

## LEVELS OF LL-37 ANTIMICROBIAL PEPTIDE IN THE GINGIVAL CREVICULAR FLUID OF YOUNG AND MIDDLE-AGED SUBJECTS WITH OR WITHOUT GINGIVITIS

### Gingivitisin Genç ve Orta Yaşlı Bireylerde Dişeti Oluğu Sıvısı LL-37 Seviyelerine Etkisi

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#### ABSTRACT

**Purpose:** LL-37 is an antimicrobial peptide which plays an important role in the innate immunity. The aim of this study was to investigate the LL-37 levels in the gingival crevicular fluid (GCF) of middle-aged and young adults who have either gingivitis or healthy periodontal tissues.

**Materials and Methods:** Forty middle-aged adults (20 healthy controls and 20 with gingivitis) and 41 younger adults (20 healthy controls and 21 with gingivitis) were included in the present study. Probing depth, clinical attachment level, plaque index, and papilla bleeding index were recorded. LL-37 levels in the GCF were analyzed by enzyme-linked immunosorbent assay.

**Results:** No significant differences were observed in the GCF LL-37 levels between young healthy and middle-aged healthy subjects. Also, there were no significant differences in GCF LL-37 levels between young and middle-aged gingivitis subjects. However, gingivitis groups had significantly higher GCF LL-37 levels than healthy groups ( $p < 0.001$ ). Correlation analysis demonstrated no significant correlation between age and GCF LL-37 levels neither in healthy nor in gingivitis groups.

**Conclusion:** The levels of LL-37 in GCF increase in the presence of gingival inflammation, however, this does not vary according to subjects being young or middle-aged.

**Keywords:** Cathelicidin LL-37; antimicrobial peptide; gingival crevicular fluid; gingivitis; age

#### ÖZ

**Amaç:** Bir antimikrobiyal peptid olan LL-37 doğal immunitede önemli rol oynar. Bu çalışmanın amacı gingivitis tanısı almış ya da sağlıklı dişetlerine sahip olan genç ve orta yaşlı erişkin hastalarda dişeti oluk sıvısındaki (DOS) LL-37 seviyelerini incelemektir.

**Gereç ve Yöntem:** Bu çalışmaya 40 orta yaşlı erişkin (20 sağlıklı kontrol ve 20 gingivitisli) ve 41 genç erişkin (20 sağlıklı kontrol ve 21 gingivitisli) hasta dâhil edilmiştir. Sondalama derinliği, klinik ataşman seviyesi, plak indeksi ve papilla kanama indeksi değerleri kaydedilmiştir. DOS LL-37 seviyeleri enzim bağlı immunoabsorbent testi ile ölçülmüştür.

**Bulgular:** Dişetleri sağlıklı olan genç ve orta yaşlı bireylerin LL-37 seviyeleri arasında bir farklılık izlenmedi. Benzer şekilde gingivitisli genç ve orta yaşlı erişkinlerde DOS LL-37 seviyeleri arasında da fark gözlenmedi. Her iki gingivitis grubu sağlıklı kontrol gruplarına göre daha yüksek DOS LL-37 seviyesine sahipti ( $p < 0.05$ ). Gerek sağlıklı gerekse gingivitisli gruplarda yaş ve DOS LL-37 seviyeleri arasında anlamlı bir ilişki saptanmadı.

**Sonuç:** Dişetinde enflamasyonun varlığı DOS'ta LL-37 seviyesinin artmasına sebep olmaktadır. Ancak hastanın genç ya da orta yaşlı erişkin bir birey olmasının bu durum üzerinde bir etkisi yoktur.

**Anahtar kelimeler:** Katelisinidin LL-37; antimikrobiyalpeptid; dişeti oluğu sıvısı; gingivitis; yaş

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## Introduction

The reversible inflammation of gingival tissues, gingivitis, is the most common form of periodontal diseases which is caused by the accumulation of dental plaque adjacent to gingival margin (1, 2). 54% of the United States population has been reported to have at least one site with gingivitis (3, 4). Plaque accumulation results in acute inflammatory response dominated by neutrophils. Consequently, collagen homeostasis changes, and pro-inflammatory cytokines are expressed (1). Neutrophil markers such as leukotriene B<sub>4</sub>, interleukin-8, and betaglycuronidase increase in the GCF of subjects with gingivitis (5-7). The severity of gingivitis is affected by the host inflammatory response to bacterial challenge (8, 9).

If the gingivitis is left untreated, it may progress to periodontitis in some patients (10, 11). The tissue damage caused by gingivitis is reversible. The presence of gingivitis is, on the other hand, a risk factor for the development of periodontitis (1, 12). Periodontal diseases are caused by the interactions between the microbial dental plaque and host immune response which plays an important role in the individual susceptibility for periodontitis (1, 13). The presence of dental plaque may trigger innate and adaptive responses. Antimicrobial peptides including LL-37, adrenomedullin and defensins are important contributors of the innate immune response (14).

Neutrophils and epithelial cells express LL-37 antimicrobial peptide (14-16). It is stored as an inactive precursor in the secondary granules of neutrophils (17, 18). Following neutrophil stimulation, mature LL-37 is released from neutrophils (18). LL-37 modulates the inflammatory and immune responses, accelerates the angiogenesis, promotes wound healing and re-epithelization, and neutralizes the lipopolysaccharides (14, 15, 17). It has been stated that there is an association between LL-37 levels and a number of inflammatory diseases (19-21). Although blood neutrophils do not have mature LL-37, abundant amount of hCAP18 in patients with Papillon-Lefèvre syndrome has been reported (22).

In an immunohistochemical study, Fransson *et al.* (23) demonstrated that there was a difference in the inflammatory response to plaque formation between young and old people. However, Tsalikis *et al.* (24) have shown that the age variable did not have an effect on the cytokine expression in experimental gingivitis. A positive correlation between saliva LL-37 level and age has been reported in childhood

(25). Castaneda-Delgado *et al.* (26) have detected similar amounts of LL-37 in the serum samples of both old and young healthy participants. It has been speculated that the effects age on the immune response and infection may be more severe in older subjects compared to younger people (27). Also, older people present increased susceptibility for infectious diseases (26), which might be caused by the dysregulation of immune response (27). The aim of the present study is therefore to investigate the relationship between the age and LL-37 levels in the gingival crevicular fluid (GCF) of middle-aged and young adults who have either gingivitis or healthy periodontal tissues.

## Materials and Methods

### *Study participants*

Forty-one participants with gingivitis and 40 subjects with healthy periodontal tissues were included in the present study. Sample was derived from the patients who had been recruited from Ege University, Faculty of Dentistry, Department of Periodontology between 2013 and 2015. Each patient was given detailed information about the study protocol and they signed the consent form. Ethics Committee of Ege University has reviewed and approved the protocol (# 12-2.1/4). Exclusion criteria were as follows: the presence of aggressive or chronic periodontitis, subjects with systemic or infectious diseases, pregnancy, lactation, having periodontal treatment within the past one year, subjects taking oral contraceptive drugs and/or those who have taken antibiotics in the last 3 months, and subjects with a history of smoking. Inclusion criteria were as follows: being a non-smoker who has 16 teeth at least. Subjects, who have healthy periodontal tissues or gingivitis, aged 18 to 30 years, were included in the young adult groups. Subjects aged 40 to 60 years, who have healthy periodontal tissues or gingivitis, were included in middle-aged adult groups.

Subjects aged 31 to 39 years were not included in the study. All healthy subjects were free of systemic or periodontal diseases. Healthy patients had no sites with clinical attachment level (CAL)>2 mm or probing depth (PD)>3 mm. Furthermore, they had bleeding on probing (BOP) less than 15% of the sites and no alveolar bone loss radiographically. Subjects with gingivitis had no clinical signs of periodontitis and no radiographic evidence of alveolar bone loss. Patients who had BOP at more than 50% of the examined

periodontal areas were diagnosed as having gingivitis.

Twenty middle-aged adults (aged 40 - 59 years) (12 female, 8 male) were included in the healthy middle-aged adult group (MA-Healthy). The mean age of this group was  $46.5 \pm 5.4$  years. Twenty subjects (aged 20-30 years) (13 female, 7 male) were included in the healthy young adult group (Y-Healthy). The mean age of this group was  $27 \pm 3$  years. Twenty subjects (aged 41 - 57 years) (11 female, 9 male) were included in middle-aged adult group with gingivitis (MA-Gingivitis). The mean age of this group was  $47.1 \pm 5.1$  years. Twenty-one subjects (aged 24 - 30 years) (13 female, 8 male) were included in young adult group with gingivitis (Y-Gingivitis). The mean age of this group was  $28 \pm 4.7$  years.

#### *Clinical examination*

PD and CAL measurements were performed at six sites per tooth excluding third molars. PD and CAL were measured by William's periodontal probe (Hu-Friedy, Chicago, IL, USA) in millimeter. Plaque index (PI) (28), BOP (29) and papilla bleeding index (PBI) (30) scores were also determined. Same clinician (O.T) performed all clinical examinations. The intra-examiner reliability was considered to be high since the intra-class correlation coefficient (ICC) was 0.87 and 0.85 for PD and CAL measurements, respectively.

#### *Sample collection and analysis of LL-37 levels*

GCF sampling was performed from vestibule sides of the interproximal areas of anterior teeth. In gingivitis patients, sample sites were selected from the teeth with BOP. In healthy group, sample sites were selected from the teeth having PD less than 3 mm without BOP. Samples were collected the day after the periodontal diagnosis. Supragingival plaque was moved away from the sampling sites. Area was isolated and dried, the sample was then obtained using a filter paper (Periopaper, ProFlow, Inc., Amityville, NY, USA) (31).

Periotron 8000 device (Oralflow Inc., Helwlett, NY, USA) was used to determine the GCF volume in each strip. Enzyme-Linked ImmunoSorbent Assay (ELISA) kit was used to determine LL-37 levels (Hbt Human LL-37, Hycult Biotechnology, Uden, Netherlands). All procedures were performed according to the manufacturer's recommendations. The minimum detectable limit of LL-37 was 0.14 ng/ml.

#### *Statistical analysis*

Statistical Package for Social Sciences (SPSS) software (SPSS Inc., ver. 17.0, Chicago, IL, USA) was used in this study. Post hoc power was 0.86 for LL-37 total amount. Chi square test was used for gender comparisons among the study groups. Periodontal variables calculated from the samples taken from whole-mouth and sampling sites were compared either with parametric or non-parametric tests. Mann-Whitney U test was used to compare GCF LL-37 levels between the study groups. Pearson correlation coefficient was used to analyze the relationship between the age and LL-37 levels in GCF.

#### **Results**

The distribution of the male and female participants was similar in the study groups.

#### *Periodontal variables*

Whole mouth PD, CAL, BOP, PBI, and PI scores in Y-Healthy group were similar to those found in MA-Healthy group. No significant differences were observed in whole mouth PD, CAL, BOP, PBI, and PI scores between Y-Gingivitis and MA-Gingivitis groups. Both gingivitis groups had significantly higher PI, BOP, and PBI scores of whole mouth compared to their healthy counterparts ( $p < 0.001$ ) (Table 1).

Y-Healthy and MA-Healthy groups had similar PD, PI scores and GCF volumes of sampling sites. PD, PI, PBI, and GCF volumes of sampling sites were found to be similar in Y-Gingivitis and MA-Gingivitis groups. There were significant differences in PD, PI, PBI values, and GCF volumes of sampling sites between Y-Gingivitis and Y-Healthy groups ( $p < 0.001$ ). Similarly, significant differences were observed in PD, PI, PBI values, and GCF volumes of sampling sites between MA-Healthy and MA-Gingivitis groups ( $p < 0.001$ ) (Table 2).

#### *LL-37 levels in gingival crevicular fluid*

Table 3 presents total amount and concentration LL-37 in GCF. No significant differences were observed in the total amount and concentration of LL-37 between Y-Healthy and MA-Healthy subjects. Also, there were no significant differences in total amount and concentration of LL-37 between Y-Gingivitis and MA-Gingivitis subjects. MA-Gingivitis group

*LL-37 levels in different age groups*

had significantly higher total amount of GCF LL-37 compared to that of MA-Healthy group ( $p < 0.001$ ). Similarly, Y-Gingivitis group had significantly higher GCF LL-37 total amount than Y-Healthy group ( $p < 0.001$ ). A significant difference was observed in GCF LL-37 concentration between Y-Healthy and

Y-Gingivitis groups ( $p < 0.05$ ), while there was no significant difference in the LL-37 concentration between MA-Healthy and MA-Gingivitis groups. Correlation analysis demonstrated no significant correlation between age and GCF LL-37 levels in healthy and gingivitis groups.

**Table 1.** Means and standard deviations of the clinical variables stratified by study groups (PD: probing depth, CAL: clinical attachment level, PI: plaque index, BOP: bleeding on probing, PBI: papilla bleeding index, Y: young, MA: middle aged).

| Clinical variables | Y-Healthy (n=20) | Y-Gingivitis (n=21) | MA-Healthy (n=20) | MA-Gingivitis (n=20) |
|--------------------|------------------|---------------------|-------------------|----------------------|
| PD (mm)            | 2.1 ± 0.5        | 2.5 ± 0.5           | 2.1 ± 0.4         | 2.4 ± 0.5            |
| CAL (mm)           | 0.13 ± 0.1       | 0.12 ± 0.1          | 0.14 ± 0.1        | 0.11 ± 0.1           |
| PI                 | 1.6 (0.6 - 2.8)  | 3.2(2.3 - 4.5)*     | 1.5 (0.9 - 3)     | 3 (2.2 - 4.5)*       |
| BOP                | 11 ± 5           | 78 ± 12*            | 13 ± 3            | 80 ± 14*             |
| PBI                | 0.1 (0.1 - 0.1)  | 2.1 (1 - 3.1)*      | 0.1 (0.1 - 0.2)   | 2 (1 - 3)*           |

\* indicates significant difference from healthy counterpart.

**Table 2.** Periodontal parameters of the sampling sites in the study groups (PD: probing depth, CAL: clinical attachment level, PI: plaque index, PBI: papilla bleeding index, GCF: gingival crevicular fluid, Y: young, MA: middle aged).

| Clinical variables | Y-Healthy (n=20) | Y-Gingivitis (n=21) | MA-Healthy (n=20) | MA-Gingivitis (n=20) |
|--------------------|------------------|---------------------|-------------------|----------------------|
| PD (mm)            | 2 (1-2)          | 3 (2 - 4)*          | 2 (1-2)           | 3 (2 - 5)*           |
| CAL (mm)           | -                | -                   | -                 | -                    |
| PI                 | 2 (1-3)          | 3 (2-4)*            | 2 (1-3)           | 3 (2-5)*             |
| PBI                | -                | 2 (1-4)*            | -                 | 2 (1-4)*             |
| GCF volume (µl)    | 0.2 ± 0.1        | 0.38 ± 0.14*        | 0.2 ± 0.1         | 0.42 ± 0.12*         |

\*indicates significant difference from healthy counterpart.

**Table 3.** Means and ranges of the LL-37 amount and concentration values in the gingival crevicular fluid (GCF) of the study groups (Y: young, MA: middle aged).

| GCF LL-37 level        | Y-Healthy (n=20) | Y-Gingivitis (n=21) | MA-Healthy (n=20) | MA-Gingivitis (n=20) |
|------------------------|------------------|---------------------|-------------------|----------------------|
| Total amount (ng/site) | 0.21 (0.01-1.63) | 0.93 (0.2-25) *     | 0.23(0.01-1.42)   | 0.97 (0.1-2.4)*      |
| Concentration (ng/µl)  | 1.37 (0.01-8.35) | 2.78 (0.43-8.21)* † | 2.35 (0.01-8.35)  | 3.13 (0.44-10.83)    |

\* indicates significant difference from healthy counterpart.

## Discussion

There are several studies investigating LL-37 antimicrobial peptide in periodontal diseases. However, to the best of our knowledge, there is no study evaluating the possible difference between

age groups in the presence or absence of gingival inflammation. Findings of the present study showed no difference in LL-37 in GCF levels in samples taken from healthy or inflammatory gingiva. Furthermore, no significant correlation was observed between age and GCF LL-37 levels. Nevertheless, our results

also suggest that the inflammation of the gingiva has an increasing effect on the GCF LL-37 levels, which is independent from the participants' age. It has been suggested that the inflammatory response to plaque formation in young and old people might be different (23, 27). However, Tsalikis *et al.* (24) showed that age did not affect cytokine expression in experimental gingivitis. Similarly, Castaneda-Delgado *et al.* (26) have found similar amounts of LL-37 in the serums obtained from healthy elder and healthy young subjects. Davidopoulou *et al.* (25) have investigated the relation of salivary concentration of LL-37 levels with age in young children, and they have found a positive correlation between LL-37 concentration and age. In the present study, young and middle-aged participants had similar amount of GCF LL-37 not only in the healthy group, but also in the gingivitis group.

Therefore, our data suggest that being a young or middle-aged participant as defined in this study does not affect the levels of GCF LL-37 levels in healthy, as well as inflammatory conditions of gingiva. However, in the present study, we hypothesized that age might be a factor affecting GCF LL-37 levels because of the dysregulation of immune response in older people. Davidopoulou *et al.* (25) stated that there was a positive correlation between salivary LL-37 concentration and age. These conflicting results might be explained by the age difference of the samples. Davidopoulou *et al.* (25)'s sample included children aged from 2 to 18 years old which is much younger than the mean age of our study population. In addition, Davidopoulou *et al.* (25) investigated LL-37 levels in saliva but not in GCF. Although there are several studies investigating the effect of periodontitis on LL-37 levels in GCF and saliva, a very limited number of articles have evaluated LL-37 levels in the presence of only gingival inflammation. Our findings demonstrated that gingival inflammation has an effect on GCF LL-37 levels regardless of age. Both young and middle aged subjects with gingivitis had significantly higher levels of GCF LL-37 compared to their healthy counterparts. This result is in accordance with those of Hosokawa *et al.* (32) in which the authors showed that neutrophil LL-37 expression was more prominent in inflammatory lesions than healthy gingiva by immunohistochemistry. In the present study, there were two age groups which were categorized as 18 to 30 and 40 to 60 years. The main limitation of this design is to exclude the subjects younger than eighteen and older than sixty. Therefore,

studies including younger, as well as older, subjects than those of the present study are needed to reveal the true effect of age on GCF LL-37. Additionally, further studies investigating the effect of age on GCF LL-37 levels in the presence of periodontitis would provide more information about this issue.

## Conclusion

To the best of our knowledge, this is the first study evaluating the effect of age on GCF LL-37 levels during the gingival inflammation or in the healthy state of the gingiva. Our findings indicate that the presence of gingival inflammation increased the LL-37 levels in GCF; however, this does not vary according to subjects being young or middle-aged.

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## Conflict of interest

None declared

## References

1. Kornman KS, Page RC, Tonetti MS. The host response to the microbial challenge in periodontitis: Assembling the players. *Periodontol* 2000 1997;14:33-53.
2. Loe H, Theilade E, Jensen SB. Experimental gingivitis in man. *J Periodontol* 1965;36:177-187.
3. Albandar JM, Kingman A. Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the united states, 1988-1994. *J Periodontol* 1999;70(1):30-43.
4. Tomar SL, Asma S. Smoking-attributable periodontitis in the united states: Findings from nhanes iii. National health and nutrition examination survey. *J Periodontol* 2000;71(5):743-751.
5. Heasman PA, Collins JG, Offenbacher S. Changes in crevicular fluid levels of interleukin-1 beta, leukotriene b4, prostaglandin e2, thromboxane b2 and tumour necrosis factor alpha in experimental gingivitis in humans. *J Periodontal Res* 1993;28(4):241-247.

6. Lamster IB, Holmes LG, Gross KB, Oshrain RL, Cohen DW, Rose LF, Peters LM, Pope MR. The relationship of beta-glucuronidase activity in crevicular fluid to clinical parameters of periodontal disease. Findings from a multicenter study. *J Clin Periodontol* 1994;21(2):118-127.
7. Offenbacher S, Barros S, Mendoza L, Mauriello S, Preisser J, Moss K, de Jager M, Aspiras M. Changes in gingival crevicular fluid inflammatory mediator levels during the induction and resolution of experimental gingivitis in humans. *J Clin Periodontol* 2010;37(4):324-333.
8. Gemmell E, Yamazaki K, Seymour GJ. Destructive periodontitis lesions are determined by the nature of the lymphocytic response. *Crit Rev Oral Biol Med* 2002;13(1):17-34.
9. Leishman SJ, Seymour GJ, Ford PJ. Local and systemic inflammatory responses to experimentally induced gingivitis. *Dis Markers* 2013;35(5):543-549.
10. Offenbacher S. Periodontal diseases: Pathogenesis. *Ann Periodontol* 1996;1(1):821-878.
11. Schatzle M, Faddy MJ, Cullinan MP, Seymour GJ, Lang NP, Burgin W, Anerud A, Boysen H, Loe H. The clinical course of chronic periodontitis: V. Predictive factors in periodontal disease. *J Clin Periodontol* 2009;36(5):365-371.
12. Agace WW, Hedges SR, Ceska M, Svanborg C. Interleukin-8 and the neutrophil response to mucosal gram-negative infection. *J Clin Invest* 1993;92(2):780-785.
13. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest* 1989;84(4):1045-1049.
14. Dale BA, Fredericks LP. Antimicrobial peptides in the oral environment: Expression and function in health and disease. *Curr Issues Mol Biol* 2005;7(2):119-133.
15. Dale BA, Kimball JR, Krisanaprakornkit S, Roberts F, Robinovitch M, O'Neal R, Valore EV, Ganz T, Anderson GM, Weinberg A. Localized antimicrobial peptide expression in human gingiva. *J Periodontol Res* 2001;36(5):285-294.
16. Turkoglu O, Kandiloglu G, Berdeli A, Emingil G, Atilla G. Antimicrobial peptide hCAP-18/LL-37 protein and mRNA expressions in different periodontal diseases. *Oral Dis* 2011;17(1):60-67.
17. Bals R, Wilson JM. Cathelicidins--a family of multifunctional antimicrobial peptides. *Cell Mol Life Sci* 2003;60(4):711-720.
18. Sorensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, Borregaard N. Human cathelicidin, hcap-18, is processed to the antimicrobial peptide ll-37 by extracellular cleavage with proteinase 3. *Blood* 2001;97(12):3951-3959.
19. Hoffmann MH, Bruns H, Backdahl L, Neregard P, Niederreiter B, Herrmann M, Catrina AI, Agerberth B, Holmdahl R. The cathelicidins ll-37 and rcramp are associated with pathogenic events of arthritis in humans and rats. *Ann Rheum Dis* 2013;72(7):1239-1248.
20. Hwang YJ, Jung HJ, Kim MJ, Roh NK, Jung JW, Lee YW, Choe YB, Ahn KJ. Serum levels of ll-37 and inflammatory cytokines in plaque and guttate psoriasis. *Mediators Inflamm* 2014;2014:268257.
21. Kahlenberg JM, Kaplan MJ. Little peptide, big effects: The role of ll-37 in inflammation and autoimmune disease. *J Immunol* 2013;191(10):4895-4901.
22. Eick S, Puklo M, Adamowicz K, Kantyka T, Hiemstra P, Stennicke H, Guentsch A, Schacher B, Eickholz P, Potempa J. Lack of cathelicidin processing in papillon-lefevre syndrome patients reveals essential role of ll-37 in periodontal homeostasis. *Orphanet J Rare Dis* 2014;9:148.
23. Fransson C, Mooney J, Kinane DF, Berglundh T. Differences in the inflammatory response in young and old human subjects during the course of experimental gingivitis. *J Clin Periodontol* 1999;26(7):453-460.
24. Tsalikis L. The effect of age on the gingival crevicular fluid composition during experimental gingivitis. A pilot study. *Open Dent J* 2010;4:13-26.
25. Davidopoulou S, Diza E, Menexes G, Kalfas S. Salivary concentration of the antimicrobial peptide ll-37 in children. *Arch Oral Biol* 2012;57(7):865-869.
26. Castaneda-Delgado JE, Miranda-Castro NY, Gonzalez-Amaro R, Gonzalez-Curiel I, Montoya-Rosales A, Rivas-Calderon B, Rivas-Santiago B. Production of antimicrobial peptides is preserved in aging. *Clin Immunol* 2013;148(2):198-205.
27. Honda T, Domon H, Okui T, Kajita K, Amanuma R, Yamazaki K. Balance of inflammatory response in stable gingivitis and progressive periodontitis lesions. *Clin Exp Immunol* 2006;144(1):35-40.
28. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. *J Am*

- Dent Assoc 1962;65:26-29.
29. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J* 1975;25(4):229-235.
  30. Saxer UP, Muhlemann HR. [motivation and education]. *SSO Schweiz Monatsschr Zahnheilkd* 1975;85(9):905-919.
  31. Lamster IB, Hartley LJ, Oshrain RL, Gordon JM. Evaluation and modification of spectrophotometric procedures for analysis of lactate dehydrogenase, beta-glucuronidase and arylsulphatase in human gingival crevicular fluid collected with filter-paper strips. *Arch Oral Biol* 1985;30(3):235-242.
  32. Hosokawa I, Hosokawa Y, Komatsuzawa H, Goncalves RB, Karimbux N, Napimoga MH, Seki M, Ouhara K, Sugai M, Taubman MA, Kawai T. Innate immune peptide ll-37 displays distinct expression pattern from beta-defensins in inflamed gingival tissue. *Clin Exp Immunol* 2006;146(2):218-225.

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