

ALKALINE PHOSPHATASE LEVEL MEASUREMENT TO DIFFERENTIATE MILD AND SEVERE AGGRESSIVE PERIODONTITIS

(PENGUKURAN TINGKAT ALKALIN FOSFATASE UNTUK MEMBEDAKAN PERIODONTITIS AGRESIF RINGAN DAN BERAT)

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Abstract

Individuals with aggressive periodontitis (AP) can have a heritable risk factors related to the bacterial floras, immunological responses and leukocyte functions. This study aimed to characterize indicators and differences between the two subclasses of AP. A series of clinical parameters and full mouth dental radiographs was obtained from 27 consenting AP patients. After sampling of gingival crevicular fluid (GCF) and peripheral blood, the activity of alkaline phosphatase (ALP) was measured from GCF by enzymatic method. The proportion of lymphocyte subpopulations was measured from blood by flow cytometry, and titer of immunoglobulin G against *Porphyromonas gingivalis* (Pg) fimbriae was determined from serum by ELISA. The results classified the subjects into eight subjects with mild AP (AP₁) and 19 subjects with severe AP (AP₂). From all indicators tested, we concluded that the GCF ALP was the one which was 100% specific for AP₂, though with 76.9% sensitivity and 77.8% accuracy; and that AP₁ and AP₂ are different types of early-onset periodontitis (EOP), of which AP₁ does not progress into AP₂.

Key words: aggressive periodontitis, subclass typing, alkaline phosphatase, serum

INTRODUCTION

Aggressive periodontitis generally affects systemically healthy individuals less than 30 year old. In a study conducted in Sri Lanka, 8% of the population had this rapid progression of periodontal disease or early onset periodontitis (EOP).¹ The corresponding prevalence of aggressive periodontitis (AP) is 1-15%.² The EOP comprises of a group of clinical entities with common attributes of an onset age approximately at puberty, with rapid destruction of the supporting tissues in apparently otherwise healthy individuals.^{1,3,4} This severe periodontitis has been further divided into prepubertal, juvenile, and rapidly progressive periodontitis.³

In 1999, International Classification Workshops, classified periodontitis into three major forms (chronic, aggressive, and necrotizing) and into periodontal manifestations of systemic diseases.⁵

The aggressive periodontitis (AP) includes two subclasses, localized (LAP) and generalized (GAP), depending on the amount of teeth involved. The GAP includes two levels of gingival tissue responses. One is a severe, acutely inflamed tissue, often proliferating, ulcerated, and fiery red. Bleeding and suppuration may be an important feature. In other cases, the gingival tissues may appear pink, no inflammation, and occasionally with some degrees of stippling. However, deep pockets can be demonstrated by probing.¹

The distribution of AP is familial, indicating that there are likely heritable risk factors related to the bacterial floras, immunological responses to plaque, and leukocyte functions.³ The altered functions of leukocytes are PMN phagocyte abnormalities and hyper-responsive macrophage phenotype including elevated production of prostaglandin E₂ (PG E₂) and interleukin-1 β (IL-1 β) in response to bacterial endotoxins.^{1,5} These risk factors frequent-

ly different between the affected individuals within the same family.^{5,6} Certain microbial species including *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans* (Aa), *Campylobacter rectus*, *Prevotella intermedia* (Pi), *Treponema denticola* and *Capnocytophaga ochracea* tend to be predominant in severe and medium AP lesions. *P. gingivalis* is a bacterial species of high virulence and most frequently detected in severe and advanced periodontitis and in active sites.^{1,7}

The lesions of AP differ from the chronic form primarily by (1) the rapid rate of disease progression seen in an otherwise healthy individual, (2) an absence of large accumulation of plaque and calculus, and (3) a family history aggressive disease suggestive of a genetic trait.⁸ The early clinical signs are periodontal pocketing and attachment loss. Attachment loss (AL) may increase rapidly and estimated that 50-75% of the attachment of affected teeth may be lost in 4-5 years.³ From radiographic evidence, osseous destructions are wider than usually seen with chronic periodontitis and range from severe bone loss (BL) associated with minimal number of teeth to advanced BL affecting the majority of teeth.¹ Despite this extreme loss, other sites in the same patient can show no bone loss. Obviously not all patients will exhibit such extremely rapid bone loss, but advanced loss is usually found before 30 years of age.⁸

Sanz et al. reported that concentration of alkaline phosphatase (ALP) in gingival crevicular fluid (GCF) from diseased sites are significantly higher than from healthy sites. A longitudinal study has associated whole-mouth ALP levels with the progression of periodontitis.⁹ The study results from Chapple et al. indicated that the GCF ALP levels may serve as a predictor for future or current disease activity.¹⁰ On other hand, some studies found that T, B, Th, Ts cells and the Th/Ts ratios of peripheral blood lymphocyte subpopulations in AP patients were not more significantly different than those of healthy control subjects. However, the number of NK cells was significantly higher than that of the control group.¹¹

Immunoglobulin G (IgG) is the predominant isotype produced locally in periodontitis patients. The serum and the gingival GCF of chronic periodontitis (CP) subjects contain elevated levels of anti-Pg antibodies. The fimbriae of Pg has been shown to be important cell structures with a role in the virulence by facilitating bacterial attachment to the host cells. Therefore it has been suggested that

the fimbriae antigen could be an important marker of a microbial infection. The serum IgG against Pg fimbriae could be used to indicate the current disease activity, as a predictor of future disease progression in a patient with evidence of previous destruction, and as a predictor of future disease initiation in a previously healthy mouth.¹¹

This study did not examine the AP subclasses, but by using the subclasses based on Novak & Novak's classification. The aims of study was to determine useful indicators to identify and differentiate between the two subclasses of AP, by using measures from clinical, radiographical, and laboratory testing.

MATERIALS AND METHODS

A total of 27 subjects was participated in this study with informed consent. Subjects were deemed suitable for the study if they were diagnosed with aggressive periodontitis and fulfilled the following criteria: aged between 20-45 years; periodontal pockets ≥ 5 mm at ≥ 3 sites in all quadrants, bleeding on gentle probing; some mobile teeth, possibly including a few or more loose teeth; gingival color between pink and pale to fiery red, possibly with suppuration; radiographic evidence of bone loss in more than one third of root length or apical area affecting five to more than 14 teeth.

Subjects fitting any one of the following criteria were excluded from the study: pregnancy or any medical history deemed relevant to their periodontal condition; any regular medication or antimicrobial drug use within the previous 3 months; periodontal treatment including maintenance therapy within the previous 3 months; and factors affecting the food impaction or food retention in the sampling area.

Novak & Novak classified aggressive periodontitis to mild (AP₁) and severe (AP₂) gingival tissue responses as follows:¹

1. Mild Aggressive Periodontitis or AP₁: Gingival tissue may appear pink, no inflammation, and occasionally with some degrees of stippling, although stippling may be absent. However, despite the apparently mild clinical appearance, deep pockets can be demonstrated by probing.
2. Severe Aggressive Periodontitis or AP₂: Severe, Acutely inflamed gingival tissue, often proliferating, ulcerated, and fiery red. Bleeding may occur spontaneously or with slight stimulation. Suppuration may be an important feature.

The full mouth dental radiographs were taken including the assessment of total plaque, calculus,

bleeding on probing scores from four sites (mesial, buccal, distal, palatal/lingual). The recorded features also included the number of remaining teeth (Nrt), missing teeth (Nmt) caused by destructive process of the bone, mobile teeth (Nmo), sextants with hyperaemic and/or edematous gingivae (Hyp), the widest gingival apparent recession (Rec), and the deepest pocket depths (Pock).

To analyze the ALP activity, gingival crevicular fluid was collected with a pair of paper strips from the deepest pocket of each subject.¹¹ Briefly, each strip was successively inserted into the pocket until mild resistance was felt, and maintained *in situ* for 30 seconds. The two strips were then placed in an Eppendorf tube and the amount of GCF was assessed by weighing the tube before and after application. After adding 250 µl of 0.9% NaCl (Saline) as eluent, the tube was shaken vigorously for 1 min and then centrifuged at 2.00 g for 5 min. After removal of the strips, the supernatant was stored at -20° C until the time of analysis.

For enzyme assay, *p*-nitrophenyl phosphate was used for the determination of ALP. About 50 µl of 30 mM carbonate buffer pH 9.8 with 3 mM MgCl₂ was dispensed into microplate. About 50 µl of the specimen was added to the well. About 50 µl of penitrophenyl phosphate was then added, followed by incubation at 37°C for 3 hours. The enzymatic reaction was terminated by adding 50 µl of 0.6 N NaOH and the reading performed at 405 nm by an ELISA rewriter.¹¹

Ten ml peripheral blood was taken from antecubital vein. Three ml was used for analyzing the lymphocyte subpopulations and the remain was centrifuged to get the serum and stored at 20°C until analyzed to measure the titter of IgG against Pg fimbria of serum. Three millilitere venous blood was collected in vacutainer tube (Becton & Dickinson) containing soluble EDTA. Lymphocyte labeling was carried out using the SimultestTM Immune Monitoring Kit (Becton & Dickinson). For lymphocyte subset analysis, a flow cytometer was used, and a computer-assisted evaluation was made with a commercially available software program. Briefly, using volume and site scatter, lymphocytes were gated and specific fluorescence was quantified by a four quadrant setting of a two-color fluorescence dotplot.¹¹

The isotypes and levels of specific antibodies for Pg fimbriae in serum specimens were determined by ELISA.¹¹ To differentiate between AP₁ and AP₂ groups, diagnostic testing using infrabony bo-

ne loss was applied as the gold standard. For statistical evaluation of the results, we used Mann-Whitney U test to differentiate data of both groups.

RESULTS

The number of male subjects and the AP₂ type subjects are higher than female and AP₁ type subjects (Table 1). The initial age of AP₁ type subjects is older (25 years) than of AP₂ type patients (21 years) (Table 2).

Data collected of all variables of AP₂ types subjects are almost higher than of AP₁ types, except data of number of remaining teeth, the number of Th cells, and the Th/Ts ratio (Table 3).

Table 1. Gender distribution of the subjects

Gender	AP ₁	AP ₂	Total
Female	3	8	11
Male	5	11	16
Total	8	19	27

Exact Fisher Test: $p = 0.325$

Table 2. Range, mean and SD of age of the subjects

Age	AP ₁	AP ₂
Age range	25 - 44	21 - 45
Mean ± SD	33.0 ± 6.1	35.5 ± 7.1

Table 3. The ranges of clinical, radiographical and laboratory indicators

Variable	AP ₁	AP ₂
PI S	36 - 191	59 - 236
C S	23 - 199	9 - 291
BOP	66 - 411	93 - 458
Nrt	28 - 32	17 - 32
Nmt	0 - 2	0 - 11
Mot	2 - 18	9 - 29
Hyp	0 - 3	0 - 6
Rec (mm)	4 - 8	3 - 17
Pock (mm)	4 - 10	6 - 14
Angular b 1	0 - 17.39	4 - 52.94
Active b 1	0 - 20	0 - 78.26
GCF ALP	0.99 - 19.99	2.21 - 24.26
T cell	45 - 69	43 - 81
Th	24 - 43	21 - 44
Ts	17 - 39	18 - 46
B	8 - 18	6 - 28
NK	11 - 38	8 - 41
Th/Ts	0.71 - 1.6	0.46 - 1.5
IgG spPgF	16.67 - 2500	0 - 13333.33

Nrt = no. of remaining teeth, Nmt = no. of missing teeth due to destructive process, Mot = no. of mobile teeth

due to destructive process, Hyp = number of sextants of hyperaemic and edemateous gingiva, Rec(mm)= widest apparent gingival recession, Pock(mm)= deepest perio-

dontal pocket, Active b l = active bone loss, GCF ALP = gingival crevicular fluid alkaline phosphatase, IgG spPgF = IgG against specific Pg fimbriae.

Table 4. Diagnostic performance of the test variables (AP₂ against AP₁), using angular bony defect as gold standard

Test variable	Sensitivity (AP ₂) %	Specificity (AP ₁) %	Prediction+ (AP ₂) %	Prediction - (AP ₁) %	Accuracy %
PI S	92.31	0	96	0	88.89
C S	96.15	0	96.15	0	92.59
BOP	88.46	0	95.83	0	85.19
Nrt	92.31	0	96	0	88.89
Nmt	73.08	0	95	0	70.73
Mot	80.77	0	95.45	0	77.78
Hyp	73.08	0	95	0	70.37
Rec (mm)	80.77	0	95.45	0	77.78
Pock(mm)	76.92	0	95.24	0	74.07
Active b l	46.15	0	92.31	0	44.44
GCF ALP	76.92	100	100	14.29	77.78
T cell	46.15	0	92.31	0	44.44
Th	42.31	0	91.67	0	40.74
Ts	80.77	0	95.45	0	77.78
B	19.23	0	83.33	0	18.52
NK	50	0	92.86	0	48.15
Th/Ts	65.38	0	94.44	0	62.96
IgG spPgF	96.15	0	96.15	0	92.59

The GCF ALP test is the only test which is 100% specific to the diagnosis of AP₂, with a sensitivity of 76.9%. The positive prediction value is 100%, whereas the negative prediction value is 14.3%. The accuracy of this test is 77.8% (Table 4).

DISCUSSION

Advanced chronic periodontitis lesions are common among individuals older than 55 years, and less severe than the AP lesions. AP is a severe form of periodontitis seen in young adults, which is highly destructive to the tissues around the teeth. This disease has been established as a distinct clinical entity, and the most sufferers of EOP, from which manifest defects in either neutrophil or monocyte chemotaxis.^{1,3,4}

Porphyromonas gingivalis (Pg) has a wide range of potentially relevant virulence factors such as extracellular proteases, which can perturb the host immune response and the tissue integrity, toxic metabolites and cellular constituents, and adherence factors that promote colonization. Furthermore, Pg can efficiently invading and replicating within the primary cultures of gingival epithelial cells and the multilayered pocket epithelium.¹² Pg fimbriae exhibits a wide variety of biological activities including immunogenicity, binding to various host proteins, stimulation of cytokine production and promotion of bone resorption.¹³ As Pg

has been found to be predominant in severe and medium AP lesions, IgG against Pg fimbriae also appears at high levels in serum of AP patients.¹¹

Several studies investigating host defense factors in AP have indicated an abnormal immune response involving neutrophil functions, T- and B-lymphocytes, serum immunoglobulins, and certain complement proteins.¹¹ Therefore, we have investigated the lymphocyte subpopulations, Th/Ts ratio, IgG against-Pg fimbriae, in addition to GCF ALP of AP patients as laboratory parameters in this study.

Gender distribution in Table 1 shows that male subjects are more than females, but this was not significant ($p=0.325$). Also the range age of AP₁ group was 25-44 years and of AP₂ group was 21-45 years (Table 2). It is deemed that the 21 years old subjects in this study had severe AP, and the 44 years old subjects had mild AP. We can bear in mind that the severe condition of AP is not initiated by the mild condition of it.

The result of this study showed that the GCF ALP can be used to specify the severity of AP₂. The sensitivity of this test or the percentage of gaining the positive test is 76.92%. The specificity of this test or the percentage of negative test in patient without AP₂ is 100%. The positive prediction of this test or the percentage of an individual to suffer AP₂ if GCF ALP activity is more than 20 IU/g is 100%, and negative prediction or the percentage that someone not suffering AP₂ if GCF

ALP activity is 20 IU/g or less, is 14.29%. The accuracy of this test or the proportion of all exact positive or all exact negative tests was 77.78 %.

It is concluded that the GCF ALP test appeared to be a useful criteria to differentiate severe AP (or AP₂) from mild AP (AP₁) that are distinct disease entities. Therefore that AP₂ is not initiated from AP₁. There may be intrinsic factors that activate the two subclasses of AP in different ways, and this suggests an area of further investigation.

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