AJDS LAZHAR Al-Azhar Journal of Dental Science Print ISSN 1110-6751 | online ISSN 2682 - 3314
Vol. 27- No. 4- 571:578- October 2024 https://ajdsm.journals.ekb.eg
Orthodontic & Pediatric Dentistry Issue (Orthodontics and Pediatric Dentistry)

# USES OF HISTATIN 5 AS BIOMARKERS FOR CARIES RISK ASSESSMENT

Malek Idris Elbawendi[1*], Hamdy Mahmoud Badreldin[2], Alaa Aldeen Abdallah Ismail[3], Mona Abdalghafar Khalil[4].

## *ABSTRACT*

**Objectives:** This study was carried out to identify the antimicrobial peptides histatin as biomarkers for caries risk assessment in children. **Subjects and methods:** This study was conducted on thirty three patients with different caries risk assessment. All the patients were divided randomly in to three equal groups according to the American Dental association caries risk assessment form as the follow; the first group; Children with low caries risk assessment (Control Group), the second group; Children with moderate caries risk assessment, the third group; Children with high caries risk assessment. **Results:** The results of this study revealed that; in comparison between the three groups according levels of Histatin, results showed significant increase in histatin levels in high risk group and moderate risk group in comparison with low risk group. **Conclusion:** Based on the results of this study, antimicrobial peptides histatin may be used as good biomarkers for high caries risk patients.

**KEYWORDS**: Histatin, Antimicrobial Peptides, Salivary Biomarkers, Dental Caries, Caries Risk Assessment.

## INTRODUCTION

One of the most prevalent chronic infectious diseases affecting preschool-aged children is dental caries, which is characterized by the destruction of tooth tissues as a result of complex synergistic interactions between the acids produced by bacterial fermentation of dietary carbohydrates and susceptible host factors, such as saliva and teeth[(1)]. Dental caries is regarded as a public health issue since it has a detrimental effect on both the child's and the family's quality of life[(2)]. The etiology of dental caries in children is linked to socioeconomic variables[(3)], irregular teeth brushing[(4,5)], and dietary behaviors[(6,7)].

The clinical process of determining whether a patient will likely develop caries lesions over time, or whether there will likely be a change in the size or activity of lesions that are already present, is known as caries risk assessment (CRA). Although it is widely agreed that CRA is a crucial part of the decision-making process for effective children caries prevention and management as well as for individual timing of recall intervals[(8)], there is undoubtedly some uncertainty as to when and how to do it.

1. Masters Candidate, General Dentist, Libya
2. Lecturer, Department of Pedodontics and Oral Health, Faculty of Dental Medicine, Boys, Cairo Al-Azhar University.
3. Associate Professor, Department of Pedodontics and Oral Health, Faculty of Dental Medicine, Boys, Cairo Al-Azhar University.
4. 4- Lecturer, Department of Medical Biochemistry Faculty of Medicine, Girls, Cairo Al-Azhar University.

• ***Corresponding author*:** malekbawendi@gmail.com .

***DOI:*** *10.21608/ajdsm.2023.184280.1398*

Saliva, a complex bodily fluid made up of both organic and inorganic components, is necessary for the mouth cavity's health. The three pairs of major salivary glands—the parotid, submandibular, and sublingual glands—as well as the numerous tiny salivary glands located in the oral submucosa are the principal sources of saliva [9]. Saliva also contains germs, bronchial expectoration remnants, food particles, and desquamated cells of the oral epithelium in addition to naturally combining with gingival crevicular fluid [10].

About 99.5% of saliva is water, 0.3% is protein, and 0.2% is trace and inorganic material [11]. One of the body's natural defense mechanisms, saliva improves dental enamel through remineralization, balances low plaque pH, washes away food particles, microbes, and sugar crystallization, among other ways to preserve teeth. Saliva also has antibacterial and bacterial characteristics [12,13]. As increased frequency and severity of oral disease are frequently related with qualitative and quantitative changes of the saliva proteome, saliva protein concentration is crucial in the preservation of oral health and balance [14,15]. By having direct antibacterial effects, proteomic compounds such histatins, mucin, lactoperoxidase, defensins, proline-rich peptides, and lactoferrin regulate the microbial ecology of the mouth cavity [16,17]. Numerous proteins found in saliva are essential for defending oral tissues against viral or fungi infections [18]. Therefore, the protein composition of saliva may be a key factor in the development of dental caries and the incidence of oral diseases [16].

Saliva collection and storage are simple, painless, reasonably priced, and low risk procedures for both patients and medical personnel. Saliva with these qualities is useful for researching caries biomarkers in newborns, children, and adults. Recent studies have examined the bacterial abundance, protein identity and concentration, and buffer capacity in saliva samples to assess the occurrence of caries.

This molecule or one of those antimicrobial peptides (AMPs) serve as the first line of defense against oral microbial colonization and illness and are crucial elements of innate immunity. Cathelicidin peptide LL-37, alpha-defensins, beta-defensins, histatins, and statherin are the AMPs that are most frequently expressed in saliva.

Histatin peptides belong to a family of antimicrobial peptides that are rich in histidine amino acids. Histidine rich polypeptides have been proven to have antimicrobial and antifungal properties [19]. They are secreted by major salivary glands including parotid and submandibular glands. The concentration of histatin peptides in saliva ranges from 50 to 425 μg/ml. protective role of saliva that aids in digestion, lubrication, protection, and host defense immunization of the oral cavity [20]. The present study was carried out to identify the antimicrobial peptides histatin as biomarker for caries risk assessment in children.

## SUBJECTS AND METHODS

This study was conducted on thirty-three patients with different caries risk assessment. The age of children were 4 to 6years and they selected that attending to the Department of Pedodontics and Oral Health, Faculty of Dentistry, Al- Azhar University.

Patient's were divided randomly into three equal groups (n=11) according to the American Dental association caries risk assessment form (Age >6) [21] as the follow:

- **Group A:** 11 Children with low caries risk assessment (Control Group).
- **Group B:** 11 Children with moderate caries risk assessment.
- **Group C:** 11 Children with high caries risk assessment.

**Patients Selection:**

Selection of patients were based on specific inclusion and exclusion criteria as the follow:

**Eligibility criteria:**

***A. Inclusion Criteria:***

1. Children patients age ranges from 4 - 6 years.
2. Both genders.
3. Healthy child without any systemic diseases.

***B. Exclusion Criteria:***

1. Medically compromised child.
2. Mentally challenged child.
3. Child who are using oral mouthwashes during the period of study.
4. Child undergoing antimicrobial treatment during the course of the study or for a period of 30 days prior to the study.

**Sample Size Calculation:** [22]

Based on the previous paper by Aldhaher 2021; the difference in antimicrobial Peptides between healthy and children with caries was 13±6.5 (ng/ml). Using power 95% and 5% significance level 8 participants in each group are required. Sample size calculation was achieved using PS: Power and Sample Size Calculation Software Version 3.1.2 (Vanderbilt University, Nashville, Tennessee, USA).

**Ethical Consideration:**

This study was carried out after approval of ethical committee, Faculty of Dental Medicine, Al-Azhar University, Cairo, Boys. (NO:711/3770)

**Patient Consent:**

Consent was obtained from the children's parents.

**Preoperative Assessment:**

***A. History of the Patient:***

Complete medical and drug history as well as patient's data (name, gender and age) were collected. As regarding the medical history, all patients were free from any systemic diseases.

***B. Clinical Examination:***

- ***Extraoral examination:***

  Include examination of face and general appearance [23]

- ***Intraoral examination***

  - Soft tissue includes examination of Gingiva, tongue and floor of mouth
  - Hard tissue examination includes Teeth

**Caries risk Assessment:**

Using the American Dental Association caries risk assessment (>6).

Circle or check the boxes of the conditions that apply:

Low Risk: only conditions in "Low Risk" column present.

Moderate Risk: only conditions in "Low" and/or "Moderate Risk" columns present.

High Risk: one or more conditions in the "High Risk" column present.

**Grouping:**

Thirty-three patients between the age of 4 to 6 years were selected and divided according to ADA caries risk assessment (>6) [24], into three groups:

- Group 1: 11 Children with Low caries risk assessment.
- Group 2: 11 Children with moderate caries risk assessment.
- Group 3: 11 Children with high caries risk assessment.

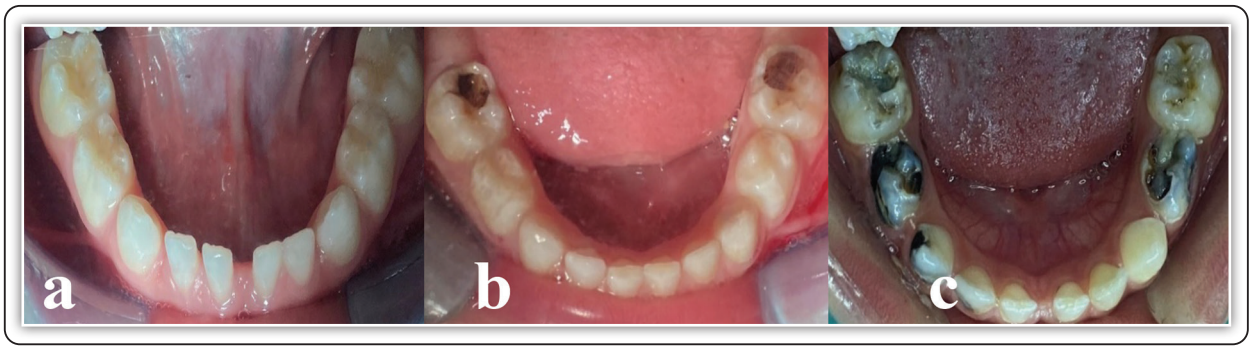


FIG (1) Different groups after caries risk assessment: a) Low caries risk assessment. b) moderate caries risk assessment. c) high caries risk assessment

**Saliva Sample:**

0.5ml of unstimulated saliva was collected by spitting method. It was collected in calibrated EPPENDORF tube 1.5ml and it should be taken to the lab within 48 hours in room temperature, or up to seven days at temperature 2-8˚C, or up to 1 month at temperature -20˚C, or up to 6 months at temperature -80˚C, with precaution of avoiding repeat freeze cycle.

**Saliva Sample Collection** [25]

Patient instructed to rinse mouth thoroughly with cold water for approximately 30 seconds.

0.5ml of unstimulated whole saliva was collected by a spitting method into calibrated EPPENDORF tube 1.5ml, which were then placed on ice box. Samples from the subjects were taken between 9:00 A.M and 11:00 A.M.

**Saliva Sample Coding:**

The EPPENDORFF tubes that contain saliva sample will divide by 3 color coding as the following:

- Green color: for the low caries risk assessment group.
- Orange color: for the moderate caries risk assessment group.
- Red color: for the High caries risk assessment group.

**Laboratory Investigation For Saliva Samples Elisa Analysis:**

The Kit used in this study (BT-Lab) Human Histatin HTN5 ELISA KIT.

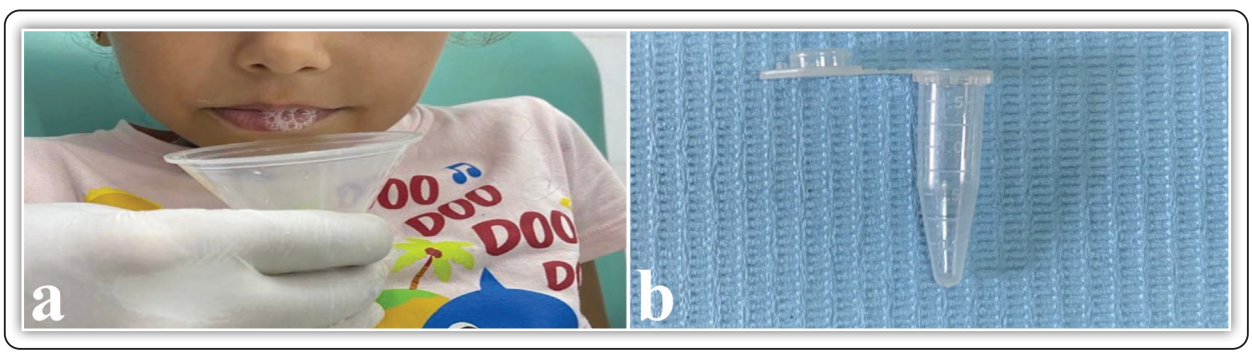


FIG (2) a) Saliva collection by Spitting method. b) Calibrated EPPENDORF Tube

***Composed of:***

(Standard Solution, Pre-coated ELISA Plate, Standard Diluent, Streptavidin-HRP, Stop Solution, Substrate Solution A&B, Wash Buffer Concentrate and Biotinylated Human Antibody).

The ELISA is a sensitive and specific analytic biochemistry assay utilized for detection and quantitative or qualitative analysis of an analyte without the requirement of sophisticated or expensive equipment [26].

**Steps of Elisa Analysis:**

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human HTN5 antibody.

1. HTN5 present in the sample is added and binds to antibodies coated on the wells.
2. Biotinylated Human HTN5 Antibody is added and binds to HTN5 in the sample.
3. Then Streptavidin-HRP is added and binds to the Biotinylated HTN5 antibody.
4. After incubation unbound Streptavidin-HRP is washed away during a washing step.
5. Substrate solution is then added and color develops in proportion to the amount of Human HTN5.
6. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450nm.

**Statistical analysis of the data:**

Data were analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 24 (SPSS Inc., Chicago, IL). Numerical data were described as mean and standard deviation or median and range. Data were explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Comparisons between 4 groups for normally distributed numeric variables were done using the ANOVA while for non-normally distributed numeric variables were done by Kruskal Wallis test. A p-value less than or equal to 0.05 was considered statistically significant. All tests were two tailed. Categorical data were described as numbers and percentages and comparisons were done by chi square test or fisher exact as appropriate.

**RESULTS**

**Demographic Data:**

Thirty-three patients were participated in this study were divided equally into three groups (11 patients in each one); Group I: Low risk (Control group), Group II: Moderate risk, Group III: High risk.

**Age and Gender distribution in both groups:**

The mean age of patients in Group (I) was 4.8±0.5 years and range (4-5.5) while in Group (II) and (III) was 5.1±0.4 years and range (4.5-5.5). There was no statistically significant difference between mean age values between the three groups (p=0.139).

Gender distribution in Group (I) involved 6 males and 5 females while in Group (II) involved 5 males and 6 females and involved 4 males and 7 females in Group (III). There was no significant difference between the studied groups for gender (p=0.693).

**Microbiology outcome:**

Data for Histatin antibodies and levels represented in table (1).

**Histatin levels:**

The mean level in low-risk group was 9.7±2.4 with range 7 to 14 while it was 21.8±3.7 with range 15.2 to 27 and 33.8±4.9 with range 26.5 to 42.9 in moderate and high-risk group respectively. This was statistically significant with p<0.001; pairwise comparison revealed that all groups are statistically significant being higher in high-risk group.

**TABLE (1)** Histatin antibodies and levels in different risk groups

| | | N | Mean | SD | Median | Minimum | Maximum | P value |
|---|---|---|---|---|---|---|---|---|
| **Histatin (Ng\ml)** | Low Risk | 11 | 9.7 | 2.4 | 9.2 | 7.0 | 14.0 | <0.001* |
| | Moderate Risk | 11 | 21.8 | 3.7 | 22.8 | 15.2 | 27.0 | |
| | High Risk | 11 | 33.8 | 4.9 | 32.8 | 26.5 | 42.9 | |

*P<0.05 is statistically significant, SD: standard deviation, analysis done by ANOVA followed by Bonferroni Post hoc test, *:all pairwise comparison are statistically significant*

**Determining cut off points in the marker:**

To discriminate high risk patients from lower and moderate risk, ROC curve was used.

For Histatin a cut-off more than or equal to 25.7ng/ml had an AUC of 0.996 to detect high risk group with a sensitivity of 100% and a specificity of 95.5%, P<0.001 (Table 1).

**TABLE (2)** Best cut off points validity of Histatin with area under the ROC curve (AUC) among the studied patients

| | | | | | | | 95% CI | |
|---|---|---|---|---|---|---|---|---|
| | | Senstivity | Specficity | AUC | SE | P value | Lower | Upper |
| **Histatin** | ≥25.7 | 100% | 95.5% | 0.996 | 0.007 | <0.001 | 0.98 | 1.00 |

*AUC: area under the curve, SE:Standrd Error, CI:Confidence interval*

## DISCUSSION

One of the most common diseases affecting children worldwide is dental caries, which affects roughly 50% of them. If untreated, it can have an impact on the child's and family's quality of life as well as their speech, smile, psychosocial environment, and mastication function. Dental disorders are incredibly expensive to treat globally, but prevention is relatively easy and efficient[27].

Unstimulated saliva is a combination of fluids that enters the mouth when no external stimuli are present. It reflects the constant, roughly 24-hour flow of saliva in the mouth cavity. Unstimulated saliva is frequently preferable over stimulated whole saliva in salivary diagnostics since the latter only carries a diluted concentration of biomarkers, which may be challenging to identify [28]. The amount of hydration, posture of the body and the location of the head during collection, exposure to light, medicines, and circadian rhythm, however, have an impact on the unstimulated saliva [29]. The methods available presently for collection of whole saliva include draining, spitting, suction and swab method.

Spitting Method: Saliva is allowed to collect on the mouth's floor before being spat out into graded or pre-weighed test tubes by the participant. The benefit of this technology is that it may be applied in situations when saliva evaporation is to be kept to a minimum and flow rates are extremely low. Due to the possibility of certain stimulatory effects, it cannot be utilized for saliva collection when not being stimulated, this agree with Lee et al [30].

Without the need for complex or expensive equipment, the ELISA is a sensitive and specific analytical biochemistry assay used for analyte detection and quantitative or qualitative analysis [31]. The analyte might be any particular molecule, such as a particular protein or a more intricate combination of many proteins.

As a methodology, ELISA is based on a few important scientific advances the most important of which is the production of antigen Specific antibodies either monoclonal or polyclonal. The development of radioimmunoassay methods has also been a major step forward. A protein may be indirectly quantified using this method by detecting the radioactivity of the detection antibodies, which can be tagged with radioisotopes. Alternately, the signal generated while using the proper substrate can be measured using antibodies that are chemically attached to biological enzymes. This method is known as indirect quantification, this in agreement with Sakai et al [26].

In comparison between three groups according levels of Histatin, results showed significant increased levels of Histatin in high-risk group and moderate risk group in comparison with low-risk group but higher in high-risk group.

This agree with Gornowicz et al [32] in which thirty-five adolescents (age 18 years) from a high school were included, divided into 2 groups. Group I was composed of 8 adolescents with DMF=3 (low intensity of dental caries) and Group II was 27 adolescents with DMF>11 (high intensity of dental caries) established the child's caries diagnosis as per caries intensity index DMF (decayed/missing/filled; D+M+F/number of the examined) which they found that histatin high in group II.

Author suggests that HST destabilize cellular membrane of bacteria by assimilating with its surface leading to cell damage. And HST decrease outflow of proinflammatory cytokines (interleukins, TNFα, metabolites of arachidonic and other acids) as a response of organism to stimulation of cell walls of Gram-negative bacteria with lipopolysaccharide for this hst consider as markers of dental caries progression at various stages of its development [33].

This is study assessed levels of Histatin as biomarkers for caries risk

## CONCLUSION

Based on the results of this study, it could be concluded that; antimicrobial peptides histatin may be used as good biomarkers for caries risk assessment.

## REFERENCES

1. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet. 2007; 369:51–9.
2. Abanto J, Tsakos G, Paiva SM, Carvalho TS, Raggio DP, Bönecker M, et al. Impact of dental caries and trauma on quality of life among 5 to 6 year-old children: perceptions of parents and children.Community Dent Oral Epidemiology.2014:1-10.
3. Scarpelli AC, Paiva SM, Viegas CM, Carvalho AC, Ferreira FM, Pordeus IAm et al. Oral health-related quality of life among Brazilian preschool children. Community Dent Oral Epidemiol. 2013; 41(4):336-44.
4. Castilho AR, Mialhe FL, Barbosa TS, Puppin-Rontani RM. Influence of family environment on children's oral health: a systematic review. J Pediatr. 2013; 89(2):116-23.
5. Corrêa-Faria P, Paixão-Gonçalves S, Paiva SM, Pordeus IA, Marques LS, Ramos-Jorge ML, et al. Association between developmental defects of enamel and early childhood caries: a cross-sectional study. Int J Paediatr Dent. 2015; 25(2):103-9.
6. Leong PM, Gussy MG, Barrow S-Y, Silva-Sanigorski A, Waters E. A systematic review of risk factors during first year of life for early childhood caries. Int J Paediatr Dent. 2013; 23(4):235-50.
7. Azevedo MS, Romano AR, Santos IS, Cenci MS. Knowledge and beliefs concerning early childhood caries from mothers of children ages zero to 12 months. PediatrDent. 2014; 36(3):95-9.
8. Twetman S, Fontana M, Featherstone JD. Risk assessment can we achieve consensus. Community Dent Oral Epidemiol. 2013; 41:64–70.

9. Lawrence HP. Salivary markers of systemic disease: non-invasive diagnosis of disease and monitoring of general health. J Can Dent Assoc. 2002; 68(3):170–174.
10. Kaufman E, Lamster IB. The diagnostic applications of saliva a review. Crit Rev Oral Biol Med. 2002; 13(2):197–212.
11. Chiappin S, Antonelli G, Gatti R. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. Clin Chim Acta. 2007; 383(1-2): 30–40.
12. Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries: role of saliva and dental plaque in the dynamic process of demineralization and remineralization (part 1). J Clin Pediatr Dent. 2003; 28(1): 47–52.
13. Dawes C. Salivary flow patterns and the health of hard and soft oral tissues. JADA. 2008; 139:18–24.
14. Scarano E, Fiorita A, Picciotti PM. Proteomics of saliva: personal experience. Acta Otorhinolaryngol Ital. 2010; 30(3): 125–130.
15. Dawes C, Pedersen AM, Villa A et al. The functions of human saliva: a review sponsored by the World Workshop on Oral Medicine VI. Arch Oral Biol. 2015; 60(6):863–874.
16. Amerongen VN, Bolscher JG, Veerman EC. Salivary proteins: protective and diagnostic value in cariology. Caries Res. 2004; 38(3):247–53.
17. Schipper R, Loof A, de Groot J. SELDI-TOF-MS of saliva: methodology and pretreatment effects. J Chromatogr. 2007; 847(1):45–53.
18. Oppenheim FG, Salih E, Siqueira WL. Salivary proteome and its genetic polymorphisms. Ann NY Acad Sci. 2007; 1098:22–50.
19. Van TH, Simoons I, Nieuw A. Synthetic histatin analogues with broad-spectrum antimicrobial activity. Biochem J. 1997; 326:39–45.
20. Wolff A, Dawes C, Pedersen AM, Villa A, Ekstro MJ, et al. A guide to medications inducing salivary gland dysfunction, xerostomia and subjective sialorrhea: a systematic review sponsored by the world workshop on oral medicine. Drugs R D. 2016; 10: 40-153.
21. ADA. Caries Risk Assessment Form (Age>6). American Dental Association. 2011.
22. Hemadi AS, Huang R, Zhou Y, Zou J. Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. Int J Oral Sci. 2017; 9(11):1
23. DEAN, Jeffrey A. McDonald and Avery's dentistry for the child and adolescent-E-book (10th ed). Elsevier Health Sciences. 2021: p6.
24. ADA. Caries Risk Assessment Form (Age>6). American Dental Association. 2011.
25. Han P, Ivanovski S. Effect of Saliva Collection Methods on the Detection of Periodontium-Related Genetic and Epigenetic Biomarkers. Ds Int J Mol Sci. 2019; 20: 4729.
26. Sakai S, Adachi R, Akiyama H, Teshima R. Validation of quantitative and qualitative methods for detecting allergenic ingredients in processed foods in Japan. J Agric Food Chem. 2013;61(24):5675–80.
27. Mathur VP, Dhillon JK. Dental caries: a disease which needs attention. Indian J Pediatr. 2018; 85(3):202–6.
28. Malamud D. Saliva as diagnostic fluid. Br Med J. 1992; 305: 207-218.
29. Priya Y, Prathibha M. Methods of collection of saliva - a review. International Journal of Oral Health Dentistry. 2017;3:149-50
30. Zimmermann BG, Park NJ, Wong DT. Genomic targets in saliva. Ann N Y Acad Sci. 2007;1098:184-91.
31. Fuller HR, Goodwin PR, Morris GE, An enzyme-linked immunosorbent assay (ELISA) for the major crustacean allergen, tropomyosin, in food. J Food Agric Immunol. 2007;17:43–52.
32. Gornowicz A, Tokajuk G, Bielawska A, Maciorkowska E, Jablonski R, Wojcicka A, Bielawski K. The assessment of sIgA, histatin-5, and lactoperoxidase levels in saliva of adolescents with dental caries. Med Sci Monit. 2014; 20: 1095-1100.
33. Gao X, Jiang S, Koh D, Hsu CYS. Salivary biomarkers for dental caries. Periodontology. 2016; 70(1): 128-41.