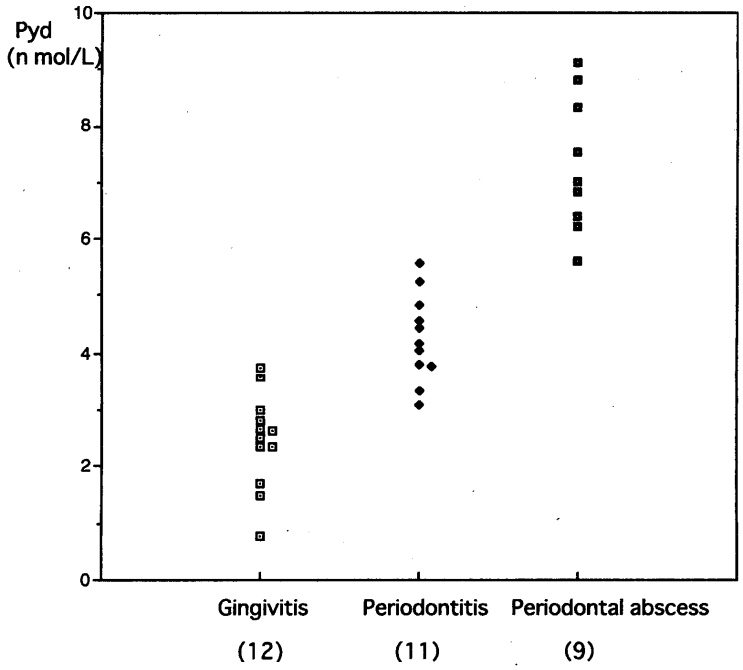

Fig 1. Pyridinoline in gingival crevicular fluid from diseased sites.
( ) Number of subject

abscess formation (6.558 ± 0.934 nmol/L, n=9, $p<0.01$, Table1).

DISCUSSION

Pyridinium crosslinks, PYD and deoxypyridinoline (D-PYD), result from post-translation modification during the maturation of collagen[10]. PYD is abundant in bone and cartilage, whereas D-PYD is present in significant amounts only in bone and dentine. The pyridinium crosslinks are not metabolized and urinary excretion of these compounds is not influenced by changes in dietary intake. Therefore, measurement of urinary excretion of pyridinium crosslinks is thought to represent a more specific indicator of bone resorption than hydroxyproline in human[5~10]. Tsukada[14] reported that urinary excretion of PYD and D-PYD following alveolar bone resorption in dogs can be measured accurately by ELISA kit designed for measurement of urinary pyridinium crosslinks levels in human. Urinary excretion levels of PYD in dogs are double those in human[11]. We have previously reported that PYD levels in GCF, which were measured by using a human urine PYD kit, correlated well with the osteoclastic activity in periodontal tissue, as measured in histochemical studies in dogs with experimentally-induced periodontitis[11]. Furthermore, we also reported in another study[12] that the progression of peri-implantitis in dogs can be monitored by measurement of serum levels of PYD, using human serum PYD kit.

In the present study, we first demonstrated that serum PYD kit could be useful for measuringg PYD levels in GCF. Previous studies reported that the normal serum concentration of PYD in healthy adults is 1.9 ± 0.4 nmol/L[13,15]. Our results also showed that PYD level in GCF should be similar to those in the serum once sufficient volumes of GCF were collected. The present study also showed that the mean concentration of PYD in GCF of patients with periodontitis was three times higher than the mean value in serum of healthy adults. PYD in GCF of patients with gingivitis was higher than that in serum of healthy subjects, suggesting borderline alveolar bone resorption in gingivitis. This conclusion is based on our previous finding of the appearance of osteoclast activity during the early period of ligature-induced periodontitis in dogs[11].

Periodontal abscess formation represents an aggressive acute inflammatory process of the periodontal tissue. Osteoclastic activity should be enhanced by inflammatory factors in areas adjacent to the alveolar bone surface. The present results showed significantly higher levels of PYD in GCF of patients with periodontal abscess compared to those of patients with periodontitis, suggesting active alveolar bone resorption.

In summary, our results suggested that PYD levels in GCF could be a potentially useful marker of alveolar bone remodeling in human periodontal diseases. Further clinical studies are necessary to develop appropriate methods for measurement of PYD levels in GCF for routine clinical check-up in pathological conditions affecting alveolar bones.

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歯肉炎，歯周炎罹患部位の歯肉溝滲出液中ピリジノリン量

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キーワード：歯肉溝滲出液，ピリジノリン，歯肉炎，歯周炎

抄録　骨コラーゲンに特有の架橋物質であるピリジノリン，デオキシピリジノリンのELISA法による微量測定が可能になり，骨吸収マーカーとして骨粗鬆症などの骨疾患の診断に用いられている．本研究は歯肉溝滲出液サンプルを採取してピリジノリンを測定し，歯槽骨吸収活性マーカーとしての評価を行うことを目的とした．

歯肉炎には本学学生12名を被験者とした．前歯あるいは小臼歯部頬側から滲出液採取を行った．歯周炎には成人性歯周炎と診断された歯周病の患者11名，急性歯周膿瘍症状を呈する9名から滲出液を採取した．

採取部位を簡易防湿後，Micro Capillary Tubeにて滲出液を採取した後，ELISA法を用いたキットにて吸光度を測定し，標準曲線よりピリジノリン値を算出した．滲出液中のピリジノリン量は，急性歯周膿瘍＞歯周炎＞歯肉炎の順で高値を示した．滲出液ピリジノリンが高い確率で歯槽骨吸収活性を示す指標になる事が判明したことから歯肉炎から歯周炎への移行を判断する有力な材料となるものと考えられる．