International Journal of Dental Research and Allied Sciences
2025, Volume 5, Issue 1, Page No: 35-41

Available online at: www.tsdp.net

ISSN: 3062-3502

Original Article

Utility of Quick Salivary C-Reactive Protein Assays in Identifying Early Oral Malignancy and Supporting Oral Cancer Detection

Yi You Huang[1], Jie-Ru You[2], Pei Chen Lin[3]

[1] Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan.
[2] Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan.
[3] Department of Otolaryngology-Head and Neck Surgery, Chang Gung Memorial Hospital, Linkou, Taiwan.

***E-mail** ✉ Peichenlin1985@yahoo.com

Received: 21 November 2024; **Revised:** 28 February 2025; **Accepted:** 01 March 2025

ABSTRACT

This research aimed to evaluate the effectiveness of a rapid, chairside salivary C-reactive protein (CRP) detection kit in distinguishing oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma from clinically normal oral mucosa, utilizing whole saliva samples. Unstimulated saliva was collected from individuals with OPMDs or oral cancer and from systemically healthy participants as controls. CRP concentrations were quantified using a newly developed colorimetric rapid assay. Statistical comparisons of the mean ± SD between study groups were made using Kruskal–Wallis ANOVA followed by Dunn's post hoc test. Receiver operating characteristic (ROC) analysis was conducted to determine the positive and negative likelihood ratios. The mean salivary CRP levels measured were 4.21 ng/mL for the oral cancer group, 2.51 ng/mL for the OPMD group, and 0.7 ng/mL for the control group. Post hoc comparisons confirmed significant elevation of CRP in both oral cancer and OPMD cases compared to healthy controls. The findings demonstrate that the novel rapid colorimetric assay effectively differentiates patients with oral lesions from healthy individuals, showing high sensitivity and potential utility as a screening tool. However, no statistically significant difference was observed between the OPMD and malignancy subgroups.

Keywords: Oral cancer, C-reactive protein, Oral potentially malignant disorders, Saliva, Biomarker

How to Cite This Article: Huang YY, You JR, Lin PC. Utility of Quick Salivary C-Reactive Protein Assays in Identifying Early Oral Malignancy and Supporting Oral Cancer Detection. Int J Dent Res Allied Sci. 2025;5(1):35-41. https://doi.org/10.51847/r3lrZGW8Wn

Introduction

Oral cancer ranks as the sixth most prevalent malignancy worldwide, associated with substantial morbidity and mortality. Despite advancements in therapeutic approaches, survival rates remain unsatisfactory, primarily due to the late-stage detection of disease—over two-thirds of patients are diagnosed only when the cancer has significantly progressed [1].

Most oral cancers develop from precursor lesions, collectively termed oral potentially malignant disorders (OPMDs), which clinically appear as white or red plaques or ulcerative lesions of the mucosa. Detecting and treating these disorders at an early stage can prevent malignant transformation in nearly 88% of cases, thereby improving prognosis and survival outcomes [2–4]. Consequently, the search for cost-efficient and accurate diagnostic markers capable of identifying early pathological changes is of paramount importance.

A strong link between chronic inflammation and tumorigenesis has been well established. Persistent inflammatory activity contributes to tumor initiation, promotion, and progression, enhancing overall cancer risk [5]. With recent advances in genomic and proteomic research, numerous biomarkers have been identified in various biological fluids—including

blood, saliva, urine, and cerebrospinal fluid—for the diagnosis and monitoring of diseases such as malignancies, autoimmune disorders, and neurodegenerative conditions [6].

C-reactive protein (CRP), first described by Tillett and Francis (1930), is a well-recognized acute-phase reactant routinely measured in serum to assess inflammatory and infectious processes. CRP facilitates innate immune defense by binding to damaged cells or foreign antigens and initiating complement activation [7]. Since serum CRP levels rise predictably following inflammatory stimuli, they are widely used in clinical diagnostics [8]. Moreover, increased CRP has been proposed as a non-invasive biomarker for detecting malignant changes [5].

While cytokines such as interleukins often show poor correlation between serum and saliva, studies indicate that salivary CRP concentrations closely reflect systemic levels [9]. Elevated CRP has been consistently reported in patients with advanced oral cancers and OPMDs. However, traditional laboratory assays for CRP are typically labor-intensive, time-consuming, and often yield results after patients leave clinical facilities [5, 10].

In contrast, salivary diagnostics provide a non-invasive, painless, and easily repeatable alternative to serum analysis. The integration of biosensor-based technologies, such as paper microfluidic systems, has enabled the creation of rapid, portable, and user-friendly diagnostic platforms suitable for point-of-care applications [11, 12].

Accordingly, this study sought to evaluate the diagnostic efficiency of a chairside colorimetric rapid salivary CRP detection kit (SpotSense, Spot Healthcare Pvt. Ltd., Jharkhand, India) in differentiating OPMDs and oral cancer from healthy mucosa using whole saliva samples. To the best of our knowledge, this represents the first investigation employing a chairside CRP colorimetric assay for the diagnostic assessment of oral premalignant and malignant lesions.

Methodology

This investigation took place in the Department of Oral Medicine and Radiology, Manipal College of Dental Sciences, Manipal, in association with Spot Healthcare Pvt. Ltd. It followed a cross-sectional diagnostic design aimed at assessing test accuracy and was implemented from May 2023 to December 2023 in compliance with STARD guidelines. Ethical clearance was secured from the Institutional Ethics Committee, Kasturba Medical College and Hospital (Approval No: 351/2022), and the work adhered to the Helsinki Declaration (2013 amendment). The study protocol was also registered with the Clinical Trial Registry of India (ID: CTRI/2023/05/067894).

Estimation of sample size

The sample size was determined using G*Power software (v3). Using a large effect size (0.4) derived from a pilot run on 20 specimens for feasibility, and setting the power at 90% with a confidence level of 95%, calculations indicated a need for at least 84 samples (28 per category). An additional 20% recruitment margin was applied to account for later confirmation through histopathological evaluation.

Inclusion and exclusion framework

All individuals attending the Oral Medicine and Radiology outpatient services were screened using predefined inclusion and exclusion measures.

Inclusion parameters

Case participants

Subjects clinically diagnosed with oral potentially malignant disorders (OPMDs) or oral carcinoma were included.

OPMD subgroup

a. Lesions identified clinically as leukoplakia, non-homogeneous leukoplakia–erythroplakia, with histopathological evidence of hyperkeratosis or parakeratosis, showing acanthosis, atrophic or inflammatory features, with or without dysplasia.

b. Confirmed cases of oral submucous fibrosis (OSMF).

c. Erosive oral lichen planus, validated both clinically and histologically.

Oral cancer subgroup

a. Oral squamous cell carcinoma confirmed by biopsy.

b. Histologically proven carcinoma in situ or invasive carcinoma.

Control group

Healthy volunteers from the same clinical setting were included if they met these requirements:

a. No use of tobacco, smoking, or alcohol.

b. Free from systemic diseases and not on long-term medication.

c. Healthy oral mucosa without inflammation, ulcers, or gingival infection and maintained oral hygiene.

Exclusion parameters

Participants were excluded if:

a. They had any systemic illness or chronic disorder.

b. Were pregnant, lactating, or on medications that could affect CRP concentrations.
c. Were undergoing chemo- or radiotherapy, or had oral ulcerations or inflammatory lesions at the time of sampling.
A standardized data form was utilized to document demographic and clinical findings, supervised by investigators KS and VP, ensuring accuracy and completeness.

Saliva collection and handling
Samples were gathered under uniform morning conditions (around 10 a.m.) using the spit collection approach. Subjects were seated upright, and any removable prosthesis or denture was taken out prior to collection. Around 2–3 mL of unstimulated whole saliva was collected in sterile Eppendorf microtubes. Samples were tested immediately without refrigeration, centrifugation, or pre-processing to maintain consistency.

Assessment of salivary CRP
CRP concentrations were determined via a quantitative colorimetric rapid immunoassay (SpotSense salivary CRP rapid test kit). This immunochromatographic system, currently in a pre-commercial phase, was initially standardized for neonatal salivary analysis [13].
Approximately 20 µL of freshly collected saliva was placed into the sample port, followed by two drops of a saline-based buffer to promote even capillary movement of the specimen across the membrane. The test result was read after 20 minutes. The appearance of both a control (C) line and a test (T) line confirmed the test's validity and presence of CRP in the specimen, respectively **(Figure 1)**.

Figure 1. Tools and devices used in the study, including the saliva collection apparatus**,** buffer reagent**,** SpotSense CRP rapid testing device, and VIEWDx analyzer for quantitative evaluation

Once the 20-minute reaction period ended, readings were obtained through a dedicated analyzer, which quantified the C-reactive protein (CRP) concentration in each saliva specimen.
All measurements were recorded in Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and subsequently processed using SPSS version 20 (IBM, Armonk, NY, USA) for statistical interpretation.
Group differences in mean and standard deviation values were determined using a Kruskal–Wallis rank-based test, followed by Dunn's post-test for multiple comparisons.
Diagnostic ability was assessed through receiver operating characteristic (ROC) analysis to compute sensitivity, specificity, and likelihood ratios (positive and negative) for detecting disease from salivary CRP levels.
A multinomial logistic regression model was further applied to examine the predictive influence of salivary CRP on the outcome variables (oral cancer or OPMDs).

Results

A total of 109 participants were included. The distribution of age and sex is summarized in **Table 1**.
Average ages were 53.50 years for individuals with oral cancer, 44.08 years for those with OPMDs, and 50.33 years in the healthy cohort.
The dataset comprised 29 histologically confirmed oral squamous cell carcinoma cases, 40 biopsy-verified OPMDs, and 40 control samples (**Table 2**).
Oral cancer lesions were most frequent in the buccal mucosa (13), followed by alveolar ridge (8), tongue (7), and alveolus + maxillary sinus (1).

Table 1. Age classification of study participants

Study Groups	Oral Malignancy (n = 29)	Precancerous Lesions (n = 40)	Healthy Controls (n = 40)
	Average	Std. Dev.	Average
Age	53.50	12.49	44.08
Gender Breakdown	Count	Percent	Count
Women	3	10.3%	7
Men	26	89.7%	33

Table 2. Median and interquartile distribution of salivary CRP concentrations

Group	Average Salivary	Std. Dev.	Middle Value	25th Percentile	75th Percentile
Oral Malignancy	4.21	7.05	1.20	0.36	4.19
Precancerous Lesions	2.51	3.19	0.68	0.41	5.99
Healthy Controls	0.70	1.20	0.31	0.25	0.64

Within the 29 malignant cases, two involved oral submucous fibrosis (OSMF) that had undergone malignant conversion (staged clinically as T3N0Mx and T3N0M0, histopathologically as T4aN2bM0 and T4aN0M0).

Another four subjects clinically labeled as leukoplakia were re-categorized as carcinoma after biopsy confirmation.

The remaining 23 were oral squamous cell carcinoma, of which 4 were stage III and 19 were stage IV.

Average salivary CRP concentrations were as follows:

- **Oral cancer** = **4.21 ng/mL** (SD = 7.05; median = 1.20; IQR = 0.36–4.19)
- **OPMD** = **2.51 ng/mL** (SD = 3.19; median = 0.68; IQR = 0.41–5.99)
- **Control** = **0.7 ng/mL** (SD = 1.20; median = 0.31; IQR = 0.25–0.64)

(**Tables 2 and 3**) show these distributions.

Statistical comparison identified a significant intergroup difference ($p < 0.001$).

Table 3. Mean salivary CRP levels among the study groups

Cohort	Average CRP Level	Standard Deviation	Participants	Average CRP Level (ng/mL)	Standard Deviation	Participants	Average CRP Level (ng/mL)	Standard Deviation	Participants	Significance Level	Follow-Up Comparison
Oral Malignancy	4.21	7.05	29							<0.001; Significant	Oral Malignancy, Precancerous Conditions > Healthy Cohort
Precancerous Conditions				2.51	3.19	40					
Healthy Cohort							0.7	1.2	40		

Further analysis revealed that both oral cancer and OPMD groups exhibited elevated CRP relative to controls ($p = 0.001$ and $p = 0.01$, respectively).

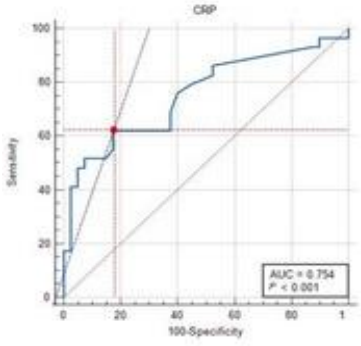
However, the two disease groups showed no measurable difference from one another ($p \geq 0.99$).

For oral cancer vs. control, sensitivity was 62.07%, specificity 82.5%, yielding +LR = 3.55 and −LR = 0.46; the AUC = 0.75 and Youden index = 0.45.

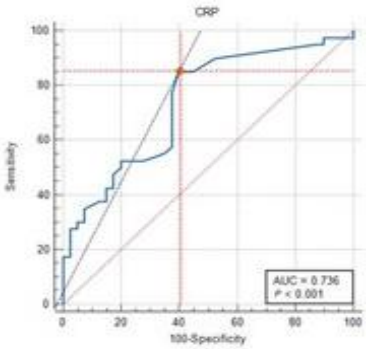
For OPMDs vs. control, sensitivity = 85%, specificity = 60%, +LR = 2.12, −LR = 0.25, AUC = 0.74, Youden = 0.45.

When both diseased groups were merged and compared to controls, sensitivity = 81.16%, specificity = 60%, +LR = 2.03, −LR = 0.31, AUC = 0.74, Youden = 0.41.

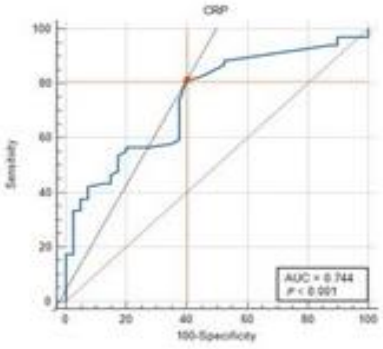
The ROC curves illustrating these findings are presented in **Figure 2**.



a. Oral Cancer vs Control



b. OPMD vs Controls



c. Cancer+OPMD vs Controls

Figure 2. (a) ROC differentiating oral cancer vs. control. (b) ROC differentiating OPMDs vs. control. (c) ROC for combined cancer + OPMD vs. control.

Table 4. ROC summary for diagnostic potential of salivary CRP in cancer, OPMDs, and combined conditions compared with healthy subjects

Cohort	ROC Curve Area (95% CI)	Diagnostic Index	True Positive Rate	True Negative Rate	Predictive Accuracy (Positive)	Predictive Accuracy (Negative)	CRP Cutoff Value	Likelihood Ratio (Positive)	Likelihood Ratio (Negative)
Oral Tumor	0.75 (0.64–0.85)	0.45	62.07	82.5	72%	75%	>0.69	3.55	0.46
Pre-Malignant Lesions	0.74 (0.63–0.83)	0.45	85	60	68%	80%	>0.33	2.12	0.25
Oral Tumor + Pre-Malignant Lesions	0.74 (0.65–0.82)	0.41	81.16	60	77.78%	64.86%	>0.33	2.03	0.31

Abbreviations: **PPV** = positive predictive value; **NPV** = negative predictive value; **AUC** = area under curve; **CI** = confidence interval; **+LR/−LR** = positive/negative likelihood ratio; **OPMDs** = oral potentially malignant disorders.

All AUC values exceeded 0.7, signifying acceptable discriminatory strength. Although positive likelihood ratios remained modest—indicating limited confirmation ability—the negative likelihood ratios demonstrated that these CRP thresholds could help exclude disease.

The multinomial regression model, adjusted for age and sex, indicated significant relationships between elevated CRP levels and disease categories (**Table 5**). Compared with healthy subjects, the odds of oral cancer were 1.77 times higher, and the odds for OPMDs were 1.67 times higher.

Because a majority of affected individuals were male—consistent with known behavioral risk factors—residual confounding by sex was likely present.

Table 5. Multinomial regression evaluating salivary CRP as a predictor of oral cancer and OPMDs, controlling for age and sex

Cohort	Variable	Significance (p-Value)	Odds Ratio (95% CI)
Oral Tumor	Constant	0.003	
	Salivary CRP	0.001	1.77 (1.25–2.5)
	Age	0.253	1.03 (0.98–1.08)
	Male Gender	0.001	14.78 (2.89–75.57)
Pre-Malignant Lesions	Constant	0.986	
	Salivary CRP	0.003	1.67 (1.19–2.36)
	Age	0.068	0.96 (0.92–1)
	Male Gender	0.007	5.19 (1.55–17.31)

Discussion

C-reactive protein (CRP) is a homopolymeric molecule synthesized by a variety of cell types, including hepatocytes, macrophages, endothelial cells, lymphocytes, and smooth muscle cells. CRP binds to compromised cell membranes, which results in an increase in serum levels during acute or chronic inflammatory states, such as malignancies and systemic autoimmune disorders. Inflammatory mediators and cytokines can influence tumor progression, growth, and immune modulation, with interleukin-1 and interleukin-6 implicated in tumorigenesis. This underscores a systemic relationship between persistent inflammation and cancer development. CRP has been established as a reliable inflammatory biomarker and is widely used in clinical practice for monitoring autoimmune conditions and visceral malignancies [6].

The salivary CRP kit employed in this study is a bedside, colorimetric lateral flow assay capable of quantitatively measuring CRP in whole saliva. It has been previously used to detect systemic and oral inflammation in both neonates and adults. The earliest study using this kit focused on predicting culture-confirmed neonatal sepsis, measuring CRP in both saliva and serum. Results demonstrated promising predictive capability, with salivary CRP cut-off values comparable to serum CRP levels [13].

Our investigation represents the second study using this innovative kit and the first application in OPMDs and oral cancer patients. The goal was to evaluate the effectiveness of the quantitative, colorimetric rapid

assay in differentiating salivary CRP in OPMD and oral malignancy patients from healthy individuals. Study results revealed significantly elevated salivary CRP levels in the OPMD group (mean = 2.51 ng/mL) and oral cancer group (mean = 4.21 ng/mL) compared to controls. The highest levels were observed in the oral cancer cohort.

Previous studies provide context for our findings. Kaur M U *et al.* reported mean salivary CRP values of 7.31 ± 3.34 mg/L in leukoplakia patients and 5.92 ± 2.76 mg/L in OSMF [10]. Similarly, Gosavi and Torkadi evaluated serum CRP in 150 participants (50 OSMF, 50 oral squamous cell carcinoma, 50 healthy) and found mean values of 5.40 mg/L in OSMF and 12.17 mg/L in carcinoma patients [14]. These trends align with our observations, showing increased CRP levels in OPMDs and malignancies versus healthy controls [10, 15]. However, direct comparison is limited due to differences in assay methodology and analytical principles. Additionally, Jablonska *et al.* demonstrated a significant correlation between serum CRP and clinical tumor stage, with higher levels in advanced oral squamous cell carcinoma, indicating tumor progression and poor prognosis [15-17]. Further longitudinal studies with larger sample sizes are needed to confirm the utility of this test for early detection of malignant transformation.

The importance of a rapid, non-invasive salivary test is highlighted by patient preference; surveys indicate that individuals are more likely to accept saliva-based screening over invasive blood sampling [12]. CRP has already been validated as a diagnostic marker in various visceral malignancies [18], and its salivary application for oral cancer has been explored by Brailo *et al.* [17]; Katakura *et al.* [18] and Sato *et al.* [19]. However, ours is the first study to utilize a chair-side rapid CRP assay.

Our data determined a diagnostic cut-off of >0.67 ng/mL for distinguishing OPMD + cancer from healthy controls, and >0.64 ng/mL for separating oral cancer from controls. While previous research has reported elevated salivary CRP in OPMDs and malignancy, none have established a diagnostic threshold. Using this cut-off, our assay achieved 81.16% sensitivity, supporting its potential as a chair-side screening tool.

Some limitations should be acknowledged. First, we did not compare salivary and serum CRP, as serum collection is invasive and has lower patient compliance. Second, the sample distribution was unequal, with more males than females, reflecting the higher prevalence of habits such as gutka, areca nut, and tobacco consumption in males in our region. Future studies should aim for balanced gender representation to reduce bias and enhance generalizability. Histopathology served as the gold standard for diagnosis, but long-term, larger cohort studies and direct comparisons with serum CRP would further validate the kit's utility for early diagnosis.

Conclusions

This study demonstrates that the rapid salivary CRP test kit effectively distinguishes healthy controls from OPMD and oral cancer patients, although it could not differentiate OPMD from malignancy based on CRP levels alone. The assay exhibited high sensitivity, indicating its potential as a screening tool. Future research with larger sample sizes is needed to define a biological cut-off capable of separating OPMDs from cancer. Additionally, studies exploring diurnal variations, tumor stage differences, and post-treatment prognosis may further elucidate its clinical applicability. This novel assay also holds promise as an educational tool for high-risk individuals, particularly tobacco users, for early identification of OPMDs and oral cancer.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Sharma D, Kudva V, Patil V, Kudva A, Bhat RS. A convolutional neural network based deep learning algorithm for identification of oral precancerous and cancerous lesion and differentiation from normal mucosa: A retrospective study. Eng Sci. 2022;18:278–87.
2. Welikala RA, Remagnino P, Lim JH, Chan CS, Rajendran S, Kallarakkal TG, et al. Automated detection and classification of oral lesions using deep learning for early detection of oral cancer. IEEE Access. 2020;8:132677–93.
3. Warnakulasuriya S, Johnson NW, Van Der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007;36:575–80.
4. Mahmood H, Shaban M, Indave BI, Santos-Silva AR, Rajpoot N, Khurram SA. Use of artificial intelligence in diagnosis of head and neck precancerous and cancerous lesions: A systematic review. Oral Oncol. 2020;110:104885.

5. Metgud R, Bajaj S. Altered serum and salivary C-reactive protein levels in patients with oral premalignant lesions and oral squamous cell carcinoma. Biotech Histochem. 2016;91:96–101.
6. Pay JB, Shaw AM. Towards salivary C-reactive protein as a viable biomarker of systemic inflammation. Clin Biochem. 2019;68:1–8.
7. Desai GS, Mathews ST. Saliva as a non-invasive diagnostic tool for inflammation and insulin-resistance. World J Diabetes. 2014;5:730.
8. Out D, Hall RJ, Granger DA, Page GG, Woods SJ. Assessing salivary C-reactive protein: Longitudinal associations with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. Brain Behav Immun. 2012;26:543–51.
9. Izawa S, Miki K, Liu X, Ogawa N. The diurnal patterns of salivary interleukin-6 and C-reactive protein in healthy young adults. Brain Behav Immun. 2013;27:38–41.
10. Uppal MK, Iyengar AR, Subash BV, Patil S, Sharma ML, Thakar S. Estimation and correlation of serum and salivary C-reactive protein in oral potentially malignant disorders. J Indian Acad Oral Med Radiol. 2021;33:47–52.
11. Davidson JL, Wang J, Maruthamuthu MK, Dextre A, Pascual-Garrigos A, Mohan S, et al. A paper-based colorimetric molecular test for SARS-CoV-2 in saliva. Biosens Bioelectron X. 2021;9:100076.
12. Narasimhan A, Jain H, Muniandy K, Chinnappan R, Mani NK. Bio-analysis of saliva using paper devices and colorimetric assays. J Anal Test. 2024;8:114–32.
13. Ramavath C, Katam SK, Vardhelli V, Deshabhotla S, Oleti TP. Examining the utility of rapid salivary C-reactive protein as a predictor for neonatal sepsis: An analytical cross-sectional pilot study. Diagnostics. 2023;13:867.
14. Gosavi SR, Torkadi AA. Serum C-reactive protein in oral submucous fibrosis and oral squamous cell carcinoma: A cross-sectional study. J Oral Maxillofac Pathol. 2020;24:46–51.
15. Jablonska E, Piotrowski L, Grabowska Z. Serum levels of IL-lβ, IL-6, TNF-α, sTNF-RI and CRP in patients with oral cavity cancer. Pathol Oncol Res. 1997;3:126–9.
16. Khandavilli SD, Ceallaigh PO, Lloyd CJ, Whitaker R. Serum C-reactive protein as a prognostic indicator in patients with oral squamous cell carcinoma. Oral Oncol. 2009;45:912–4.
17. Brailo V, Vucicevic-Boras V, Lukac J, Biocina-Lukenda D, Zilic-Alajbeg I, Milenovic A, et al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral Cir Bucal. 2012;17:e10–5.
18. Katakura A, Yamamoto N, Sakuma T, Sugahara K, Onda T, Noguchi S, et al. A screening test for oral cancer using saliva samples: Proteomic analysis of biomarkers in whole saliva. J Oral Maxillofac Surg Med Pathol. 2015;27:1–5.
19. Sato J, Goto J, Murata T, Kitamori S, Yamazaki Y, Satoh A, et al. Changes in saliva interleukin-6 levels in patients with oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 2010;110:330–6.