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RESEARCH ARTICLE

A SPECIAL REVIEW, ROLE OF ALCOHOL AND SMOKING IN THE SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK, ADVANCED DIAGNOSTIC AIDS IN ORAL CANCER

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ABSTRACT

Oral cancers are one of the most common cancers worldwide today. They are usually neglected by the common population when compared to systemic cancers such as the lung cancer, colon cancer, Betel quid chewers, alcoholism, and tobacco etc. These are the most common malignancies in South and Southeast Asian countries. Oral premalignancies are also very common in betel quid chewers and about 10% of these undergo malignant transformation .Alcohol and tobacco are responsible for a very large proportion of chronic disease and some tumors in particular may be the result of interactions between the two risk factors. The present systematic literature review was conducted to judge combined effects of alcohol drinking and tobacco, as well as and genetic polymorphisms on alcohol-related cancer risk. However, they also may be extremely fatal if left untreated even at a very initial stage of the lesion. Early detection and treatment gives the best chance for its cure. The five-year survival rate of oral cancer still remains low and delayed diagnosis is suggested to be one of the major reasons. The detection and diagnosis are currently based on clinical examination, histopathological evaluation of the biopsy material and molecular methods. Several diagnostic aids have been developed over the years for early detection of oral cancer. The purpose of this article is to review the advanced available diagnostic adjuncts for the detection of oral cancer.

INTRODUCTION

In our oral cavity, oral cancer is a life threatening disease (Burkhardt, 1985). It is a part of group of head and neck cancer which may arise as a primary lesion in any part of the oral cavity or oropharynx by metastasis from a distant site of origin. Oral cancer most commonly involves the tongue, floor of the mouth, buccal mucosa, gingiva and lips. In many Asian countries, especially India, chewing betel, paan and Areca are known to be risk factors for developing oral cancer. Several studies have been done in the past regarding the factors behind the diagnostic delay of Oral Squamous Cell Carcinoma (OSCC) but early detection of it still remains disappointingly constant over recent decades Oral cancer is one of the most common cancers in South and Southeast Asian countries, in contrast to accounting foronly 1 to 4% of the total malignant tumors in Western societies (Field and Spandidos, 1987). The age-adjustedrates of oral cancer incidence are 24.2/100,000 for males and 11.2/100,000 for females in Kerala, India (Nair *et al*.,1988) and 10.2/100,000 for males and 4.1/100,000 for females in Sri Lanka (Randeniya, 1987). There are strong indications for an association of the habit of betel quid chewing with cancers of the mouth, oropharyngealcavity, and upper parts of the digestive tract (Dave *et al*., 1992; Jayant *et al*., 1977; IARC Monograph, 1985). Moreover, chewing and smoking habits interact synergistically for these cancers (Jayant *et al*., 1977). Although some pathological (Pindborg, 1980), epidemiological (Boyle *et al*., 1990; Gupta *et al*., 1996; Hirayama, 1966; Mehta *et al*., 1981) and genetic (Chiba *et al*., 1998; Kashiwazaki *et al*., 1997) studies on oral cancer and pre-cancer have been already reported, the incidence of oral cancer is still very high in these countries and only limited efforts have been carried out for its prevention. An early detection of these cancers helps in better and faster treatment for improving the prognosis to some extent and the available advanced diagnostic adjuncts aid as a helpful tool for the early diagnosis of oral cancer to the medical practitioners in treating patients suffering from it. The methods for the inclusion of scientific articles in this review are liberal in nature. This review does not aim to be wholly comprehensive of all the literature on OPC; rather, it highlights the most relevant articles in the literature, and discusses the most important finding in each study. No exclusion criteria were applied when deciding which scientific articles would be included in this review. The search engines PubMed (Knobloch *et al*., 2009) and Google Scholar were used to locate the most relevant articles pertaining to oral and pharyngeal cancers, regardless of year published. Thus, this review includes studies from 1988 to 2009. Furthermore, a wide time (Collin *et al*., 2010). Frame was used to determine

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whether later studies differed in their results than earlier ones, and to assess how OPC related research has changed over time. Keywords in these searches included 'oral' and 'pharyngeal' and 'cancers'. Other keyword searches included 'oral and pharyngeal cancers', 'OPC malignancies', 'OPC risk factors', and 'OPC disparities'. Also, unlike conditions such as the metabolic syndrome (MetS) where the definition is not wholly agreed upon or standardized (Colin Hopper, Joel Epstein, 2008). There was no evidence in the literature that this was the case for OPC. The International Classification of Diseases, Tenth Revision (ICD-10) defines OPC as any malignant neoplasm of lip, oral cavity (tongue, major salivary glands, gum, floor of mouth, other and unspecified parts of mouth), and pharynx (oropharynx, nasopharynx, and hypopharynx) (Casto *et al*., 2009). OSCC can be a small problem in numerical terms, but it is considered as a highly lethal disease in world population (Binnie and Rankin, 1984). Lack of awareness in the public of the various signs, symptoms and risk factors for oral cancer are all believed to be responsible for the diagnostic delay in a long venture (Stefano, 2009). They are often difficult to diagnose by routine clinical examination. Diagnosis of these diseases is mostly based on the microscopic study of cells and tissues (Richard *et al*., 2002). Maryland, a District of Columbia has seventh highest overall mortality rate for oral cancer in the state, due to lack of information regarding educational materials and interventions for the public to promote oral cancer (Horowitz *et al*., 2002). Most of the oral cancers are OSCC. In past three decades, the five-year survival rate has improved but still remains in the range of 53% to 60%. Most OSCC is not diagnosed until an advanced stage, which has been one of the major reasons for minimally improved survival rate over the years (Jemal *et al*., 2009; Yi-Shing, 2011). Historically, the screening of patients with signs and symptoms of oral cancer and various precancerous lesions has usually been relied upon the conventional pattern, that is, oral examination (Lingen *et al*., 2008). Application of immunohistochemistry has proved to be a useful tool, laboratory diagnosis in oral cancer by the use of antigens and antibodies (Pettigrew, 1989).

Risk factors

Tobacco and alcohol

Tobacco use is understood to be the most important risk factor for the development of OPC (Peterson PE 2009). Much of the literature on OPC risk factors is focused specifically on tobacco use. For example, Rodriguez *et al* (2004) analyzed data from two case-control studies including 137 cases of OPC and showed that the multivariate odds ratios (OR) for having OPC for heavy smokers was 20.7 in young adults from Italy and Switzerland. Rodriguez *et al* found that the OR for heavy drinkers for OPC was 4.9 (Boyle *et al*., 1990). However, when the categories of heavy drinkingand smoking were combined, an OR of over 48 was observed. The authors also found that tobacco accounted for 77% of OPC cases in the examined population, alcohol for 52%, low vegetable consumption for 52%, and the combination of the three for nearly 85% of all OPC (Rodriguez *et al*., 2009). A separate case-control study performed on Italian and Swiss men also found large risk increases for oral cancer (OR = 228) and pharyngeal cancer (OR = 100) for the highest level of drinking (≥ 77 drinks/week) and smoking (≥ 25 cigarettes/day) combined (Gallus *et al*., 2009). The authors of this study found there to be synergistic effects of smoking and alcohol consumption on OPC. The authors found interesting independent factors in that if alcohol consumption increased while smoking levels were stable, the increase in oral cancer would be greater than the increase in pharyngeal cancer. This was shown to be the case in this study because the authors explain that the ratios of ORs between oral cancer and pharyngeal cancer was about 2-times greater for oral cancer than for pharyngeal cancer for each combined level of smoking and drinking. In addition, alcohol consumption has been shown to be associated with increased odds of OPC among never smokers (Fioretti *et al*., 1999) examined 42 cases of OPC among never smokers and found that the major risk factor for OPC in never smokers was alcohol consumption, with an OR three-fold higher in drinkers than non-drinkers (Franseschi *et al*., 2009). Another case-control study that confirmed a multiplicative synergism between smoking and alcohol consumption on OPC examined 1,114 cases and 1,268 controls. Similar results were found as the above studies in that among those that smoked two or more packs of cigarettes and four or more drinks per day, there was an observed 35-fold increase in the risk of OPC.

The authors estimate that smoking and drinking combined account for about 75% of all OPC in the United States (Blot McLaughlin Winn *et al*., 1988). A similar study in a Spanish male population found that tobacco smoking was associated with OPC with an OR of 27.7 (Varela-Lema *et al*., 2009). These studies combined confirmthe magnitude that smoking and alcohol consumption have on OPC. Moreover, a study examining the separate effects of alcohol in non-smokers and smoking in non-drinkers also found results similar to the above studies cited. This study was performed essentially to test the relative independence that smoking and alcohol consumption has on the development of OPC. The ORs were 1.5 for 14-55 versus 0-13 drinks per week, and 2.2 for 56 drinks or more in non-smokers. The ORs for non-drinkers who smoked were 3.8 for smokers of < 15 cigarettes per day and 12.9 for ≥ 15 cigarettes per day (Talamini *et al*., 1990). This is an important study in the literature because it establishes the independence that smoking and drinking have on the development of OPC. Interestingly, two studies found that among male current smokers, those that used filter cigarettes had a reduced risk of oral cancer than those who used non-filter cigarettes (Kabat *et al*., 1994). However, little research has been performed on this topic, thus the results should be interpreted with caution.

Dietary risk factors

Less research has been focused on risk factors besides smoking and drinking and their relationship with OPC. However, there is some evidence that meat, vegetable, and vitamin intake may be related to OPC. A case- control study on meat consumption in Uruguay among nearly 4,000 cases showed a significant increase in the odds of having OPC (OR = 3.65, 95% CI 2.21-6.01) with a high intake of red meat (Aune *et al*., 2009). Several studies have also found significantly protective effects of fiber intake on OPC (OR = 0.29, OR = 0.40).Dietary vitamin C consumption of > 745 mg/week was also shown to protect subjects from developing OPC (OR = 0.39).Another study also found that vitamin C had a similar protective effect (OR = 0.63) (Negri *et al*., 2000).

One study, however, found that vitamin C was protective of OPC, but noted that vitamin C's effect was difficult toseparate from the effects of fruit and vegetables intake (Bosetti *et al*., 2009). Vitamin E supplements (i.e., "ever regularly used") have also been found to be associated with a significantly reduced OPC risk with an adjusted OR of 0.5 (95%CI 0.4-0.6). Other studies have also shown that consumption of fruits (OR = 0.2), raw (OR = 0.3) and cooked vegetables (OR = 0.1), and fish (OR = 0.5) had inverse risks and protective effects on OPC (Conti *et al*., 2000). Similar results were found among 414 cases in Shanghai, China, in that the risks of OPC development decreased with an increased intake of oranges, tangerines, other fruits, and some dark yellow vegetables, and white radishes. The authors found that men in the category with the highest intake of fruits and vegetables had an OR of about 0.5-0.7 when compared to men in the lowest group of fruit and vegetable consumption (Rossi *et al*., 2009). Vitamin D intake was also found to have an inverse risk on OPC (OR = 0.76). The same study found that the OR for OPC among heavy smokers with low dietary vitamin D intake was 10.4 (95%CI 6.9-15.5) and an OR of 8.5 (95%CI 5.7-12.5) among heavy alcohol drinkers with low dietary vitamin D intake (Schoenberg *et al*., 1992). When looked at as a whole, these studies provide substantial evidence that dietary factors play a very important role in OPC development.

Betel Quid Chewing Habits

Oral cancer was described in the Sushruta Samhita, a treatise on Indian surgery written in Sanskrit around 600 B.C. In addition, literary references to the habit of chewing betel quid (betel leaf, areca nut and lime) in India are atleast 2,000 years old. Tobacco was introduced around the sixteenth century. It is estimated that at least 200 million individuals consume areca nuts in one form or another worldwide. The habit is now widespread in Southeast Asia and the South Pacific islands and in people of Indian origin elsewhere in the world. The betel quid chewing habit is in fact found all over the world wherever Indians have settled. There is some confusion in the reporting of "betel quid" and tobacco chewing habits. At a recent workshop in Kuala Lumpur (Zain *et al*., 1997; Zain *et al*., 1999), it was recommended that "quid" be defined as "a substance, or mixture of substances, placed in the mouth or chewed and remaining in contact with the mucosa, usually containing one or both of the two basic ingredients, tobacco and/or areca nut, in raw or any manufactured or processed form." There are several types of chewing habits in India featuring use of betel quid (fresh betel leaf, fresh areca nut, slaked lime, catechu and tobacco), pan masala (areca nut, slaked lime, catechu, condiments and tobacco), mainpuri (tobacco, slaked lime, areca nut, camphor and cloves), mawa (areca nut, tobacco and slaked lime), khaini (tobacco and slaked lime), gutka (an industrially manufactured food item) and other smokeless tobaccos (mishri, gudhaku, bajjar etc.). In Sri Lanka, the betel quid is composed of fresh betel leaf, fresh areca nut, slaked lime and tobacco, and they are introduced together inside the mouth. In Papua New Guinea, betel quid chewing is practiced in a different way as compared to the rest of Southeast Asia. Areca nut, betel leaf and slaked lime are introduced into the oral cavity separately; however, tobacco is never included in these preparations (IARC Monograph, 1985). There are many reasons for chewing betel; it causes euphoria, increases salivation, satisfies hunger, relieves tooth pain and ameliorates nausea in pregnant women. Because of these reasons, it is very difficult to persuade people to quit betel quid chewing.

Carcinogens in Betel Quid Ingredients

The major areca nut alkaloids are arecoline, arecaidine, arecolidine, guvacoline and guacine (IARC Monograph, 1985). Arecoline (1, 2, 4, 5, -tetrahydro-1-methylpyridinecarboxylic acid; molecular weight 155.19) is the most abundant alkaloid of areca. These alkaloids undergo nitrosation and give rise to N-nitrosamines (Hoffmann *et al*., 1994). It has been suggested that metabolic activation may involve the cytochrome p450 system (Sundqvist *et al*., 1991; Wary and Sharan, 1991). The nitrosation of are coline may produce a variety of betel quid-specific nitrosoamines (BQSN). The BQSN interact with DNA, proteins or other targets forming adducts to exert its carcinogenic activity. The introduction of tobacco from European countries reinforced this practice, and now almost all habitually chewed betel quid's include tobacco. A comparison of the carcinogenicity of the habit of chewing betel quid with and without tobacco has been attempted through a reassessment of the available epidemiological evidence on the etiology of oral cancer and pre-cancer; however, the role of tobacco use in the carcinogenicity of betel chewing is still unclear (Gupta *et al*., 1982). Analytical studies should clearly distinguish between chewing habits with tobacco and without tobacco. Slaked lime is also included in betel quid. It causes inflammation in the sub-mucosal area and Nair *et al*. (1990) have reported that the calcium hydroxide content of lime in thepresence of the areca nut is primarily responsible for the formation of reactive oxygen species that might cause oxidative damage in the DNA of buccal mucosa cells of betel quid chewers (Nair *et al*., 1990). In addition to these ingredients of betel quid, tooth attrition caused by the chewing action is also important for the establishment and development of oral cancers. The oral mucosa could thus be injured by the keen edges of teeth resulting in facilitated exposure and regeneration.

Oral Lesions Caused by Betel Quid Chewing

Oral cancer occurs more commonly among men than women depending upon the extent and type of tobacco habits prevalent. Betel quid chewing is the major risk factor for buccal mucosal and gingival cancer. For the tongue cancer most frequent in Western countries smoking is the major risk factor. Leukoplakia is one of the commonest lesions in betel quid chewers. The WHO has classified these into two groups, homogeneous and non-homogeneous. Among nonhomogeneous leukoplakias, nodular leukoplakia tends to show the highest rate of malignant transformation. The relative risk compared with individuals with tobacco habits but without any precancerous oral lesion was also found to be the highest for nodular leukoplakia (Gupta *et al*., 1989). Oral Submucous Fibrosis (OSMF) is a chronic condition characterized by mucosal rigidity of varying intensity due to fibroelastic transformation of the juxta-epithelial layer (Murti *et al*., 1995). OSMF is a high-risk precancerous condition (Pindborg *et al*., 1984) with a malignant transformation rate of about 7.6% (Murti *et al*., 1985). Areca nut chewing could be one of the

most important etiologic factors in OSMF (Sinor *et al*., 1990). Oral lichen planus may be important for malignant transformation, although its nature remains unclear (Murti *et al*., 1986). It has been categorized as a "probable precancerous condition (Mehta and Hamner, 1993)."

Genetic Alterations

Little is known of the genetic events involved in the progression of precancerous status to oral cancer (Pillai *et al*., 1991). Moreover, most molecular studies have been performed on populations from developed countries in which the rates of incidence of oral cancers are relatively low. Genetic events related to betel quid chewing have, however, been reported for some genes.

p53 gene

Results of immunohistochemical analysis of p53 are controversial. Some researchers have reported p53 expression in oral cancer of betel chewers (Kaur *et al*., 1994; Kuttan *et al*., 1995; Ranasinghe *et al*., 1993). p53 expression could be correlated with malignant potential of precancers and prognosis; however, there are some reports that there is no significant relationship with their likelihood of malignant transformation (Murti *et al*., 1998). Rather than immunohistochemistry, mutational analysis of the p53 gene using PCR-SSCP, a yeast functional assay and sequencing is informative (Kashiwazaki *et al*., 1997). Using these methods, we detected mutations in 43% of oral cancers in betel quid chewers in Sri Lanka (Chiba *et al*., 1998). Moreover, these mutations were clustered in exon 5, suggesting that this site could be one of the specific targets for some carcinogens in betel quid ingredients (Chiba *et al*., 1998).

Ras Genes

Mutations in the H-ras gene are more frequent in oral cancers of betel quid chewers (Saranath *et al*., 1991) than those in Western countries (Chang *et al*., 1991; Warnakulasuriya *et al*., 1992; Xu *et al*., 1998; Yeudall *et al*., 1993) and Japan (Matsuda *et al*., 1996; Sakai *et al*., 1992; Sakata, 1996). Ki-ras mutations have also been reported in oral cancers of betel quid chewers in Taiwan (Kuo *et al*., 1994). These observations suggest that there are genetic and etiological differences in oral cancers between populations from these geographical areas.

Field Cancerization

In 1953, Slaughter *et al*. (Slaughter and Southwick, 1953) demonstrated "field cancerization" from the pathological point of view. Chronic exposure to alcohol and tobacco causes field cancerization. This concept should be considered in molecular biological studies of oral cancer (Waridel *et al*., 1997) reported that multiple biopsies of histologically normal tissue from the upper aero-digestive tract were tested and clonal p53 mutations were identified in 76% (38/50) of biopsies from patients presenting with multiple tumours compared with 32% (38/117) of biopsies from patients presenting with single tumours. (Jang *et al*., 2001) reported that lesions in the majority of multiple oral cancers and precancers arise from clonally independent cells affected by field cancerization. "Normal" oral mucosa as well as the precancer or cancer of betel quid chewers could be genetically affected by betel quid ingredients.

Glutathione-S-Transferases and Cytochrome p450s

Molecular epidemiological examinations have provided evidence that oral cancer susceptibility is also mediated by genetic and epigenetic factors. Although betel quid chewing is clearly established as the main risk factor for oral cancer, only a small proportion of betel quid chewers develop significant lesions, suggesting the presence of inherited differences in the genes of enzymes which detoxify or activate carcinogens. Glutathione S-transferases (GSTs) are involved in detoxification of carcinogens and homozygous deletions are associated with higher risks of cancer. Homozygous deletion of the GST μ class isozyme (GSTM1) gene has been shown to occur in approximately 50% of populations of various ethnic origins (Kiyohara, 2000), while homozygous deletion of the GSTT1 gene occurs in between 10 and 64% in various ethnic groups. In oral cancers, an increased risk for developing premalignancies in null genotypes of GSTM1 and T1 has been reported (Nair and Bartsch, 2001; Nair *et al*., 1999). Cytochrome p450s regulate the expression of enzymes that convert procarcinogens to their ultimate carcinogenic forms (Sundqvist *et al*., 1991; Wary and Sharan, 1991). The nitrosation of arecoline, which contains a 3- ethylenic bond at the 3-4 position on the pyridinium ring, may produce a variety of betel-nut-specific nitrosoamines (BSNA). Although cytochrome p450 CYP2D6, CYP1A1 and CYP2E1 loci have been examined for oral cancer patients and control individuals, there are no differences between them in the frequencies of presumed risk genotypes (Matthias *et al*., 1998).

Advanced Diagnostic Aids for Oral Cancer

Vital Tissue Staining–Toludine Blue Staining & Lugol's Iodine

Oral carcinoma in situ and early invasive oral carcinoma shows affinity for toluidine blue dye. Lugol's iodine and toluidine blue have been used together in the detection of early carcinomas and other oral lesions. Toluidine blue is an acidophilic meta chromatic dye which selectively stains acidic tissue components, thus staining DNA and RNA. As it binds to nucleic acids (DNA or RNA) , it helps in better visualization of high risk areas especially with rapid cell proliferation of OSCC and premalignant lesions (Pegah *et al*., 2012). It stains mitochondrial DNA, cells with greater than normal DNA content or altered DNA seen in dysplastic and malignant cells. Lugol's solution is used for delineation of the malignant change which produces a brown black stain when the iodine reacts with the glycogen content. The use of toluidine blue and Lugol's iodine serves as a useful adjunct in the diagnosis of patients who are at risk and for selecting the site for biopsy with wide field cancers prior to treatment (Sujata and Ajit, 2006).

Vizilite

Vizilite is a nontoxic chemiluminescent light. Today, vizilite Plus examination, in combination with the regular visual examinations, provides a comprehensive oral screening procedure for those patients who are at increased risk for oral

cancer. Vizilite Plus technology helps in identifying soft tissue abnormalities which is shined inside the mouth. This shows glowing of abnormal tissue different from that of normal tissue thus making it more visible. The technique is painless and fast and can help in saving life (Sujata and Ajit, 2006). To improve early detection of oral cancer, the use of a dilute acetic acid rinse and observation under a chemiluminescent light such as ViziLite is usually recommended (Oh and Laskin, 2007).

Brush Cytology

Brush cytology (Oral CDX), developed in 1999 and has become popular in dental practice today. In the past decades, adjunctive technique has facilitates the early detection of Oral Premalignant and Malignant Lesions (OPML). In that context, Oral CDx is useful in the assessment of dysplastic changes in various suspected lesions especially in oral cancer (Patton *et al*., 2008). As majority of oral cancers are squamous cell carcinomas, Cytological study of oral cells is a relatively inexpensive, simple, noninvasive and also risk-free technique which is well accepted by the patient and medical practitioner today (Smaroula *et al*., 2009). The oral cells can be obtained by the use of a cytobrush. With brush cytology, sensitivity for detecting oral epithelial dysplasia or Oral squamous cell carcinoma is high (Yi-Shing and John, 2011). But, the technique has attracted lots of controversies and more incidences of false negative results with this technique (Sujata and Ajit, 2006) has been encountered.

VEL scope

The VEL scope Vx is one of the most powerful tools available today for assisting in oral abnormalities especially oral cancer. The distinctive blue-spectrum light causes the soft tissues of the mouth to naturally fluoresce. The use of VEL scope Vx is a safe and simple technique and the entire examination can be done in about two minutes. However, it is a relatively new device and so far only a limited number of studies have been done on its effectiveness as a diagnostic adjunct for oral cancer (Yi-Shing and John, 2011).

***In-Vivo* Confocal Microscopy**

Confocal microscopy is an imaging technique for various researches in cell biology with an advantage of optical sectioning and high resolution imaging. *In vivo* confocal images from the oral cavity show the characteristic features such as nuclear irregularity which is used to differentiate OSCC from normal oral mucosa. However, further optimization of the instrument is still needed to rate it a promising non-invasive tool for the early detection of oral cancer (Yi-Shing and John, 2011).

Saliva-based oral cancer diagnostics

The concept of saliva to diagnose OSCC is a latest concept. Oral fluid or the saliva is a noninvasive, accessible and highly efficient diagnostic medium today. The utility of salivary transcriptome diagnostics are helpful in the detection of oral cancer (Li *et al*., 2004). Promoter hypermethylation patterns of TSG p16, O6- methyl guanine-DNA-methyltransferase, and death associated protein kinase are identified in the saliva of head and neck cancer patients and high salivary counts of *Capnocytophagagingivalis*, *Prevotellamelaninogenica*and *Streptococcus miti*sis found in patients with OSCC. However, it is still difficult to support the suggestion that this could be a reliable diagnostic indicator (Crispian *et al*., 2008). Today, saliva testing for genetic patterns which are linked with oral cancer is gaining interest of research but still it has not been incorporated as a commercial product, but researchers are hopeful that this technology will be readily available in the market very soon (Tricia and Suzie, 2008).. The use of saliva for the detection of oral cancer has proved to be a historical goal that has to be reached to the population in the future for better and faster management of OSCC (Wong, 2006).

DNA Ploidy & Quantification of nuclear DNA content

DNA ploidy is the measurement of nuclear DNA content that provide a measurement of gross genetic damage. If the chromosomes are not uniformly distributed to the daughter cells during mitosis or if some parts of chromosomes become detached, the chromosomal segregation becomes unbalanced and aneuploidy is seen which is commonly observed in many cancers. DNA image cytometry shows high sensitivity and serves as a non-invasive method for cancer (Crispian *et al*., 2008). Pre-malignant lesions such as oral leukoplakias, the nuclear DNA distribution patterns can be analyzed by flow-cytometry, showing different rates of dysplasia, however the quantity of specimens should be more for the examination (Grässel-Pietrusky *et al*., 1982). Even cytology with DNA-cytometry has emerged as a highly sensitive and non-invasive method for the early diagnosis of oral epithelial neoplasia and hence in oral cancer (Maraki *et al*., 2004).

Tumor Markers & Bio Markers

Tumor markers may be present in blood circulation, body cavity fluids, cell membranes and cell cytoplasm when released by cancer cells or produced by the host in response to cancerous substances. They are used in identification of a cancerous growth (SujataSatoskar and AjitDinakar, 2006). Tumour Suppressor Genes, oncogenes, cell proliferation markers, angiogenic markers and cell adhesion molecules are some of the potential tools which help in prediction for the prognosis of patients with OSCC. According to a study, use of cytokeratin markers are also used in detecting OSCC by the help of analyzing the altered keratin expression in the oral site especially the buccal mucosa (Vaidya *et al*., 1989).

PCR-Based diagnostic aids

The Polymerase Chain Reaction (PCR) is a scientific technique in molecular biology which can be used in the diagnosis and study of infectious diseases and malignancies associated with microorganisms. PCR helps in the study of cancer and provide clearer understanding of the pathogenesis of neoplasia. PCR can be used to detect mutations in cancer-associated oncogenes (e.g., K-ras, Nras), tumor suppressor genes (e.g., p53, p16) etc. and aids as an important detection tool (Richard *et al*., 2001; O'Leary *et al*., 1997). With the introduction of Polymerase Chain Reaction (PCR), reverse transcriptase PCR (RT-PCR) and other molecular techniques,

the diagnosis and prognosis of other lesions such as chronic myelogenous leukemia has also been useful (Glassman, 1998).

Auto fluorescence Spectroscopy

Auto fluorescence spectroscopy has emerged as a promising tool for oral cancer detection. The system consists of a small optical fiber which produces various excitation wavelengths and a spectrograph which receives and records on a computer and analyzes it with the help of software, the spectra of reflected fluorescence from the tissue. Overall, it seems to be very accurate for distinguishing lesions especially malignant tumors from healthy oral mucosa, with a high sensitivity and specificity (Stefano Fedele, 2009). It is a non-invasive aid in the detection of various alterations in the structural and chemical compositions of cells indicating the presence of a diseased tissue. It can be useful in guiding the clinician in identifying the optimal location for biopsy (Sujata Satoskarand and Ajit Dinakar, 2006). According to a study, on using violet excitation light, camera-based auto fluorescencephoto detection technique has presented as a highly promising tool for the diagnosis of oral malignancies (Betz *et al*., 1999).

Fluorescence Photography

Fluorescence photography is non-invasive, rapid, simple and reproducible method in detection of oral cancer. Fluorescence positivity can show enlargement of carcinomas and progression of the disease. The system is usually used in the diagnosis of squamous cell carcinoma. However, biopsies are still necessary (Sujata and Ajit, 2006). According to a study, fluorescence photography has shown as a useful tool for the diagnosis of oral cancer, especially in patients with squamous cell carcinoma (Onizawa *et al*., 1996).

PET scan

Our oral cavity is one of the cancer sites in the head and neck which is accompanied by a high incidence of regional metastasis. Due to cervical lymph node metastasis, there is significantly reduction in the survival of the patient and which is an important prognostic factor for scientific debate today. Positron Emission Tomography (PET) with fluorodeoxyglucose (FDG) is increasingly day by day as a useful tool in preoperative staging of cancer patients (Bart *et al*., 2006). The predictive value in determining lymph node status and thus helping in screening and early diagnosis of oral cancer in affected patients. Standardized uptake value (SUV) of the tumor mass helps in prognosis for overall survival of the affected patient (Gregory *et al*., 2010).

Oral Cancer Prevention Trials in Asia

Primary Prevention

Intervention studies for primary prevention of oral cancer in India by Gupta *et al*. have been very enthusiastic (Gupta, 1991; Gupta *et al*., 1989; Gupta *et al*., 1986; Gupta *et al*., 1992; Gupta *et al*., 1990; Gupta and Mehta, 2000; Gupta *et al*., 1995). Randomized intervention trials to evaluate oral visual inspection for the early detection and prevention of oral cancer started from 1986 in India. Trained health workers identify, recruit, examine and refer subjects with suspected lesions for confirmation and management (Mehta *et al*., 1986). Subjects with confirmed oral precancers are advised to quit using tobacco, and encouraged to undergo removal of excisable non-homogeneous oral leukoplakias, if present. A total of 36,471 tobacco chewers and smokers were selected from the rural population (Gupta *et al*., 1986). People in the intervention cohort were encouraged to give up their habits by personal advice and via the mass media. No educational intervention was provided to the control cohort. A reduced incidence of oral pre-cancerous lesions was reported after a primary prevention trial for 12,212 betel quid chewers and smokers was carried out (Gupta, 1991; Gupta *et al*., 1992).

Chemoprevention

A randomized intervention trial to evaluate the cancer preventive potential of vitamin A in subjects with nonhomogeneous oral precancerous lesions in Kerala, India, has been performed (Stich *et al*., 1989; Stich *et al*., 1988). Short-term administration of vitamin A is effective for oral precancer as the lesions were reversed; however, severe side effects were detected. A study involving supplementation of multiple micronutrients to 169 subjects with OSMF in Karachi, Pakistan, resulted in a significant relief of symptoms such as intolerance to spicy food, burning sensation, and difficulty in mouth opening, was observed (Maher *et al*., 1997).

Conclusion

To conclude this review, regardless of all the type of oral cancer, it is still unknown what really causes its development. Recent studies show that usage of tobacco may be the leading cause of oral cavity and oropharyngeal cancer development and it is believed that smokers are more likely to develop oral cancer than nonsmokers. Carcinogens, which are present in high concentration in tobacco and its products and are also the leading cause of cancer in lungs, esophagus and several other organs. Screening and early detection of oral cancer using various diagnostic aids mentioned herewith decrease the risk morbidity and mortality associated with oral cancer. There has been a dramatic increase in the development of many potential oral cancer screening techniques in last few years and still many researchers are on the look for any better and faster aids of diagnosing these life threatening cancers.

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