REVIEW ARTICLE

Detection and Screening of Oral Cancer and Pre-cancerous Lesions

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Oral cancer is a fatal disease, accounting for the fourth highest incidence of malignancy in males and the seventh in females in Taiwan. The relatively high prevalence of oral cancer in Taiwan is mainly because there is a high-risk group of 2.5 million people with the habit of smoking and betel nut chewing. Unfortunately, 50% of new cases in our medical center who present with TNM stage III or IV lesions have a shorter than 5-year survival after treatment. This highlights the need for: (1) early treatment of fresh oral cancer cases; (2) screening of the high-risk population to detect new lesions; (3) careful follow-up of cases after treatment; and (4) detection of occult early neck nodal adenopathy in surgical cases. It is generally accepted that prevention and screening of oral cancer are equally important to treatment due to its location. In this review article, we describe the nature of oral cancer and highlight the various conventional and novel methods of screening for this disease and ongoing important related research. Related literature is reviewed and future work that needs to be done is detailed. [*J Chin Med Assoc* 2009;72(5):227–233]

Key Words: betel nut, cytology, marker, oral cancer, prevention, screening

Introduction

Oral cancer is the fifth most common cancer in the world, accounting for 412,000 new cases and 262,000 deaths annually in 1985, four-fifths of which occurred in developing countries. Epidemiologic differences exist in South Asia, where oral cancer ranks first among all types of cancers in male patients and third in female patients.[1,2] Oral cancer is associated with chronic irritating factors such as tobacco, smoking, alcohol, and betel quid (BQ) use. While cigarette smoking and alcohol drinking are the major risk factors in Western countries, BQ use and smoking are major factors in the causation of oral cancer in South Asia, Southeast Asia and Taiwan.[1–3] Oral cancer is a fatal disease, accounting for the fourth highest incidence of malignancy in males and the seventh in females in Taiwan. Oral squamous cell carcinoma (OSCC) accounts for >95% of all oral malignancy. The relatively high prevalence of oral cancer in Taiwan is mainly because there is a high-risk group of 2.5 million people with the habits of smoking and betel nut chewing. Oral mucosa diseases such as leukoplakia, oral submucosa fibrosis, oral precancer lesions and oral cancer have been strongly associated with the use of BQ.[3,4] Unfortunately, around 50% of new cases at their first visit to our medical center often present with advanced TNM stage III or IV lesions.[4,5] It is generally accepted that prevention and screening of oral cancer are equally important. In this review article, we describe the nature of oral cancer and highlight the various conventional and novel methods of screening for this disease and important related research.

Importance of Early Treatment

Not only oral cancer, but also BQ-associated mouth diseases such as mucositis, submucous fibrosis, severe tooth attrition, and periodontitis have long been a tough challenge in general health care. About 50% or more of oral cancer patients have stage III or IV lesions

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at their first visit to our medical center in Taiwan. Unfortunately, the overall 5-year survival rate of these patients is poor despite recent advances in surgery, radiotherapy and chemotherapy.[5] Here, we give an example to highlight the importance of early treatment.

A 50-year-old patient at his first visit to our department presented with a growth measuring 4×5 cm in his right buccal mucosa-gingivae area and cervical lymph adenopathy. OSCC T4N1M0 stage IV was diagnosed after incision biopsy. The patient received a commando operation with partial mandibulectomy leaving a huge transbuccal defect. Radio-forearm fibular and anterior lateral tight free flaps were performed for reconstruction. The patient received a 24-hour long surgery, followed by a week of intensive medical care. The final pathologic report confirmed the previous tentative diagnosis. Afterwards, combination treatment with concurrent chemotherapy and radiotherapy was given. He suffered severe hair loss and radiotherapy-burned facial skin. Unfortunately, the patient was diagnosed with distant lung metastasis 2 years after surgery. Following consultation, chemotherapy and radiotherapy were given as palliative treatment for 2 months to control the metastatic neoplasm. Finally, the patient died due to treatment failure. The expense of treatment for this stage IV oral cancer patient was far more than that for treating a stage I or II oral cancer patient.

Screening the High-risk Population

Oral cancer can be cured if treated early enough. Oral cancer is one among the few human cancers with a vast potential for prevention. Programs for detecting oral cancer have been supported by our government for many years. To cope with this program, funding has been distributed in several directions, including to general health auxiliaries in public first-line health care institutes, dentists and ENT doctors in medical centers.[3,4] However, the long-term effect remains to be seen. Previous reports revealed that 90% of male oral cancer patients were both BQ chewers and smokers. It is undoubtedly the case that this high-risk group, accounting for one-tenth of the population in Taiwan, should be screened with priority. Meanwhile, a follow-up system should be established to recall and monitor the cancer patients and patients with precancerous lesions who have been treated in hospital.[5–8]

Premalignant lesions predisposing to oral cancer

Attention should be paid specifically to precancerous lesions.[6–9] Leukoplakias are among the most common potentially malignant oral lesions. Some are idiopathic, while others are related to habits such as tobacco, alcohol or BQ use. About 80% of leukoplakias are benign, with no evidence of dysplasia and no tendency to malignancy, but biopsy is clearly indicated to define the remaining 10–20% that are either dysplastic or already changed to invasive carcinoma.[6] Unfortunately, there is currently no histologic or other means of reliably predicting whether those leukoplakias will indeed progress to malignancy. Overall, the rate of malignant transformation of leukoplakias is about 3–6% over 10 years, but rates that are much higher have been reported. Medical management of leukoplakias includes reducing or quitting those habits relating to risk factors, increasing the intake of fruits and vegetables in the diet, and possibly the use of active agents.[7] Retinoids, carotenoids and topical cytotoxic agents inducing apoptosis show promise, and newer therapies are on the horizon.[7,9,10] Also, matrix metalloproteinase-13 (MMP-13) could be a potential tumor marker for OSCC. Epigallocatechin-3-gallate, a component of green tea, has also been demonstrated to inhibit oral cancer through the modulation of MMP-13.[11]

Roles of Health Care Workers

Health care workers need to clearly understand their roles in cancer screening. It is sometimes argued that oral cancer screening is not necessary because routine dental examinations should include a full oral mucosal examination. However, apart from the fact that more than 50% of the over-45-year-old population do not attend a dentist annually, there is evidence that many cases are missed, even by dental practitioners. This is probably because early lesions are not specifically looked for or may appear to be innocuous and are ignored. Thus, other professions or specialists may also need to be included in the screening program. Screening for oral cancer is a simple, noninvasive procedure that can be easily integrated into the comprehensive assessment of older patients who account for the majority of oral cancer patients. Further, geriatricians might feel more comfortable performing an oral cancer screening examination. Since 5-year survival rates are far greater in individuals with localized lesions than in those with distant metastases, the detection of early oral cancer can make a significant positive contribution to prognosis.[12–14] Elderly persons at risk for oral cancer visit their dentist far less frequently than they visit their physician. Primary physicians look at sore throats every day, and taking a few extra minutes to do a thorough oral examination could benefit the patient. If primary

care physicians joined in routinely screening for oral cancer, long-term survival rates would undoubtedly improve.

Methods of Screening

There are many reports on the miscellaneous methods of oral cancer detection and screening.[8,12–16] Physical examination includes self-examination and clinical examination. Clinicians have a responsibility to perform a thorough head and neck examination as part of the physical assessment of their patients. It takes less than 2 minutes to perform. The goal of examination is to detect any nodules, swellings, mucosal alterations (ulcerations, textural or color changes) and unexplained neck lymph nodal adenopathy. While many routines exist for an oral examination, each clinician must develop his or her own method, use it in all patients, and carefully document positive findings. Toluidine blue staining is a simple method, with the dye having an affinity to cancer cells. Commercial kits with protocol are available for large-scale screening of high-risk populations or in clinical patients by topical application or mouth rinsing. For subjects having panoral field cancerization, rinsing or gargling is recommended. However, a significant percentage of false-negatives and false-positives exist.[8,15,16]

Meanwhile, a biopsy is still needed before a diagnosis can be confirmed. An excision biopsy is definitely sufficient for pathologic analysis of small 0.5–1.0-cm lesions. However, multiple incision biopsies are more appropriate for a large lesion, which may also be stained with toluidine blue to define highly suspected locations. It is common to find cases with no definite lesion location but rather a picture of panorally premalignant cancerization in high-risk patients with a history of BQ use and smoking for 20–30 years.[5,8]

Exfoliative cytology in screening for oral cancer has never achieved the same success as it has for diagnosing cancer of the uterine cervix. Oral exfoliative cytology enjoyed much attention in the 1960s, but eventually fell from favor, due largely to the subjective nature of its interpretation, and also because the field cancerization led to it being extremely difficult to tell where exactly cancer cells came from in early cancer cases. The recent application of quantitative and immunocytochemical techniques has, to some extent, refined its potential role. However, the absence of a marker, present in all malignant lesions but never in benign lesions, limits its clinical utility.[8,16] An additional drawback of exfoliative cytology for screening is that the lesion still had to be identified or anatomically located. Technical problems may also easily be encountered in cases with field cancerization in betel nut chewers.[15–17]

Saliva containing exfoliated cells from scraping or natural exfoliation combined with Cytospin may be another approach. Utilizing Cytospin preparation from saliva may potentially increase the collected cellular contents for analysis. Some squamous cell carcinoma cytokeratin markers have been detected occasionally at a higher level within serum in a few cancer patients. However, unlike α-fetoprotein for hepatoma or some important markers for prostate or other cancers, there is no one specific marker that can be universally used for the detection of oral cancer. Lacking profound hepatic sinusoid circulation, the superficially located oral lesions have not given a promising result so far.[6,8,12] Current molecular biology techniques may improve the cytology screening method. The experimental data of Lin et al showed initial success in detection of 3q26-27 oncogene amplification in laser-captured-microdissected samples in the brushed buccal cells of betel chewers.[18] David Wong from UCLA dental school has proposed a new method for using pure saliva secreted from the major or minor salivary glands without cellular content for cancer detection.[19] This technique is powered by nanotechnology, proteomics, and high throughput microarray. Previously, the saliva has not been proven to have any contribution for cancer detection. Two salivary proteins, interleukin (IL)-8 and thioredoxin, can be used to discriminate between the saliva of oral cancer patients and that of control subjects. IL-8 is significantly elevated in the saliva of oral cancer patients and is highly discriminatory for detecting oral cancer in saliva at a cut-off of 600 pg/mL. Similarly, oral cancer patients have significantly higher salivary IL-8 mRNA concentrations than control subjects. Both elevation of IL-8 and IL-8 mRNA can discriminate inflammatory disease such as periodontitis from oral cancer.[19] It is accepted that oral exfoliative cytology can not only assume a greater role by providing samples of DNA for genetic analysis but can also provide a useful tool for screening. The pendulum is swinging from the morphologic picture towards the molecular level. We may yet see a new role for exfoliative cytology. Greater understanding of genetic aberrations may predict not only the biological behavior of the tumor but also its likely response to both traditional and novel forms of therapy. It remains to be seen if exfoliative cytology can progress from being a research tool to being used in routine clinical practice.[12,13] The future role for oral exfoliative cytology—bleak or bright? It may well be that a more scientific and efficient method for oral exfoliative cytology might enjoy greater success

based on the understanding of the molecular mechanism and characteristics of cancer development.

Mechanisms of Oral Cancer Formation

Similar to the well-established colorectal carcinoma model, oral cancer is also considered to be a multi-hit process involving a number of aberrant genetic events culminating in malignant transformation at the molecular and biological levels. It is known that following the action of various carcinogens (chemical, physical, biological) on normal cells in humans, a long period (latency) of several months to years (~10 months to 30 years) occurs between the development of precancer cells and their transformation into cancer cells. However, the molecular and biological events that take place within the precancer cells during this quiescent stage are not yet fully understood. Recent studies revealed that preneoplastic cell development and transformation into cancer cells is determined initially by genetic changes (oncogenes, antioncogenes), with sequential multiple somatic mutations, and later by epigenetic or environmental cell factors such as hormones, growth factors, cytokines, vitamins, and prostaglandins. These factors can markedly change the evolution of preneoplastic cells by enhancing, retarding, or inhibiting their transformation into cancer cells, or even reversing them to a normal phenotype.[1,2,9] These effects act on DNA, RNA, and protein synthesis, as well as on cell replication, cell cycles, cell surfaces, and intercellular communication. Therefore, these abnormal DNA, oncogenes or tumor suppressor genes, and ultrastructural intracellular or cell surface antigenic determinants as potential biomarkers are essential for early detection of preneoplastic cells and cancer cells. A significant recent advance is the gradual understanding of the molecular mechanism of oral cancer formation.[20–33] Although a universal tumor marker is still lacking for oral cancer, a combination of several markers may be useful and more accurate.

BQ-associated Chemical Carcinogenesis

BQ users as a group are seen as being at highest risk of oral cancer in Taiwan. This is because the areca nut (AN) contains areca alkaloids, polyphenols and tannins. Considerable evidence suggests that areca alkaloids are the major factors for AN toxicity. AN is reported to contain more than 4 alkaloids, including arecoline, arecaidine, guvacoline and guvacine.[34] Oral keratinocytes and fibroblasts appear to be the major target cells attacked by BQ ingredients. BQ ingredients have been shown to induce cytotoxicity, DNA strand breakage, and DNA-protein cross-linkage of oral keratinocytes and fibroblasts.[34–36] Repeated and continuous exposure of oral mucosal cells to BQ ingredients will lead to impairment of cellular defense systems such as antioxidants, glutathione peroxidase, and superoxide dismutase. The induction of DNA damage and the inhibition of DNA repair will promote the fixation of mutated nucleotides, leading to the formation of initiated cells.[37,38] BQ ingredients increase mtDNA mutation in human oral tissues, and that accumulation of mtDNA deletions and subsequent cytoplasmic segregation of these mutations during cell division could be an important contributor to the early phase of oral carcinogenesis.[39] Also, BQ induces the chromosomal imbalances that occur in oral carcinoma and is associated with their clinical implications. The preliminary findings of a lower incidence of loss of 4q and gain of 8q in BQ-associated tumors compared to non-BQ-associated tumors might provide insight into the carcinogenic effect of BQ.[40] Many studies have been designed to directly analyze the carcinogenicity of BQ ingredients in experimental animals. Repeat brushing of the hamster cheek pouch with a dimethyl-sulfoxide (DMSO) extract of AN 3 times/week for 21 weeks led to the development of tumors in 38% of test animals, and leukoplakia in 90% of test animals. Direct painting of DMSO extract of AN also induced early malignant changes in the hamster cheek pouch.[41–43]

New Markers and Tools for Oral Cancer Detection and Treatment

The last 10 years has seen a shift in diagnostic methods from the histopathologic to the molecular level.[44–49] With advances in modern molecular biology techniques, many new markers for oral cancer have been found and studied. Significant momentum has been seen in the exploration of P53, P16, telomerase, and so on in many cancer research groups. For example, using *in situ* hybridization (ISH) and telomeric repeat amplification (TRAP) assay, a gradual increase in telomerase activity was observed in the malignant transformation process of oral cancer.[39] Loss of retinoic acid receptor (RAR)-β expression in the malignant transformation of oral cancer has been reported by analyzing the expression of RAR-β using ISH of RAR-β antisense riboprobe in oral cancer and adjacent noncancerous matched tissues to correlate with their clinicopathologic features.[47–49] With the great advancement in disclosing pieces of the puzzle of cancer

development, the next generation of cancer screening methods will favor a more efficient and reliable tool based on previous contribution of scientists. The newly developed microarray/gene chip technology with more reliable/predictable tumor markers will encourage us to seek a new approach to cancer screening.[50] Current formal diagnosis of oral cancer is still based on the pathology report from biopsy. Beyond just being used for staging, we expect microscopic observations to provide more information about potential early nodal metastasis, tumor behavior, and clues to fine-tune treatment modality or even to predict prognosis.

By revealing the story of oral carcinogenesis, the normal oral epithelial cells should go through the steps from abnormal cells to precancer or cancer cells *in situ*, stroma invasion, vascular permeation and metastasis. What we see as a huge tumor in the oral cavity can be further microscopically dissected based on many molecular events equivalent to various pathological covariates.[51,52] These pathological covariates might potentially provide some clues for treatment planning. The following are some interesting findings proposed by our research team. RAR-β is an important differentiation marker of oral epithelium. We used ISH to discover that the loss of expression in buccal squamous cell carcinoma could relate to a more advanced histopathologic grade of tumor.[49] We investigated the significance of histopathologic factors on clinical outcome in squamous cell carcinoma of buccal mucosa and found that perineural invasion and lymphovascular permeation significantly affects local recurrence. Tumor thickness significantly relates to staging of tumors and the survival of cases. We further investigated the pathologic risk factors affecting nodal metastasis in tongue cancer. We found that differentiation, invasion depth, perineural invasion, and lymphovascular permeation significantly affect nodal metastasis in tongue cancer. Therefore, patients in early stages I or II with such pathologic covariates may need more appropriate early neck treatment.[53]

We also found differential expression of adhesion molecules E-cadherin in metastatic lesions compared to primary OSCC. This implies that disintegration of cellular junctions of cancer should correlate significantly to the degree of malignancy and its ability to enter into blood and lymphatic vessels.[54] Not only the cancer cells themselves but also the stroma tissue was thought to be important in oral carcinogenesis. The functional single nucleotide polymorphism (SNP) in the MMP-3 gene is associated with oral submucosal fibrosis susceptibility. The MDM2 SNP and p53 codon 72 SNP are independent and useful prognostic factors in OSCC patients receiving postoperative radiotherapy. The genotype of these cases may have higher resistance to radiotherapy. The functional SNP of the MMP-9 gene is associated with risk of OSCC in younger male AN users. Young AN chewers with this genotype may have a higher oral cancer risk. Correlation between functional genotype in the MMP-1 promoter was found to be associated with the risk of OSCC.[23,55–57]

Due to the lack of unique molecular markers in oral cancer, a diversified phenotype/genotype of OSCC cases needs more powerful tools to demonstrate its gene expression profiles linking to their clinical behaviors.[7,58,59] Conventional TNM staging obviously does not provide all the needed information.[53] If we could further analyze the gene expression profiles underlying the pathologic covariates of what we see under the microscope, it would aid greatly in directing more appropriate treatment modalities such as the need for neck dissection, postoperative radiation chemotherapy or even target therapy.

Future Work

OSCC is the fourth leading malignancy in men in Taiwan due to the popularity of BQ chewing. There are more than 2.5 million BQ chewers in our country who are at high risk for OSCC. Despite advances in cancer treatment and diagnosis in the past decades, the prognosis for OSCC remains dismal. Most OSCC patients die of recurrence or metastasis. Therefore, much remains to be done to elucidate the pathogenesis associated with BQ chewing and to improving the therapies for OSCC. Among multiple AN ingredients, AN extract (ANE) was classified as a group I carcinogen by the International Agency for Research on Cancer. Our previous studies have shown that ANE modulated signaling activation, leading to karyotypic alterations and increase in the aggressiveness of oral cancer cells. The gene expression signatures associated with metastasis of BQ-associated OSCC have been established in our previous research.[24,29,31,34,36,41] We also adopted brushing samples to identify copy number amplification of oncogenes in the oral epithelial cells from BQ chewers.[18] On the basis of previous achievements, the ongoing pathogenetic projects are the following: (1) to characterize the oncogenic potential of several ANE-modulated genes, and to specify their roles in OSCC recurrence and metastasis; (2) to investigate the impact of ANE on the microenvironment for the genesis and progression of OSCC; and (3) to develop molecular analysis for oral brushing and serum samples from high-risk BQ chewers or OSCC patients in identifying markers for early diagnosis and prognostic

prediction. We believe that by the devotion of clinicians and scientists, a new approach to oral cancer will no longer be a dream.

The program of cancer detection and screening is like a war, and should be backed by full government support, cooperation of school education, news media, medical services, and general awareness from the whole population. Care should especially be taken in policy and strategic planning and in performing the cancer detection/screening examinations. As health care workers, we need to know the important role of our profession in the screening and detection of oral cancer.

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