An Overview of Detection and Screening of Oral Cancer in Taiwan

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Oral cancer is a fatal disease, which accounts for the fourth highest incidence of malignancy in males and the seventh highest in the general population of Taiwan. About 95% of oral cancer is oral squamous cell carcinoma (OSCC). The relatively high prevalence of OSCC in Taiwan is mainly because a high-risk group of the population exists, made up of 2.5 million people and who exhibit habits of betel nut chewing as well as cigarette smoking. Unfortunately, about 50% of the new OSCC cases found in medical centers presented with TNM stage III or IV cancer lesions leading to a low 5-year survival. Therefore, it is generally accepted that the prevention and screening of OSCC at early stages or premalignant levels in the high-risk group of the population is as equally important as treatment. In this review article, we describe the current status of OSCC in Taiwan regarding epidemiology. Furthermore we research and highlight the importance of various conventional and novel methods in the detection of this disease.

Key words: *betel nut, cigarette smoking, detection, OSCC, prevention*

Oral cancer was the fifth most common cancer in the world, accounting for 412,000 new cases and 262,000 deaths annually in 1985, four-fifths of what occurred in the developing regions. Epidemiological differences exist in South Asia where oral cancer ranks as the most critical amongst all types of cancers in male patients and third most critical in female patients[1,2]. About 95% of oral cancer is oral squamous cell carcinoma (OSCC). OSCC is associated with chronic irritant 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, betel quid chewing and cigarette smoking are major causes of OSCC in Taiwan and both South and Southeast Asia[1-3]. Almost 2.5 million people are BQ users in Taiwan. A higher rate of incidence of OSCC and its mortality were found to be associated with the increasing prevalence rate of betel chewing in this area. For more than a few hundred years betel chewing has been generally accepted as a social custom or behaviour. According to an epidemiological study by Ko et al in Taiwan, the incidence of OSCC in BQ users and smokers is about 100 times higher than that in the general population[3,4]. A number of studies have been carried out to clarify the roles of BQ ingredients in relation to carcinogenesis. It had been extensively explored for more than a decade and there was general agreement that BQ may potentially damage the oral mucosa to induce genotoxic or non-genotoxic effects, which may be related to the initiation, promotion and progression of OSCC[3,5-10]. Various ingredients of BQ including areca nuts, nitroso-derivatives, arecoline, safrole, lime and so on have been studied and been shown to correlate with carcinogenic effects, co-carcinogenic effects and tumour promotion[11-15]. Until now, more updated and evidence-based studies have disclosed the roles of each ingredient in BQ, in relation to carcinogenesis[16-19]. The other major

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co-predisposing factor of OSCC progression is cigarette smoking. It was further demonstrated that the nicotine from cigarette smoking had a strong contribution to the onset of OSCC[3,20-23].

Importance of early detection and treatment

Not only OSCC but diseases associated with betel chewing in the oral cavity such as mucositis, submucous fibrosis, severe tooth attrition and periodontitis challenged the work of general oral health care practitioners. The OSCC accounts for the fourth highest incidence of cancers in the male population of Taiwan, and the first in the 40-year-old age group of the male population. We may name it as a cancer located at the front of the body trunk because of its easily seen location of occurrence. Yet, its location does not comply with the current status of this disease being found in the hospital. About 50% or more of OSCC patients were found to have their tumours ranked as stage III or stage IV during their first visit to the medical center in Taiwan, leading to a low overall 5-year survival rate, despite recent advances in surgery, radiotherapy and chemotherapy[24]. This highlights the priority for early detection, diagnosis, and treatment to increase the survival. Not surprisingly, the cost for the treatment of a stage III or stage IV OSCC patient was a lot higher than treating a stage I or II OSCC patient. According to a retrospective study on the analysis of 703 OSCC cases in Southern Taiwan by Chen et al, the 5-year survival rate in the patients of advanced stages III and IV was far less than that of those in the early stages of I and II[4]. A retrospective analysis to compare the outcome of all tumour stages between cases treated before 1996 and cases after 2004 at Taipei-Veterans General Hospital by Chen et al, indicated there was approximately a 20% increase (77.2% vs 56.3%) in the 5-year overall survival rate, regardless of tumour stage. We considered that the improved result should be attributed to the increased understanding of the clinicopathological features of this disease, fine-tuned surgical techniques, more appropriate treatment modalities and generally much improved systemic patient care following on from a decade of advancement in the cancer management. Another reason should be attributed to the patients' self-awareness regarding the prevention of OSCC that had been intensively propagated through the mass media over the last 10 years[24,25]. These results again strongly suggest the importance and effective outcomes from early diagnosis and treatment of OSCC.

Globally all cancers had an average increase in the 5-year survival rate from 50% in 1975 to 66% in 2003. During this period, the 5-year survival rate of prostate cancer in the male population even had an increase from 69% to 99%. Surprisingly compared to other major cancers, it has been 40 years since OSCC presented a much less improved 5-year survival rate, from 53% in 1975 to 59% in 2003. This was even seen in developed Western countries for example USA and UK[26]. This further highlights the significance of exploring the mechanism of malignant transformation in order to find out novel ways of early diagnosis and effective therapy. OSCC certainly could be cured if it were treated early enough at stage I and II or even at the pre-cancer stage[24,25]. Out of the few human cancers OSCC is one which has a vast potential for prevention. Due to a lack of previously reliable epidemiological data on the survey relating to OSCC prevention in Taiwan, so far, the value and potential of prevention and screening is largely based on the fact that the oral cavity is an organ which allows visual examination and ease of access. The current status of the prevalence of OSCC in Taiwan has been highlighted by the health and welfare ministry[27]. Programmes broadcast through the mass media to address the importance of detecting OSCC have been supported by the state for more than a decade. In the meantime, funding has been distributed through several channels including general health auxiliaries in first-line public health care institutes, dentists in general practice, oral surgeons and Ear, Nose and Throat (ENT) doctors in the medical center[3,4]. Furthermore, constant public funding support is needed to aid both basic research scientists and clinicians of academic institutes, in order to develop new approaches for OSCC detection, diagnosis and therapy. The long-term goal of investing funding into increasing the survival of OSCC patients remains to be seen. Previous reports revealed that 90% of male OSCC patients were both smokers and chewers of betel quid [3,4,24,25]. Clearly the high-risk group accounting for one tenth of the population in Taiwan should receive a periodic screening with priority. Meanwhile, a follow-up system has recently been established to record and monitor the cancer patients and patients with pre-cancer lesions being treated in the hospital[27,28].

Premalignant lesions and chemoprevention

Attention should specifically be focused on premalignant lesions[29-32]. Although the potential malignant effects of the erythroplakia has been reported to be a lot higher than that of the leukoplakia, which are amongst the most commonly seen premalignant oral lesions. Some are idiopathic but most are related to habits of BQ use, cigarette smoking and alcohol use. Reviews from Western countries revealed that 80% leukoplakias are

benign with no evidence of dysplasia and no tendency to malignancy, but clearly a biopsy is required to define the remaining 10% to 20% that are either dysplastic or are already invasive carcinomas[30]. However in the past there was no histological means or other method for reliably predicting which leukoplakias are indeed potentially malignant. Scientific evidence-based research reports strongly supported the fact that the tumour transformation from normal epithelial cells to leukoplakias with aberrant gene expression was closely linked to betel chewing[5-19,33-35]. Overall the rate of malignant transformation of leukoplakias is about 3% to 6% over 10 years but much higher rates have been reported. The medical management of leukoplakia includes reducing or quitting habits related to risk factors, increasing the intake of fruit and vegetables in the diet and possibly the use of active agents[31]. Retinoids, carotenoids and topical cytotoxic agents inducing apoptosis, use to show potential but were given up largely due to their side effects or uncertain prognosis[31,36-38]. However, newer therapies are on the horizon. Recently, ingredients of green tea and curcumin have demonstrated promising potential for the chemoprevention of OSCC[39-42].

Role of health care workers

Health care workers need to clearly understand their roles in cancer screening. It is sometimes argued that OSCC screening is not necessary because routine dental examinations should include a full oral mucosa examination. However, apart from the fact that more than 50% of the population aged over 45 do not attend the dentist on an annual basis, there is evidence that many cases are missed, even by dental practitioners[32,33]. This is probably because early lesions are not specifically looked for or may appear to be innocuous and are ignored. Therefore, other professions or specialties may also need to be included in the screening programme. Screening for OSCC is a simple, non-invasive procedure, which can be easily integrated into the comprehensive assessment of the high-risk population of betel users and smokers, who account for the major part of OSCC patients in Taiwan. Furthermore, geriatricians might feel comfortable performing an OSCC screening examination[31,32]. Since 5-year survival rates are over four times greater in individuals with localised lesions than in those with distant metastases, the detection of early OSCC can make a significant contribution[21]. The aged population at risk of OSCC visit their dentist far less frequently than they see their physician[20]. The primary physicians looking at sore throats every day, could take a few extra minutes in order to carry out a thorough oral examination, which could benefit the patient. If primary care physicians carried out routine screening for OSCC, long-term survival rates may show potential improvement[43-45].

Methods of screening

Abundant reports have been documented regarding miscellaneous methods of OSCC detection and screening over a decade[32,43-47]. Physical examination includes a self-examination and clinical examination by health care workers. 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 min to perform an oral examination by a specialist. The goal of the examination is to detect any nodules, swellings, mucosa alterations (ulcerations, textural or colour changes) and unexplained lymph adenopathy[43-46]. Whilst many routines exist during an oral examination; each clinician must develop his or her own method; use it in all patients and they must carefully document positive findings for biopsy or referral. The toluidine or methylene blue staining is an easy method, which uses the dye that has affinity to cancer cells[48,49]. Commercial kits with protocols are available for large scale screening of high-risk populations, either by topical application or by mouth rinsing. Yet for candidates with oral field cancerisation, the rinsing or gargling of the staining dye is recommended instead of using the topical spray. Meanwhile, a biopsy is still needed before a diagnosis can be confirmed. An excision biopsy is definitely sufficient for analysis of a small 0.5 to 1.0 cm lesion. However, multiple incision biopsies are more appropriate in lesions with areas larger than 3 cm. The large and superficial cancerised area may also be stained with dye to define highly suspected locations which take priority of biopsy. In a clinical study using methylene blue stain as a diagnostic aid in the detection of cancer and pre-cancer, there was no doubt that the staining dye was, to some extent, useful to health care auxilliaries in the large scaled screening programme. It provided a 90% sensitivity rate and a 75% positive predictive value. However, a 10% false negative still existed in true cancer cases[48,49]. Often the cases encountered had no definite location of lesion but presented with a picture of panoral premalignant and field cancerisation in high-risk patients with a history of BQ use and who had smoked for 20 to 30 years[29,32]. It can be difficult to perform an incision biopsy at precise locations of suspected regions under such panoral field cancerised conditions. Therefore, some researchers or clinicians claimed using an alternative method of screening by exfoliative cytology, which was collected

by tissue scraping. However, exfoliative cytology in the screening for OSCC had never achieved the same success as it did for diagnosing cancer of the uterine cervix. Oral exfoliative cytology gained much attention in the 1960s, eventually losing validity, due largely to the subjective nature of its interpretation[46]. 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 of cancer detection swings from the morphological picture towards the molecular level, whereby we foresee a new role for exfoliative cytology. Greater understanding of genetic aberrations may predict not only the biological behavior of the tumour but its potential response to both traditional and novel forms of therapy. Previous thoughts regarding the absence of a marker, present in all OSCC lesions but never seen in benign lesions or normal epithelium, limited its clinical utility in the past[32,50,51]. The other drawback of exfoliative cytology for screening OSCC was that the lesion still had to be identified or anatomically located for biopsy confirmation before a surgery could be initiated. Technical problems may also easily be encountered in cases with field cancerisation in the BQ chew group. The experimental data provided by Chang and Lin's report showed their initial success in the detection of certain oncogenes 3q[26,27] in the brushed buccal cells of betel chewers[40,52]. The recent application of techniques, based on molecular biology has to some extent, refined its potential role. It remains to be seen if exfoliative cytology can be developed from research tools into tools used in routine clinical practice[53,54].

Pure saliva secreted from the major or minor salivary glands without cellular content used to have no significant contribution for OSCC detection. However, saliva containing the exfoliated cells from scraping or natural exfoliation combined with cytospin may provide a new approach. Utilising cytospin preparation from the saliva may potentially increase the collected cellular contents for analysis. Some oral cancer cytokeratin markers have been detected occasionally at a higher level within serum in OSCC patients. Recently, two important biomarkers miR-21 and miR-31 were shown to increase in the plasma and saliva of oral pre-cancer and cancer patients. The level of miR-21 and miR-31 correlates well with a tumour decline, in other words, the detected level of miR-21 and miR-31 decreased after tumour ablative surgery[55,56]. The newly developed microarray/gene chip technology which offers more reliable tumour markers will encourage us to seek a new approach for cancer screening. Furthermore, a breakthrough in the early diagnosis of oral pre-cancer/cancer by detecting a specific biomarker from the saliva or plasma may not be far away in the future[19,55-59]. Another choice of visual technique for OSCC detection is the imaging tool with the potential for the detection of primary, recurrent or secondary primary SCC in the oral cavity or hypopharynx. The predisposed relapse or recurrent site of OSCC tends to still occur in the oral cavity. Chu et al successfully used Narrow Band Imaging (NBI) for early detection of pre-cancer and recurrent lesions in treated OSCC cases[60,61].

Mechanisms of OSCC formation

Similar to a well-established colorectal carcinoma model, OSCC is also considered to be a multi-step process involving a number of aberrant genetic events culminating in malignant transformation at the molecular level[1,2]. It is widely understood that following the action of various carcinogens (chemical, physical, biological) on normal cells, a long period (latency) of several months to years (10 months to 30 years) in humans occurs between the development of pre-cancer cells and their transformation into cancer cells[5-8,11-15,22,23]. From the viewpoint of cancer prevention, the molecular and biological events that take place within the pre-cancer cells during this quiescent stage certainly warrants investigation. It is well known that preneoplastic cell development and transformation into cancer cells is determined initially by genetic (oncogenes, anti-oncogenes) changes, with sequential multiple somatic mutations, and later by epigenetic or environmental cell factors such as hormones, growth factors (GFs), cytokines, vitamins, and prostaglandins (PGs). These factors can markedly change the evolution of preneoplastic cells by enhancing, retarding or inhibiting their transformation into cancer cells; or even reversing them into a normal phenotype[1,2]. These effects act on DNA, RNA, and protein synthesis, as well as on cell replication, cell cycles, cell surfaces and intercellular communications. Therefore, these abnormal DNA, oncogenes or tumour suppressor genes, and ultra-structural intracellular or cell surface antigenic determinants acting as potential biomarkers, are essential for early detection of preneoplastic cells and cancer cells[7,10,22]. A significant recent advancement revealed the gradual understanding of the molecular mechanism of cancer formation. Although, a universal tumour marker might still be lacking, a combination of several markers may be useful and more accurate than ever. In particular, the last 10 years has seen a shift in diagnostic methods from the histopathological to the molecular level[11-13,53,54]. Several recent novel findings related to the malignant transformation

of OSCC include epithelial-mesenchymal transition (EMT), which may play a key role in OSCC progression. It was found that the down regulation of adhesion molecules and the upregulation of mesenchymal markers in OSCC cells are controlled through a cascade of gene expression and regulation events in the Twist, Snail, SIP1 pathway. This allowed cancer cells to transform more invasively from carcinoma in situ (CIS) to invasive carcinoma. Therefore, we may develop novel approaches in this pathway to target EMT, to intercept the progression of OSCC and tumours opposed to therapy, to focus on exploring the molecular mechanisms of EMT, to look at the cancer contributing to local invasion and treatment resistance (core phenotype) and further to discover novel biomarkers and new treatment strategies for clinical trials[62-64].

Conclusion

With advancements in modern molecular and cell biology at the knowledge and technique level, a lot more new markers in OSCC were studied and found. Significant momentum has been devoted to the exploration of the molecular mechansim of the malignant transformation of oral epithelial cells in relation to ras oncogenes, p53, p16 tumour suppressor genes; and the abnormality of other genes such as cyclin-D1, retinoids or retinoic acid nuclear receptors, telomerase, cancer stemness, EMT and so on. A new generation of oral cancer screening methods will favour a more efficient and reliable tool, based on previous contributions of scientists. The final goal of the research on oral cancer will be to identify new approaches for early detection, diagnosis and novel therapy to decrease the overall mortality of OSCC cases. However, the program of detection and screening of OSCC should be based on full government support, the cooperation of school education, the mass media, medical services and the general awareness of the whole population. Care should be taken in the policy or strategy planning for the cancer detection/screening program. As a health care worker, we need to know the important role of our profession in the screening and detection of oral cancer.

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