Advances in the Diagnosis of Oral Premalignant and Malignant Lesions

• **Joel B. Epstein,** DMD, MSD, FRCD(C) •
• **Lewei Zhang,** DDS, PhD, FRCD(C) •
• **Miriam Rosin,** PhD •

Abstract

The diagnosis and treatment of oral premalignant lesions and squamous cell carcinoma are currently based on histopathologic features, site of involvement and stage of disease. Recent advances in techniques for detecting lesions and predicting their progression or recurrence are reviewed here. Adjuncts for detection of lesions and selection of biopsy sites include vital tissue staining (with toluidine blue) and exfoliative cytology. Advances in diagnosis and staging at the molecular level are expected to affect choice of treatment and patient outcomes. Oral health care providers should be aware of these advances in the evaluation and diagnosis of oral premalignant lesions and squamous cell carcinoma.

MeSH Key Words: *carcinoma, squamous cell/diagnosis; loss of heterozygosity; mouth neoplasms/genetics*

© J Can Dent Assoc 2002; 68(10):617-21
This article has been peer reviewed.

Oral squamous cell carcinoma (SCC) is the most common cancer of the head and neck. Each year it accounts for more than 300,000 cases worldwide, more than 30,000 cases in the United States and more than 3,000 cases in Canada. The 5-year survival rate for oral SCC has remained at approximately 50% for the past several decades.[1] A key factor in the lack of improvement in prognosis over the years is the fact that a significant proportion of oral SCCs are not diagnosed or treated until they reach an advanced stage. This diagnostic delay may be caused by either patients (who may not report unusual oral features) or health care workers (who may not investigate observed lesions thoroughly),[2-4] and it is presumed that such delays are longer for asymptomatic lesions. The prognosis for patients with oral SCC that is treated early is much better, with 5-year survival rates as high as 80%; in addition, quality of life improves after early treatment,[5] because cure can be achieved with less complex and less aggressive treatment than is necessary for advanced lesions. Furthermore, many oral SCCs are believed to develop from oral premalignant lesions, and early detection and diagnosis of these premalignant lesions should be possible. Identification of high-risk oral premalignant lesions and intervention at premalignant stages could constitute one of the keys to reducing the mortality, morbidity and cost of treatment associated with SCC. In addition, certain patients are known to be at high risk for head and neck cancer, specifically those who use tobacco or alcohol and those over 45 years of age. Such patients can be screened by physical examination, and early-stage disease, if detected, is curable. This paper reviews recent advances in techniques for detecting lesions early and predicting their progression or recurrence.

Molecular Change in Carcinogenesis

Several potential markers of molecular changes in oral premalignant and malignant lesions have been studied, and interest in the genetic changes associated with these lesions is increasing.[6–21] Progression from benign to malignant disease is a genetic process that later becomes evident at the cellular level (phenotypic change) and ultimately at the clinical level. Recent studies have identified a molecular (genetic) profile of risk of malignancy in premalignant oral lesions.[6–10,16–20] These studies assessed microsatellite markers (short DNA sequences repeated throughout the genome) to detect imbalance or loss of heterozygosity (LOH) in the genetic sequence of specific chromosomes in these tissues (**Figs. 1a**, **1b** and **1c**). These studies showed a

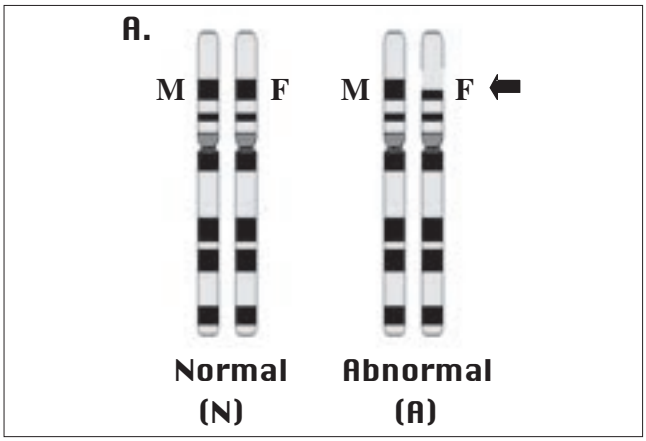

Figure 1a: *Diagrammatic representation of 2 pairs of chromosome from a patient, with alleles from both parents (M = maternal allele, F = paternal allele). One pair of the chromosome is from normal cells (N) and the other pair is from abnormal cells (A) (either dysplasia or squamous cell carcinoma) of the same patient. The chromosome pair from the abnormal cells shows a loss of a chromosome region (loss of heterozygosity, LOH) that contains tumor suppressor genes on the paternal allele (F) (see arrow).*

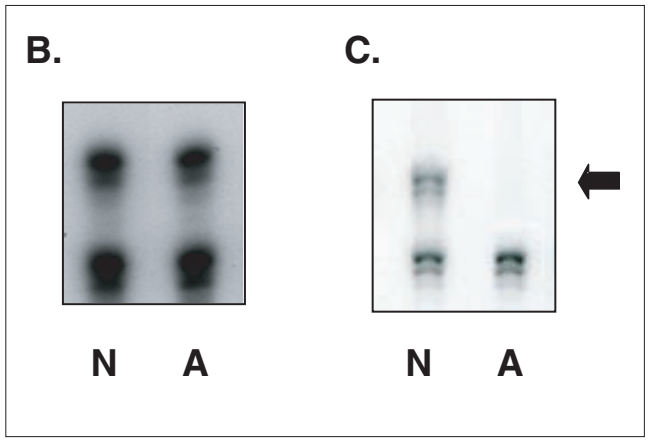

Figure 1b: *Autoradiograph of DNA bands in a polyacrylamide gel. DNA isolated from normal tissue cells (N) have 2 bands (the upper band is the paternal band and the lower band is the maternal band, as shown in Fig. 1a). The abnormal cells (A) also display 2 bands, indicating no chromosome loss at the region (no loss of tumor suppressor genes).*

Figure 1c: *Autoradiograph of DNA bands in a polyacrylamide gel. The normal tissue cells (N) have both the upper paternal band and the lower maternal band. In contrast, DNA isolated from abnormal cells (A) show only the lower maternal band with a loss of the upper paternal band (arrow), indicating chromosome loss at the region (loss of tumor suppressor genes).*

progression of genetic change, with early changes occurring at 2 specific chromosome sites (sites 3p14 and 9p21).[7] The risk of progression to cancer is low when no genetic change is seen, intermediate if there is genetic loss on the short arms of chromosomes 3 and 6 (3p and 9p) and high if there is 3p and 9p loss accompanied by genetic loss on additional chromosome arms (including 4q, 8p, 11q, 13q and 17p)[7,10,11] (**Fig. 2**). Lesions with LOH on 3p or 9p (or both) and genetic loss on 4q, 8p, 11q, 13q or 17p have a 33-fold greater risk of progression to malignancy than lesions with no genetic loss.[6,19] High-risk lesions, as indicated by LOH, may progress to cancer over a 5-year period in up to 50% of cases, whereas low-risk lesions progress to cancer in only 2% of cases.[8,11] These findings have led to a molecular model of carcinogenesis (**Fig. 2**).

Results of studies of LOH in premalignant lesions are consistent with those of studies of head and neck SCC, which document accumulation of molecular changes.[20] After excision of both malignant and premalignant lesions, the margins may appear clinically and histologically within normal limits but they may retain the genetic markers of increased risk. There may be a greater risk of recurrence if some genetically abnormal cells are left untreated or if there was advanced genetic change within the lesion. Therefore, molecular evidence of clear margins may be essential in confirming adequate management of premalignant and malignant lesions, and margins may in future become the markers of adequate treatment.[6,8,9–11,20]

Common risk factors for oral cancer, including tobacco and alcohol use, may result in broad areas of change in the oral mucosa (what is known as field cancerization). Molecular change may be present in these areas before cellular phenotypic changes become detectable by light microscopy. A recent study showed that leukoplakia at high-risk oral sites exhibited more advanced molecular changes (LOH) than were suggested by the histologic findings, which were similar to those of lesions from lower-risk sites in the oral cavity.[21] In such cases, management is difficult, and multiple and recurrent lesions may develop. Oral white and red lesions (leukoplakia, erythroplakia and erythroleukoplakia) is considered a premalignant lesion, even though risk of malignant change is small and unpredictable. Examination of molecular changes may advance our understanding of which lesions are a greater risk of progressing to malignancy. In SCC, molecular markers can be used to predict lesions at greater risk of recurrence, and extension to lymph nodes and bone, and metastatic spread.[12,19,20] Molecular change may be local, resulting in single lesions with accumulation of genetic change over time, or regional, involving all at-risk tissue exposed to carcinogens. Molecular markers may allow intermediate measurement of the outcome of therapy, as molecular change occurs before histologic change.[22]

Clinical Assessment and Diagnostic Sampling

The principal methods for assessing mucosal changes include recognition of risky behaviours and high-risk individuals. Patients at highest risk are those who have had previous cancer of the upper aerodigestive tract, of whom 10% to 22% will experience recurrence of the cancer or

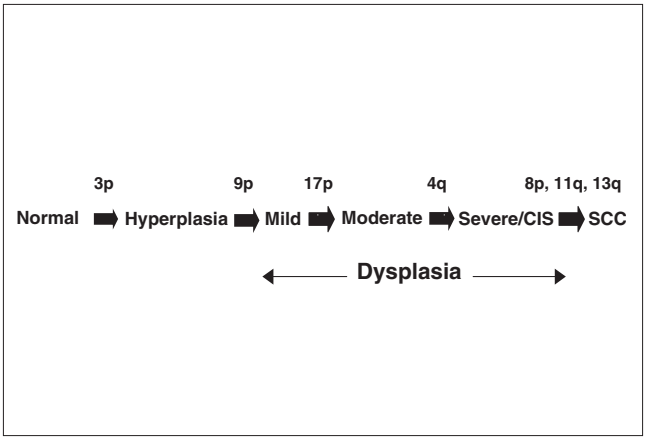

Figure 2: *Molecular model of oral carcinogenesis. The diagram shows the genetic progression from dysplasia to squamous cell carcinoma (SCC), through changes in the p or q arm of chromosomes 3, 4, 8, 9, 11, 13, and 17. CIS = carcinoma in situ.*

development of a second primary cancer within 2 years of treatment.[2,23–27] Those who use tobacco products and alcohol are also at greater risk.

Clinical Examination

Clinical examination for oral premalignant lesions and SCC should include a thorough head, neck and intraoral examination, with examination of the cervical lymph nodes and visual examination and palpation of the oral mucosal surfaces. Erythroplakia, leukoerythroplakia, verrucous lesions and ulcerative lesions may represent higher risk, whereas homogeneous leukoplakia carries a lower risk of dysplasia or malignancy at diagnosis. The location, size, border, colour and surface characteristics of any lesion should be recorded so that future changes can be recognized. A clinical classification of leukoplakia has been described to facilitate continuing research and to assist in determining the need for biopsy and treatment of a mucosal lesion.[28,29] The diagnosis of leukoplakia, erythroplakia and irregular lesions is challenging because the clinical appearance alone is not diagnostic; for example, frictional keratosis may resemble leukoplakia, and inflammatory lesions may look like erythroplakia.[28,30,31] When a biopsy is performed, site selection is critical, as the histologic features may vary in non-uniform lesions. If only areas of less severe cellular change are sampled, the less severe cellular pattern observed may be interpreted as representative of the lesion as a whole (even if there are other areas of more severe cellular change), and appropriate treatment may not be given. Similarly, histologic interpretation is itself a subjective science, and interpretation varies among pathologists; this variability can also lead to inappropriate diagnoses and treatment.

Vital Tissue Staining

Vital tissue staining has been identified as an adjunct to the early recognition of malignant lesions. Toluidine blue (tolonium chloride) is a metachromatic dye that stains mitochondrial DNA, cells with greater-than-normal DNA content[10] or altered DNA in dysplastic and malignant cells.[32] In experienced hands, at institutions where large numbers of cancer patients are seen, topical application of toluidine blue assists in identifying sites of malignant change and possible high-grade dysplasia. These studies have shown high sensitivity, no false-negative results and good positive predictive values.[33–37] A recent study showed biospy guided by toluidine blue in patients previously treated for head and neck cancer revealed LOH in all patients who had had SCC and in 82% of those who had had carcinoma in situ.[32] In that study, LOH was observed in 59% of histologically benign specimens from lesions that had previously been described as false-positive on the basis of toluidine blue staining; therefore, these lesions actually demonstrated the molecular changes associated with progression to cancer although their histologic features appeared benign.[32] However, widespread application of toluidine blue should be undertaken with caution, as there are no studies assessing its use in nonspecialty centres or assessing the practices of individuals with less experience in interpreting results. If this dye is felt to be appropriate as an adjunct to visual examination, especially for patients with suspicious lesions, referral to a centre or individual with extensive experience in head and neck cancer is recommended. Toluidine blue has also been reported as an aid in selecting biopsy sites and in delineating the margins of lesions.

DNA Content

A recent study assessed the DNA content of oral leukoplakia and followed 150 patients for a mean of 8.6 years.[10] Of the dysplastic lesions, the risk of progression increased with greater DNA content: 70% were low-risk diploid lesions of which 3% progressed to cancer during follow-up, 13% were intermediate tetraploid lesions of which 60% progressed, and 17% were high-risk aneuploid lesions of which 84% progressed. The degree of cellular atypia (dysplasia) did not correlate with DNA content or cancer risk. However, it is possible that lesions that appeared to be at greater risk were treated with wider excision, which would reduce the actual risk.

Exfoliative Cytology

Oral biopsy represents the gold standard for determining the nature of a mucosal lesion and for diagnosing SCC, and exfoliative cytology has, until recently, been discounted as a tool for assessing oral mucosal lesions. However, techniques have now been reported that include evaluation of exfoliated oral epithelial cells and comparisons of these methods with biopsy techniques. Exfoliative techniques have the advantage of being minimally invasive, and they

do not require local anesthetic. Use of a cytobrush reportedly allows sampling of the full thickness of stratified squamous epithelium of the oral mucosa.[38] Full-thickness sampling is essential if histomorphologic evaluation of the collected cells is to yield representative findings. For example, many dysplastic lesions are first identified in the basal epithelial layers, and the diagnostic histomorphologic findings may be lost as the cells mature and parakeratin and keratin are produced.

A recently reported technique includes computerized assessment of exfoliated cells for screening purposes, followed by evaluation by a pathologist if the computer analysis identifies any abnormality.[38] In that study, sensitivity for oral sampling was 100%. However, biopsy was not performed for all lesions, so negative results could not be assessed. In addition, cell collection for several specimens was described as "inadequate," and these specimens were not included in the calculations of sensitivity and predictive values. Caution in using this technique is recommended for several reasons: the findings are based on a single study, the study had several limitations, the technique has not been evaluated by general practitioners, and reports of false-positive and false-negative results have been posted on the Web site of the Bulletin Board for Oral Pathology (www.sdm.buffalo.edu/bbop/). Further study is continuing.

Molecular Analysis of Exfoliated Cells

Exfoliated cells can be subjected to additional analysis. As noted above, changes occur at the molecular level before they are seen under the microscope and before clinical changes occur. Molecular changes in the progression to SCC include common changes at chromosome sites that lead to changes in RNA and subsequent protein production. LOH and other molecular changes, including changes at p16, p53 and cyclin D, can be assessed in exfoliated cells.[6,7,22] Molecular analysis of cells collected by rinsing the mouth has shown the same changes as are present in tumor biopsy specimens.[39] Exfoliated cells from oral lesions had LOH that was highly correlated with biopsy findings from the same sites; there were no false-positive results.[40] Examination of exfoliated cells for molecular markers may allow assessment of the progression of change and the outcome of therapy, including preventive studies.[41] Thus, molecular assessment of exfoliated cells may make it easier to diagnose lesions and assess their progress during treatment and follow-up.

Further studies of both exfoliative techniques and use of toluidine blue are under way. Ultimately, studies of the use of these techniques by less experienced clinicians will be necessary.

Discussion

Several adjuncts to visual examination, specifically application of toluidine blue and exfoliative cell collection, may lead to advances in the recognition of lesions and may guide selection of biopsy sites. Toluidine blue and exfoliative techniques are now clinically available, and it is expected that molecular evaluation of oral lesions will eventually become clinically available. Molecular techniques are expected to aid in diagnosis and staging of disease, and in providing intermediate markers to assess treatment interventions. In addition, advances in knowledge may lead to new therapies, ultimately improving the management of at-risk lesions once they are identified, as well as improving the prevention and management of SCC.

In the past, exfoliated cell collections did not sample the full thickness of the epithelium, which led to a large number of false-positive and false-negative results, relative to diagnosis by biopsy. Collection of exfoliated epithelial cells by cytobrush may yield more complete sampling of the epithelium, but the data obtained will still be less than that available through biopsy, as the relationship between epithelial cells and the connective tissue cannot be assessed from exfoliated cells. Combining information from molecular markers with exfoliative techniques may overcome some of the current limitations of exfoliative cytology. These combined techniques may prove to be sensitive and specific procedures that can be performed sequentially over time and perhaps as screening methods for at-risk lesions already identified.

Molecular markers are expected to become essential in the diagnosis and management of patients with oral cancer; they will guide future study and clinical care and will ultimately lead to new interventions directed at the molecular changes of cancer. Use of molecular markers allows earlier diagnosis and staging of tissue change, before changes in cell morphology occur and certainly before tissue changes become clinically visible. Ultimately, the use of molecular markers in diagnosis may lead to better survival and less treatment-associated morbidity through early recognition of and intervention for at-risk oral lesions. Thorough examination and appropriate selection of biopsy sites, coupled with expert histopathologic evaluation, are required for diagnosis of oral leukoplakia and erythroplakia. Clinicians should start thinking at the molecular level as advances in our understanding of the pathogenesis of cancer and in the diagnostic armamentarium at our disposal continue. ◆

Dr. Epstein *is medical staff, division of dentistry, British Columbia Cancer Agency, Vancouver, B.C., and professor, department of oral medicine, University of Washington, Seattle, WA.*

Dr. Zhang *is associate professor, division of oral medicine and pathology, faculty of dentistry, University of British Columbia, Vancouver, B.C., and pathologist, department of laboratory medicine and pathology, Vancouver Hospital and Health Sciences Centre.*

Dr. Rosin *is a senior scientist in the cancer control research program, British Columbia Cancer Agency, Vancouver, B.C., and a full professor, cancer prevention laboratory, school of kinesiology, Simon Fraser University, Burnaby, B.C.*

Correspondence to: *Dr. Joel B. Epstein, Cancer Control Agency, 600 West 10th Ave., Vancouver, BC V5Z 4E6. E-mail: joelbep@u.washington.edu*

The authors have no declared financial interests.

References

1. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. *N Eng J Med* 1993; 328(3):184–94.
2. Allison P, Locker D, Feine JS. The role of diagnostic delays in the prognosis of oral cancer: a review of the literature. *Oral Oncol* 1998; 34(3):161–70.
3. Kowalski LP, Franco EL, Torloni H, Fava AS, de Andrade Sobrinho J, Ramos G, and others. Lateness of diagnosis of oral and oropharyngeal carcinoma: factors related to the tumour, the patient and health professionals. *Eur J Cancer B Oral Oncol* 1994; 30B(3):167–73.
4. Wildt J, Bundgaard T, Bentzen SM. Delay in the diagnosis of oral squamous cell carcinoma. *Clin Otolaryngol* 1995; 20(1):21–5.
5. Silverman S. Oral Cancer. *Semin Dermatol* 1994; 13(2):132–7.
6. Rosin MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J, and others. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin Cancer Res* 2000; 6(2):357–62.
7. Mao L, Lee JS, Fan YH, Ro JY, Batsakis JG, Lippman S, and others. Frequent microsatellite alterations at chromosome 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. *Nat Med* 1996; 2(6):682–5.
8. Partridge M, Pateromichelakis S, Phillips E, Emilion GG, A'Hern RP, Langdon JD. A case control-study confirms that microsatellite assay can identify patients at risk of developing oral squamous cell carcinoma within a field of cancerization. *Cancer Res* 2000; 60(14):3893–8.
9. Lee JJ, Hong WK, Hittelman WN, Mao L, Lotan R, Shin DM, and others. Predicting cancer development in oral leukoplakia: ten years of translational research. *Clin Cancer Res* 2000; 6(5):1702–10.
10. Sudbo J, Kildal W, Risberg B, Kopang HS, Danielsen HE, Reith A. DNA content as a prognostic marker in patients with oral leukoplakia. *N Engl J Med* 2001; 344(17);1270–8.
11. Zhang L, Poh CF, Lam WL, Epstein JB, Cheng X, Zhang X, and others. Impact of localized treatment in reducing risk of progression of low-grade oral dysplasia: molecular evidence of incomplete resection. *Oral Oncol* 2001, 37(6):505–12.
12. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma 3: clinico-pathological applications. *Oral Oncol* 2000; 36(5):404–13.
13. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma 2: chromosomal aberrations. *Oral Oncol* 2000; 36(4):311–27.
14. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma (SCCHN): 1. Carcinogen metabolism, DNA repair and cell cycle control. *Oral Oncol* 2000; 36(3):256–63.
15. Zhang L, Epstein J, Band P, Berean K, Hay J, Cheng X, and other. Local tumor recurrence or emergence of a new primary lesion? A molecular analysis. *J Oral Pathol Med* 1999; 28(8):381–4.
16. Califano J, Westra WH, Meininger G, Corio R, Koch WM, Sidransky D. Genetic progression and clonal relationship of recurrent premalignant head and neck lesions. *Clin Cancer Res* 2000; 6(2):347–52.
17. Cairns P, Sidransky D. Molecular methods for the diagnosis of cancer. *Biochim Biophys Acta* 1999; 1423(2):C11–8.
18. Ahrendt SA, Sidransky D. The potential of molecular screening. *Surg Oncol Clinics N Am* 1999; 8(4):641–56.
19. Mao L. Can molecular assessment improve classification of head and neck premalignancy? *Clin Cancer Res* 2000; 6(2):321–2.
20. Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, andothers. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *New Engl J Med* 1995; 332(7):429–35.
21. Zhang L, Cheung KJ Jr, Lam WL, Cheng X, Poh C, Priddy R, and others. Increased genetic damage in oral leukoplakia from high-risk sites: potential impact on staging and clinical management. *Cancer* 2001; 91(11):2148–55.
22. Lippman SM, Lee JJ, Sabichi AL. Cancer chemoprevention: progress and promise. *J Natl Cancer Inst* 1998; 90(20):1514–28.
23. Tepperman BS, Fitzpatrick PJ. Second respiratory and upper digestive tract cancers after oral cancer. *Lancet* 1981; 12;2(8246):547–9.
24. Jovanovic A, van der Tol G, Schulten EA, Kostense PJ, de Vries N, Snow GB, and other. Risk of multiple primary tumors following oral squamous-cell carcinoma. *Int J Cancer* 1994; 56(3):320–3.
25. Jones AS, Morar P, Phillips DE, Field JK, Husband D, Helliwell TR. Second primary tumors in patients with head and neck squamous cell carcinoma. *Cancer* 1995; 75(6):1343–53.
26. Haughey BH, Gates GA, Arfken CL, Harvey J. Meta-analysis of second malignant tumors in head and neck cancer: the case for an endoscopic screening protocol. *Ann Otol Rhinol Laryngol* 1992; 101(2Pt1):105–12.
27. Khuri FR, Kim ES, Lee JJ, Winn RJ, Benner SE, Lippman SM, and others. the impact of smoking status, disease stage and index tumor site on second primary tumor incidence and tumor recurrence in the head and neck retinoid chemoprevention trial. *Cancer Epidemiol Biomarkers Prev* 2001; 10(8):823–9.
28. Axell T, Pindborg JJ, Smith CJ, van der Waal I. Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18-21 1994. International Collaborative Group on Oral White Lesions. *J Oral Pathol Med* 1996; 25(2):49–54.
29. van der Waal I, Schepman KP, van der Meij EH. A modified classification and staging system for oral leukoplakia. *Oral Oncol* 2000; 36(3):264–6.
30. Bouquot JE, Whitaker SB. Oral leukoplakia – rationale for diagnosis and prognosis of its clinical subtypes or "phases". *Quintessence Int* 1994; 25(2):133–40.
31. Silverman S Jr, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. *Cancer* 1984; 53(3):563–8.
32. Guo Z, Yamaguchi K, Sanchez-Cespedes M, Westra WH, Koch WM, Sidransky D. Allelic losses in OraTest-directed biopsies of patients with prior upper aerodigestive tract malignancy. *Clin Cancer Res* 2001; 7(7):1963–8.
33. Epstein JB, Oakley C, Millner A, Emerton S, van der Meij E, Le N. The utility of toluidine blue application as a diagnostic aid in patients previously treated for upper oropharyngeal carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997; 83(5):537–47.
34. Rosenberg D, Cretin S. Use of meta-analysis to evaluate tolonium chloride in oral cancer screening. *Oral Surg Oral Med Oral Pathol* 1989; 67(5):621–7.
35. Epstein JB, Scully C, Spinelli J. Toluidine blue and Lugol's iodine application in the assessment of oral malignant disease and lesions at risk of malignancy. *J Oral Pathol Med* 1992; 21(4):160–3.
36. Warnakulasuriya KA, Johnson NW. Sensitivity and specificity of OraScan (R) toluidine blue mouthrinse in the detection of oral cancer and precancer. *J Oral Pathol Med* 1996; 25(3):97–103.
37. Mashberg A, Samit A. Early diagnosis of asymptomatic oral and oropharyngeal squamous cancers. *CA Cancer J Clin* 1995; 45(6):328–51.
38. Sciubba JJ. Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. U.S. Collaborative OralCDx Study Group. *J Am Dent Assoc* 1999; 130(10):1445–57.
39. Spafford MF, Koch WM, Reed AL, Califano JA, Xu LH, Eisenberger CF, and others. Detection of head and neck squamous cell carcinoma among exfoliated oral mucosal cells by microsatellite analysis. *Clin Cancer Res* 2001; 7(3):607–12.
40. Rosin MP, Epstein JB, Berean K, Durham S, Hay J, Cheng X, and others. The use of exfoliative cell samples to map clonal genetic alterations in the oral epithelium of high-risk patients. *Cancer Res* 1997; 57(23):5258–60.
41. Mao L, El-Naggar AK, Papadimitrakopoulou V, Shin DM, Shin HC, Fan Y, and others. Phenotype and genotype of advanced premalignant head and neck lesions after chemopreventive therapy. *J Natl Cancer Inst* 1998; 90(20):1545–51.