CHAPTER IV

DISCUSSION

4.1. Subjects and Method Validity

This study was performed in three groups; (i) 40 normal subjects whose age between 20 to 51 years, (ii) 40 gingivitis patients whose age ranged between 21 to 49 years, and (iii) periodontitis patients whose age between 20 to 75 years. The standardized classification in clinic was measured with the three issues; laden with plaque, bleed on probing and the depths of probing as in the method of the previous study (Grbie et al., 1991). Among three groups of this study they are not differences in the flow rate of saliva secretion (1 ml/min) and the pH (6.7), similar to some previous studies (Suddick et al., 1980; Ben-Aryeh et al., 1986). The protein concentration that determined by the method of Henskens (1992) has shown only higher in the periodontitis group, which compared to the other groups.

The determination of proteinase and inhibitor activity was modified from the method of Koritsas VM, et al (1995) by digesting the biotinylated gelatin on microtiter plate. This modified method is sensitive to detect the proteolysis activity with required a minimal of sample volume, and represented in nanogram quantity. Proteinase activity can be readily quantified by measuring the reducing in the amount of substrate after incubation with enzyme. In protocol for study the inhibitory effect can be determined by preincubating the boiled saliva with proteinase, and adding substrate on the plate. After incubation, the mixture on the plate was washed with buffer to reduce interference.

In this study, elastase and its inhibitor activity were detected by using the HPLC and spectrophotometric methods. The specific substrate (SAAVNA) was cleaved by elastase and the end product was analyzed. The sensitivity of the HPLC assay allows

for quantification of low levels of enzyme. The successful implementation of the HPLC assay with its high precision and accuracy was due to the sensitivity and relative specificity of electrochemical detection. Moreover, the absolute amount of enzyme detectable by the HPLC assay was much less than the amount required from the spectrophotometric assay (Table 3.3). Because in the spectrophotometric assay may have interference from compounds which have spectra similar to *p*-nitroaniline.

4.2. The proteolytic enzyme and enzyme inhibitors

The results in saliva samples showed significant higher median values of total protease specific activity (μg/mg protein) in periodontitis group (346.3), which compared with the normal (156.0) and gingivitis groups (60.0). The total protease inhibitors (μg/mg dry saliva) were significant difference. The median values of periodontitis (4.9) and gingivitis group (5.4) were significant lower than the normal group (29.6). From this study, the total protease activity in saliva of periodontitis patients showed high concentration as indicated the previous studies (Armitage et al., 1994, Atilla et al., 1996). The higher level of total proteinase and lower level of its inhibitor reflected the systemic inflammatory component of periodontal tissue. The higher concentration of proteinase can degrade tissue and can be detected in the saliva. The different concentration of total proteinase within saliva of periodontitis via gingivitis groups may reflect the severity of disease and duration of inflammation in different patients. The novel of this study could represent the concentration of total proteinase in weight per volume of sample, whereas any previous studies had not been reported.

The mean values of cysteine proteinase specific activities (μg/mg protein) in the normal (2.0), gingivitis (3.5), and periodontitis groups (2.9) were not statistically significant difference. The total cystatin (μg/mg dry saliva) in three groups were statistically significant differences. The cystatin level in saliva from gingivitis (0.7) and periodontitis group (1.0) had lower than the normal group (8.3) that was similar to those obtained by Aguirre et al (1999) and Shomers et al (1982). Cysteine proteinase generally

plays a role in tissue destruction (Kunimatsu et al., 1990) and its biological activity may be regulated by cystatin (Barrett et al., 1986). Cystatin in the serum is very important in disease as in the study of Skaleric et al., (1989) who found an inverse correlation between the levels of cystatin C in gingival homogenates and the degree of gingivitis and periodontitis.

The elastase specific activities (μg/mg protein) in this study that detected by HPLC. The mean value of the periodontitis groups (3.6) was higher than the normal (0.6) and gingivitis groups (0.4). In addition, the level of elastase activity, which detected by spectrophotometic method, was as same as evaluation with densitometry in the study of Neiminea et al. (1995). The results in the levels of elastase inhibitor (μg/mg dry saliva) found there were not statistically significant difference in the normal (4.1), gingivitis (5.1), and periodontitis groups (4.6).

The results showing the higher level of elastase in periodontitis group compared with other groups was confirmed by using SDS-PAGE method and it showed the darker band in the periodontitis group.

The study of total proteinase and their inhibitor activity showed high level of total protease and elastase within saliva of periodontitis group, while as represented a low level of cystatin. Therefore, determination of total proteinase, elastase, and cystatin levels in saliva should be applied to classify the patients who may be progressive to the periodontal disease and they will be potential biochemical markers in dentistry the clinical study.

4.3. Proteinase inhibitor from Thai-herbs

For this study, the gelatinolysis method was also applied to test the several of proteinase inhibitor of water extracts from Thai-herbs. Using the preexisting gelatin as a substrate on x-ray film and adding various concentration of proteinase performed the method. This method was quick, simple and adequate to detect the clear zone that depended on the proteinase activity. It was also detect for the inhibitory activities from reduction of clear zone. Extracted compounds from betel nut, guava, black tea, emblie

myrabolan, clove tree and soybean which were rich in inhibitor of trypsin, elastase and papain were performed (Table 3.5). They were also tested for their inhibitory activity by using the specific substrate method. The extracted compound from betel nut showed higher inhibitory activity on the trypsin, elastase and papain than black tea and clove respectively (Table 3.6).

4.4. Inhibition of proteinase activity in saliva of the periodontitis group

The IC 50 dilution of extracts from this study about the Thai – herb inhibitor activity was tested on the proteolysis activities in saliva of the periodontitis group. The result of enzyme inhibitors (μg /mg dry weigh) in this study indicated that the elastase inhibitors in periodontitis saliva with adding betel nut (13.4), black tea (16.9) or clove (18.9) extracts were increase the elastase inhibitor in saliva of periodontitis group (7.5). Wu-Yuan and colleagues (1988) have identified the inhibitory function of an extract of Chinese nut gall (*M. Chinensis*) and found that the active compound is a polyphenolic group that is similar to gallotannin. Therefore, their study supported this study about the active compounds of nut. Notice in this study, the SDS-PAGE bands illustrated that the activity of elastase in the saliva pretreated with tannin was more inhibited than in the saliva without-tannin. The mechanism of tannin on enzyme inhibition is very interesting about its active compounds for study in the future.

For the conclusion, the determination for the concentration of total proteinase, cysteine proteinase, and elastase in saliva show increases in groups of both gingivitis and periodontitis patients but the low in the concentration of total proteinase inhibitor, cystatin, and elastase inhibitor. They may imbalance of enzyme and inhibitor mechanism for host defense against pathogens. Therefore, they prove useful in the diagnosis and monitoring of periodontal diseases. More pathological samples with different progressive activities need for further investigation. In this study, twenty-eight kinds of Thai herb extracts (Table 3.5), specially, betel nut, black tea and clove can inhibit proteinase activity. In addition, they may reduce virulence of proteinase activity by inhibiting their ability to damage gingival tissue and to degrade host-derived protein.