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Review Article

Prognostic value of molecular markers of oral pre-malignant and malignant lesions

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ABSTRACT

Background: *The representation of oral cancer and precancerous lesions is often undetected until at later stage and the survival rate of oral cancer has remained essentially unchanged over the past three decades. Over 90% of these tumors are squamous cell carcinoma. The American Cancer Society estimates that among 28,900 new cases of oral diagnosis in 2002, nearly 7,400 people will die from this disease. Oral pre-malignant and malignant lesions have multi-step process both at phenotype and genetic levels that influence tumor behavior and genetic mutations.* ***Purpose:*** *The aim of this presentation was to review the current knowledge of prognostic value of tumor marker in order to achieve early detection, prognostic value, proper and accurate treatment of oral cancer.* ***Reviews:*** *Technological advances in molecular biology have greatly increased the number of new molecular markers that can be detected by molecular analysis such as immunohistochemistry (IHC), polymerase chain reaction (PCR) and surgical margin analysis that may increase prognosis and treatment of oral cancer. The result of most valuable tumor markers is twenty nine divided into four groups according to their function such as enhancement of tumor growth, tumor suppression and anti tumor defense, including immune response and apoptosis, angiogenesis, tumor invasion and metastatic potential, including adhesion molecules and matrix degradation.* ***Conclusion:*** *In general the conclusion is that the location of markers within the tumor and not the quantitative assessment is as same as emphasized. Especially, the analysis of new molecular markers have been used to be of great importance for early detection, surgical margin analysis, prognostication and treatment of oral pre-malignant and cancerous lesion.*

Key words: *tumor markers, oral pre-cancer, oral cancer, surgical margin, prognostic*

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INTRODUCTION

Oral and oro-pharynx cancers are found in 28,900 new cases and 7400 victims were dead in 2002 in United States, which 3% found on men and 2% found on women among all malignancy of the body.[1,2] It was reported that in the United States, one patient of oral cancer with oral squamous cell carcinoma dead every hour in every day, so the number of recurrences, morbidities, and mortalities tends to increase.[3] If the oral cancer is detected in the early stage, the percentage of live expectation will be about 80–90% while if detected in the advanced stage the percentage will be about 50% within five years. Unfortunately, there is still no current information about the prevalence of oral cancer from Badan Registrasi Kanker Indonesia. However, based on medical records of RSUD Dr. Sutomo Surabaya in Poli Kepala Leher RS Dr. Sutomo from 1983 to 1992, there are 3.3% patients of head and neck tumor.[4] The number of mortalities is relatively high in all parts of the world, there are 5.1% of oral cancer cases in early stage and 76.3% in advanced stage.[4] The high rate of mortality, morbidity, and the worse prognosis of oral cancer are actually the world's and clinician's problem of cancer nowadays.[4,5]

Unfortunately, the basic molecular pathogenesis of oral squamous cell carcinoma through multi-step process is still clinically not fully understood, so the early detection of oral cancer is only based on clinical diagnosis. The histopathology examination (HPA) also causes mistakes in diagnosis, recurrence, and mistakes in therapy.[4-6]

The other problem is surgical margin analysis. In this approach, in order to obtain proper surgical margin of tissue that is free of tumor by using frozen section examination microscopically, the different results between clinical diagnosis and HPA diagnosis always occur. It indicates that it does not guarantee whether it will have no tumor recurrence or will worsen oral cancer prognosis. Thus, it can increase the rate of morbidity, mortality and five years survival rate into 50%. It means that there has not been any significant progress in the last two decades.[3,6]

Actually, the new approach of oral cancer treatment has radically changed in the last two decades since there were more understanding about molecular changing in oral carcinogens through multi-step process and advancement of technology in molecular biology so the new diagnosis can be obtained through immunohistochemistry (IHC), polymerase chain reaction (PCR) and surgical margin analysis. As a result, the target of molecular markers obtained is about 29 molecular markers in patients of either pre-malignant lesion or oral carcinoma. Those molecular markers are classified into four based on the functions like: enhancement of tumor growth, including acceleration of proliferation and cell cycle; tumor suppression and anti tumor defense, including immune response and apoptosis; angiogenesis; tumor invasion and metastatic potential including adhesion molecules and matrix degradation. Besides, nowadays the surgeons, especially oral and maxillofacial surgeons tend to use surgical margin analysis to make molecular diagnosis that is by using molecular marker, p53, so that the surgical margin that is free of tumor can be determined in order not only to eliminate tumor recurrence and the rate of morbidity and mortality, but also to use as prognostic indicator and therapy of pre-malignant lesion, especially for leukoplakia and oral cancer.[3,7,8]

Epidemiology

Based on epidemiology study, leukoplakia in oral cavity, signed by white spots, is found in about 5–15%; dysplasia is found increasing about 31.4%. They are potentially considered to be oral cancer. Meanwhile, oral cancer prevalence especially oral squamous cell carcinoma in some countries including Indonesia is about 3–20% of all cancer cases.[9]

The etiology of leukoplakia pre-malignant lesion is related to tobacco use (severe smokers), betel-chewing, marijuana use in young patients, severe alcohol consumption, and candida albicans.[10] On the other hand, the etiology of oral cancer is still not identified clearly. Nevertheless, the main risk factor is multi-factorials caused by external factors like tobacco, alcohol consumption, carcinogenic materials, radiation, and virus, especially HPV 16 (about 22%) and HPV 18 (about 14 %).[11] Dietary factors like low fruit and vegetable consumption can increase the risk factor of oral cancer.[12]

The internal factors like multi-steps and complex genetic change cause the variation of clinical type oral cancer. It depends on the high risk factor in patients such as age, topography location, and race.[13,14] However, with the growth of molecular biology in the last three decades and supported by the techniques of genetic engineering, now cancer has obviously been proven that it has genetic basis. The etiologic factors of oral cancer is not only showed by the changing of molecular controlling through many lines, especially for G1-S phase of cell cycle, but also correlated with the description of phenotype, clinical description, and histopathology.[15]

Diagnosis of pre-malignant lesions and oral cancer

The description of pre-malignant lesions is related to erythroplakia and leukoplakia. Erythroplakia is divided into granular and non-granular erythroplakia. However, erythroplakia are rarely found, so pre-malignant lesions is focused on leukoplakia which is divided into 2 types, homogenous and non homogenous. It is divided again into thin leukoplakia, thick leukoplakia, granular leukoplakia, verruciform leukoplakia, verrucous proliferative leukoplakia, and speckled leukoplakia.[16] Some researchers, furthermore, point out that the frequency of dysplasia lesion or cancerous changing leukoplakia lesion is about 15.6–39.2%, showing dysplasia degree started from mild, moderate, and severe. Non homogenous type of leukoplakia like nodule, erythematous, and/or followed by verrucous components tends to be more cancerous than that from homogenous type of leukoplakia. Meanwhile, verrucous proliferative leukoplakia shows type of aggressive lesion that almost tends to be cancerous.[16] Even though the risk of non homogenous type of leukoplakia is four to five times to be malignant compared to the risk of homogenous type of leukoplakia, only 5% of cases have just been reported. Thus, leukoplakia cases considered to be malignant need more accurate alternative diagnosis than histopathology examination, an analysis of molecular marker detection either with immunohistochemical technique or with the most sophisticated molecules nowadays.[16]

The process of malignancy transformation takes a long time to detect pre- malignant tumor. Besides, there is a multi-step theory under laying the malignancy process in oral epithelium either in phenotypic level or genetic level, so it can detect malignancy stage with different differentiation degree in pre-malignant lesion and the early stage of oral cancer that is difficult to detect by clinical observation.[17]

Molecular changing in carcinogens

Cancer occurs by accumulation of genetic changing within a cell. The genetic changing shows a degree of genetic destruction which reflects a degree of tissue destruction because of the stimulus of carcinogenic materials in long term.[17]

Carcinogen of oral cancer is a multi-step process involving many genetic events so that it can change normal function of oncogene and tumor suppressor gene. Besides, this multi-step process can improve the production of growth factors or a number of nucleolus cell receptor, and then the increasing of intracellular messenger signs and production

of transcription factors. Genetic events influenced by inactivity of tumor suppressor genes, moreover, shows a capability of cell phenotype that can cause the increasing of cell proliferation signed by the lost of cell formation, infiltration in local tissue, and tumor spread to the further location from its primer tumor.[18]

Therefore, the study in other to obtain some potential molecular markers must be related with the malignancy growth like pre- malignant lesions and oral cancer, so it can predict the degree of recurrence, the cancer spreading to lymphoid gland and bone, and further metastases.[19]

Molecular marker of patients with oral cancer

Identification of molecules that can potentially do malignancy transformation shows that the increased number of molecular marker has correlated with gradation, the severity of tumor, prognosis and cause of cancer. Molecular marker for cell proliferation has been applied as molecular indicator for analyzing severity without clinically analyzing tumor description or behavior. The malignancy characters detected by the molecular marker hopefully can clinically improve the understanding of many variations of tumor description and behavior while can help the prognosis estimation of patients with oral cancer. Then, the molecular marker relating with malignancy transformation hopefully can also show the possibility of conducting therapy without any surgery. Molecular target of this treatment is achieved by conducting anti sense therapy or gene therapy. Actually, the number of the studies concerning with molecular marker is very high, but they are still for the diagnosis and therapy of oral cancer, and there is also lack of knowledge about prognosis values. The number of molecular marker in the recent studies concerning with molecular marker is temporarily about 29 molecular markers, classified into 4 groups with different functions, a) enhancement of tumor growth; b) tumor suppression and anti tumor defense; including immune response and apoptosis; c) angiogenesis; d) tumor invasion and metastasis potential; adhesion molecules and matrix degradation.[5,20]

The group of molecular markers relating with enhancement of tumor growth consists of 9 molecular markers, which are a) epithelium growing factor (EGF) and receptor of epithelium growing factor (EGFR, c-erb-1 or Her-2/neu); b) cyclin (Cyclin A, B_1,D_1 E); c) proliferation cell nucleus antigen (PCNA); d) Ki 67/ MIB; e) argyrophylic nucleolar organizer-region associated protein (AgNOR); f) skp2; g) bcl2/BAG-1; h) Heat shock protein (Hsp27 and HSP70); and i) telomerase. The gene group relating with tumor suppression and anti tumor defense consists of 7 molecular markers, which are a) Protein retinoblastoma (pRb); b) cyclin dependent kinase inhibitors (CDKIs)-p15, p16, p21, p27; c) p53; d) Bax; e) Fas/FasL; f) ζ–chain (Zeta chains); and g) dendrite cells S 100/p55. Next, the group relating with angiogenesis consists of 3 molecular markers, which are a) vascular endothelial factor/receptor (VEGF/ VEGF-R); b) Nitric oxide synthase type 2 (NOS2); c) platelet-derived endothelial cell growth factor (PD-ECGF). And, the group relating with tumor invasion and metastasis potential consists of 6 molecular markers, which are matrix-Metallo-Protease (MMPs); cathepsines; integrins; cadherins and catenins; desmoplakin/plakoglobin; and Ets-1.[7,21]

Furthermore, the characterization of molecular markers in pre-malignant lesions and oral cancer, that are increasing related with the recurrence intensity, progression, and oral cancer prognosis that has not still finished yet and has many variations of molecular marker improvement, shows that carcinogenic process with genetic changing factors that are multi-steps and complex acquiring system control of activity and molecular function through the number of arrangements for cell behavior and cell coordination within a tissue or organ with cancer.[7,21] Nevertheless, some of other researchers tend to use surgical margin analysis with PCR technique in order to determine the surgical margin that is free from tumor and not detected by HPA examination using p53 as molecular marker detector since in that case there is recurrence in local and regional tissue. In other words, the use of molecular marker, p53, and other tumor markers is to detect whether there is gene mutation as the malignancy indicator or not. Thus, the use of tumor marker p53 and other markers is can be useful as the marker for the surgical margin analysis of the pre-malignant lesions that is potential to be oral cancer. The application of molecular technique with molecular marker can also be used optimally in order to obtain the result as fast as frozen section analysis technique.[22-24]

Procedure of immunohistochemistry technique (IHC), polymerase chain reaction (PCR), and surgical margin analysis on oral pre-malignant and malignant lesions

Immunohistochemistry technique (IHC) examination on proliferated cells using proliferating cell nuclear antigene (PCNA) shows 100% cells of positive tumor. The increased PCNA expression has correlation with other proliferation markers like the percentage of S. Phase friction, Ki 67, and mitotic count. However, some of researchers do not have the same opinion because they think that PCNA is involved in the process of DNA repair for some tumors. Because of this, PCNA remains controversial and it needs to be considered as proliferation marker in particular tumors.[20,21]

Identification of immunohistochemistry on tumor marker of specimen is taken from oral mucosa swab. Specifically it is gained from cytokeratin. Profile of cytokeratin expression taken from cytology examination indicates that there is information on differentiation status of cell especially cytokeratin markers such as K8 and K19 which are useful for definitive indicators of undetected malignancy by other DNA profiles.[22] Analysis of immunohistochemistry applies proliferation index of PCNA and AgNOR on each cytology specimen to evaluate the presence of Ki-67 expression before and after radiotherapy using dosage 24 gray on 43 patients of oral squamous cell carcinoma. The result of Ki-67 expression shows that there are 10 cells of positive tumor and label index of proliferation varies from 0.1% to 0.01%. Analysis toward the number of cells and nucleus

activity (AgNOR) is applied on patients who have high risk to be infected oral cancer (severe smokers). Besides, it is reported that AgNOR analysis on cytology specimen can be used as method of regular examination to diagnose oral cancer.[23] Findings of recent molecular markers by IHC analysis on saliva indicate that Cyfra 21-1, TPS and CA 125 are significantly increased on oral cancer.[23]

Huang *et al*.[24] applies PCR technique to amplify DNA from samples of exfoliation cytology KSSRM. Furthermore restriction-fragment length polymorphism (RFLPs) analysis is applied. This analysis has found that the occurrence of *loss* of heterozygote (LOH) is 66% in one position at sequence p53. Meanwhile, another occurrence of LOH is 55% in several places. Analysis of PCR and RLFPs has been applied as marker detection of microsatellite which is like short repetition of sequence p53. Gene mutation using microsatellite and presence of LOH is alteration of characteristic molecular from carcinoma of squamous cell in such areas as head and neck. In addition, the gene mutation can also be used as molecular marker of malignancy in oral cavity. Nunes *et al*.[25] applies micro satellite analysis on samples of carcinogen of oral cavity and samples of oro-pharynx which are taken by gargling and exfoliation cytology. Since LOH as much as 84% is found by various differentiations of cell, it can be used to diagnose or observe any malignancy as early as possible.

Recently surgical margin analysis has been used by some surgeons especially specialist of oral and maxillofacial surgeon to diagnose molecular using molecular marker p53. The molecular marker p53 was firstly used to detect the surgical margin in 1995 on patients of cancer. It should be noted that the cancer is located in the head and the neck. In 2002 specialist of oral and maxillofacial surgeon found that if the HPA examination was applied, 13 of 25 patients of oral cancer were diagnosed negative to have carcinogen. In contrary, if the molecular detection p53 was applied, the result was positive. Five patients were positive toward lokoregional occurrence but they were negative if HPA examination was applied. The use of molecular marker p53 is very helpful to evaluate regular HPA examination, immunohistochemistry, and gene mutation on surgical margin analysis of patients of oral cancer and LOH as molecular marker for oral pre-malignant lesions.[26]

DISCUSSION

Data of epidemiology for pre-malignant lesion and oral cancer are needed because the prevalence of pre-malignant lesion is increased from 5-15% to 31.4% in United States.[9,10] Meanwhile, for oral cancer and oro-pharynx cases, the number of patients were increased from 28,900 in 2002 to 34,000 in 2007. There are 481,000 new cases of oral cancer every year in the world. Most of patients belong to oral squamous cell carcinoma. They die every hour and day in the United States.[3,9] This leads to high mortality. However, the latest data of epidemiology on patients of pre-malignant lesion and oral cancer can not be gained from Badan Registrasi Kanker Indonesia. The data of epidemiology contain prevalence, sex, etiology, location, clinical picture of pre-malignant lesion, oral cancer, types of leukoplakia such as thin leukoplakia, thick leukoplakia, granular leukoplakia, verruciform leukoplakia, proliferative verrucous leukoplakia, speckled leukoplakia, diffuse leukoplakia, and erythroplakia including granular and non granular erythroplakia.[16] These all are correlated with the presence of genetic factor which is multi steps and complex. The alteration of gene has caused clinical form of oral cancer vary depending on factors; age, topography, and race of patients.[13,14]

Diagnosis of pre-malignant lesion and oral cancer is still applying examination of pre-malignant lesion and oral cancer by traditional technique that is false negative and its sensitivity is low. Because the sample withdrawal is less adequate and interpretation diagnosis or procedure is subjective,[18] more sophisticated technique is needed by applying immunohistochemistry and molecular biology examination such as polymerase chain reaction (PCR).[19] On sample of cytology, particular examinations of immunohistochemistry technique are used like analysis of proliferation index, the number of keratin cell, nucleus activity with molecular marker Ki-67. Besides, these examinations are applied on patients of pre-malignant lesion and oral cancer. As consequence, they can be used as regular method of examination to diagnose cancer in oral cavity.[20,21] Identification of immunohistochemistry using marker tumor K18 and K19 on specimen from oral mucosa swab i.e. cytokeratin has been effective as definitive indicator of malignancy that can not be detected by other examinations like DNA profile.[22] The findings of recent molecular markers by IHC analysis on saliva indicate that Cyfra 21-1, TPS and CA 125 are significantly increased on oral cancer.[23]

There are 29 groups of the molecular marker. They are divided into four different groups based on their ability to control and arrange molecular. The first group is enhancement of tumor growth including the proliferation and the cell cycle. The second is tumor suppression and anti tumor defense: Immune response and apoptosis. The third is angiogenesis. The fourth is tumor invasion and metastasis potential: Adhesion molecules and matrix degradation.[7] There are 29 tumor markers of oral cancer. They are EGF, EGFR (EGFR, c-erb-1 or Her-2/neu), Cycline (Cycline A, B_1, D_1, E); PCNA,Ki 67/MIB, AgNOR, skp2, bcl2/BAG-1, HSP (Hsp27,70), telomerase, pRb, CDKIs (p15, p16, p21, p27), p53, Bax, Fas/FasL, ζ – chain (Zeta chains), S 100/p55, VEGF/VEGF-R , NOS2 , PD-ECGF, MMPs, Cathepsines; Integrins, Cadherins and catenins; Desmoplakin/ plakoglobin dan Ets-1.[7] The use of molecular marker has been very important since it could be used to detect the molecular alteration. This alteration can be seen before the morphological alteration of malignancy can be observed on clinical symptoms of pre-malignant lesion and oral cancer. In addition, it can be used to detect the

surgical margin of patients of oral cancer. If those patients are examined by HPA, the result will be negative. If they are examined by molecular detection p53, the result will be positive. The use of molecular marker p53 and other markers is effective to evaluate regular HPA examination. Besides molecular marker p53, immunohistochemistry examination and gene mutation can be applied as molecular marker on patients of oral cancer and LOH for surgical margin of pre-malignant lesion.[3,7] It is expected that the use of all examinations will continuously be applied as markers for surgical margin of pre-malignant lesion. As a result, molecular technique can be optimized to gain rapid result as same as analysis technique of frozen section. The common molecular markers which are commonly applied are PCNA, Ki 67, genetic ploidy, oncogene cmyc, gene mutation of tumor supression p53 for pre-malignant lesion and oral cancer detection with immunohistochemistry and PCR technique. These markers are applied through aneuploidy cell, cell mutation, anaplasia, cell invasion, metastasis potential.[2,3,7,8]

In conclusion, there is an increase of molecular marker either for pre-malignant lesion or oral cancer. There are 29 molecular markers for surgical margin analysis with IHC and PCR technique. The surgeon especially oral and maxillofacial surgeon tend to apply PCNA, Ki 67, AgNOR, genetic ploidy**,** oncogene cmyc especially gene p53. It is recommended that there should be date base of molecular marker which can be used as treatment standard for pre-malignant lesion and oral cancer. Later, it will be applied to reduce the prevalence, increase early detection, diagnosis, prognosis estimation with IHC and PCR technique, accurate therapy such as gene therapy. Finally, it will decrease the morbidity and mortality rate on pre-malignant lesion and oral cancer.

REFFERENCES

1. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. N Eng J Med 1993; 328 (3): 184–94.
2. Lippman SM, Hong WK. Molecular marker of the risk of oral cancer. N Engl J Med 2001; 344 (17): 1323–6.
3. Hill BR. Oral cancer foundation; histopathology, biology and markers. 2007. Available at: http:// www. oralcancerfoundation.org/about/index. htm. Accessed January 24, 2008.
4. Agus P. Analisis molekuler patogenesis karsinoma sel skuamosa rongga mulut berdasarkan pola mutasi gen p53 dan p16. Dissertation. Surabaya: Pasca Sarjana Universitas Airlangga; 2004. p. 1–177.
5. Ramli M. factors affect the treatment of oral cancer. Warta IKABI Ropanasuri 1999; 23(1): 61–70.
6. Lippman SM, Sudbo J, Hong WK. Oral cancer prevention and the evolution of molecular-targeted drug development. N Engl J Med 2001; 344(17): 1323.
7. Schliephake H**.** Prognostic relevance of molecular markers of oral cancer—A review. Int J Oral And Maxillofac Surg 2003; 32: 233–45.
8. Natkunam Y, Mason YD. Prognostic immunohistologic markers in human tumors: why are so few used in clinical practice? Laboratory Investigation 2006; 86: 742–7.
9. Sudbo J. Novel Management of oral cancer: A paradigm of predictive oncology. Clinical Medicine & Research 2004; 2(4): 233–42.
10. Lee JJ, Hong WK, Hittelman WN, Mao L, Lotan R, Shin DM, *et al*. Predicting cancer development in oral leukoplakia: Ten years of translational research. Clinical Cancer Research 2000; 6: 1702–10.
11. Sugerman PB, Shillitoe EJ. The high risk human papillomaviruses and oral cancer. Evidence for and against a causal relationship. Oral Dis 1997; 3: 145–7.
12. Winn DM, Ziegler RG, Pickle LW. Diet in the etiology of oral and pharyngeal among woman from the southern United States. Cancer Res 1984; 44: 1216–22.
13. Chen GS, Chen CH. A study on survival rates of oral squamous cell carcinoma. J Kao-Hsiung-I-Hsueh-Ko-Hsueh-Tsa-Chih 1996; 12: 317–25.
14. Todd R, Donoff RB, Wong DTW. The molecular biology of oral carcinogenesis. Toward a tumor progression model. JOMS 1997; 55: 613.
15. Suryohudoyo P. Ilmu kedokteran molekuler. Jakarta: Kapita Selekta, CV. Sagung Seto; 2000. p. 102–9.
16. Reibel J. Prognosis of oral pre-malignant lesions: Significance of clinical, histopathological, and molecular biological characteristic. Crit Rev Oral Biol Med 2003; 14(1): 47–62.
17. Williams HK**.** Molecular pathogenesis of oral squamous carcinoma. J Clin Pathol Mol Pathol 2000; 53:165–72.
18. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002; 52: 195–215.
19. Bouquot JE. Oral precancer: Dysplasia, molecular biology. Microbiology 1994. Available at: http:// www. Maxillofacialcenter.co/precancerDysplasia.html. Accessed June 1, 2009.
20. Van Diest PJ, Brugal G, Baak JP. Proliferation markers in tumours: interpretation and clinical value. J Clin Pathol 1998 October; 51(10): 716–24.
21. Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions, Mol Cancer 2006; 5: 11.
22. Ogden GR, Cowpe JG, Chisholm DM, Lane EB. DNA and keratin analysis of oral exfoliative cytology in detection of oral cancer. Oral Oncol Eur J Cancer 1994; 30B: 405–824.
23. Nagler RM, Bahar G, Shpitzer T, Feinmesser R. Concomitant analysis of salivary tumor markers—a new diagnostic tool for oral cancer, Clinical Cancer Research, 2006; 12: 3976–84.
24. Huang MF, Chang YC, Liao PS, Huang TH, Tasy CH, Chou MY. Loss of hetrozygosity of p53 gene of oral cancer detected by exfoliative cytology. Oral Oncol 1999; 35: 296–301.
25. Nunes DN, Kowalski LP, Simpson AJ. Detection of oral and oropharyngeal cancer by microsatelite analysis in mouth washes and lesion brushings. Oral Oncol 2000; 36: 525–8.
26. Eipstein BJ, Zhang L, Rosin M. Advances in the diagnosa of oral premalignant and malignant lesions. J Can Dent Assoc 2002; 689(10): 617–21.