Diagnostic Potential of Epithelial Biomarkers in Oral Diseases-
Immunohistochemical Basis

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Abstract: The malignant potential of many epithelial disorders remains obscure till date. Several studies have shown greater inter and intra - observer variability study in the assessment of potentially malignant disorders. Although generally moderate or severe dysplasias (or in-situ carcinomas) show a greater predilection for malignant transformation than mild or non-dysplastic cases, carcinomatous transformation may also take place in non dysplastic cases. Analysis of mutations in tumors, particularly the mutations of p53 gene, PCNA and values of AgNORs are important in assessment of pre-malignant and early malignant disorders. Some epithelia markers, like ki-67, MIB-1 and cytokeratins are expressed more in potentially malignant disorders. Recently, role of non-collagenous bone proteins has been evaluated. This review article discusses various aspects of immune-histochemical biomarkers in evaluation of potentially malignant disorders.

Key Words: Bone proteins, Cytokeratins, Epithelial biomarkers, Immuno-histochemistry, Prognosis

I. Introduction

According to WHO definition (1978), premalignant lesion is defined as “a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart. Examples: leukoplakia, erythroplakia, palatal keratosis associated with reverse smoking. WHO (1978) defined an oral precancerous condition as “a generalized pathological state of oral mucosa associated with a significantly increased risk of cancer.” Examples: Oral submucous fibrosis, Lichen planus, DLE, Epidermolysis bullosa. Warnakulasuriya et al. have recommended abandoning the distinction between the terms “potentially malignant lesions” and “potentially malignant conditions” and to use the term “potentially malignant disorders” instead. According to them, the term “potentially malignant disorders” means that not all abnormalities may transform into cancer, but some may have an increased potential for malignant transformation. The WHO Collaborating Centre for Oral Pre-cancerous Lesions, established in Copenhagen in 1967, recognized three major problems that were attached to the importance of epithelial dysplasia in predicting malignant development: (1) The final diagnosis was essentially subjective. (2) Not all lesions showing dysplasia would eventually become malignant, and some even regressed. (3) Carcinoma developed from lesions in which epithelial dysplasia had not been diagnosed in previous biopsies. The problems in evaluating diagnosing oral pre-malignant lesions and conditions generally arise from: Lack of objectivity, arbitrary division of the grading, lack of calibration of criteria and grading, and evaluating the malignant potential on the basis of histological studies.

II. Malignant Transformation Of Various Disorders

Leukoplakia is most common premalignant, potentially malignant or precancerous lesion of the oral mucosa. The word leukoplakia is derived from two words, leuko meaning white and plakia meaning patch. Recently, cumulative role of genetic and environmental factors is implicated in its pathogenesis. The malignant potential varies from one place to other. According to Amagasa et al. (2006), the percentage of leukoplakia that progresses to invasive squamous cell carcinomas ranges from 0.13 to 17.5 percent and the rates of five year cumulative malignant transformation ranges from 1.2 to 14.5 percent. Gangadharan et al. (1971), in the study of 626 leukoplakias found malignant transformation rate to be 10 percent over a mean period of 8.9 months. Mehta et al. (1972) in a 10 year follow-up study of 117 leukoplakias found mean transformation rates of 0.9 percent only. Gupta et al. (1980) observed malignant transformation rate higher among women than men, and more in persons chewing tobacco. In their study they also found that patients with oral leukoplakia carry a fivefold higher risk of developing oral cancer than controls. Burkhardt et al. (1985) reported that mild, moderate and severe dysplasias have malignant potential of about 3%, 4% and 43% respectively. Zhang et al. (2001) analyzed 71 epithelial dysplasias from the floor of the mouth, ventro-lateral tongue, and soft palate, designated as high-risk sites and 56 epithelial dysplasias from other sites of the oral cavity, designated as low-risk sites. The results were not influenced by gender or smoking. They found that epithelial dysplasias from high-risk sites had a higher frequency of loss of heterozygosity and a pattern of loss associated with an increased risk of progression to malignancy. Bouquot et al. (2006) reported that severe dysplasia has an overall malignant transformation rate of about 16% with 7-50% range. Speight et al. (2007)
reported that moderate dysplasia have malignant transformation potential of 3-15%, whereas mild epithelial dysplasia show a very low risk (<5%). Silverman et al. (1976) in a study found that 11% of lesions altered clinically and exhibited increased tendency towards. Banoczy et al., found 13% of lesions undergoing malignant transformation.

Various prospective and retrospective epidemiological studies in several countries suggested that patients with oral lichen planus present a higher risk for developing squamous cell carcinoma than the general population. Van der Mei et al. in 1999, suggested that two thirds of the malignant transformation cases of oral lichen planus were not sufficiently documented to be considered and their study revealed an annual malignant transformation rate of 0.27%. Since 2005, WHO classifies oral lichen planus as a potentially malignant disorder. Various studied have indicated Oral submucous fibrosis (OSMF) as premalignant disorder. Murti et al. followed sixty-six patients with oral submucous fibrosis for a period of 17 yr (median observation 10 yr) in Ernakulam District, Kerala, India. Oral cancer developed in five (7.6%) patients. The malignant transformation rate in the same sample was 4.5% over a 15-yr observation period (median 8 yr). These findings impart a high degree of malignant potential to this condition. Dayal et al. (1979) in their study found OSMF associated with pemphigus and squamous cell carcinoma.

The progression rates for oral epithelial dysplasia are varied. Several studies have shown greater inter and intra - observer variability study in the assessment of various grades of dysplasias. Although generally moderate or severe dysplasias (in-situ carcinomas) show a greater disposition for malignant transformation than mild or non-dysplastic cases, carcinomatous transformation may also take place in non dysplastic cases. Very small lesions have been found to exhibit dysplastic changes.

III. Role Of Biological Markers

Various biological markers are used for the assessment of cancer risk. Identification of these molecular markers has shown promise as these are used for the diagnosis and future prognosis of malignancies. The recognition of some of the markers are thought to be important with reference to dysplasia’s since the reactivity and recognition of these molecules were positively related to different grades of epithelial dysplasia. The best-characterized markers for determining future cancer development in oral pre-malignant lesions could be divided into:

1. Genomic markers that include DNA content (ploidy), chromosome aberrations (allelic loss or gain) and changes in the expression of oncogenes and tumor suppressor genes
2. Proliferation markers
3. Differentiation markers including keratins and carbohydrate antigens.

Analysis of mutations in tumors, particularly the mutations of p53 gene, is useful tool in the epidemiology of human cancer for several reasons. P53 mutations are common in most types of cancer and p53 proteins typically have much longer half-life than the wild-type protein, the diagnosis of cancers that harbor mutant p53 is feasible by immunohistochemical detection of its accumulation within the cell. Iwasa et al., (2001) the proportion of lesions positive for PCNA, p53 and values of AgNORs parameters steadily increased from hyperplasia to mild, moderate and severe dysplasia, and SCC. Piattelli et al., (2002) investigated the expression and relationship of p53, bcl-2, MIB-1 and the apoptotic index (AI) in normal oral epithelium, leukoplakia and oral squamous cell carcinoma. They found a strong correlation between p53 over expression and cell proliferation (MIB-1) and the AI. An inverse relationship was found between bcl-2 expression and MIB-1 and AI. A significant inverse relationship was also found between p53 and bcl-2. A good positive correlation was present between AI and MIB-1 expression. Kovess et al., (2002) observed immunohistochemical reactions of Ki-67 and p53 in 15 leukoplakias and 3 OSCC. They examined that the severity of dysplasia, mitotic and apoptotic index and expression as well as distribution of Ki-67 and p53 were related to the clinical appearance of leukoplakia. They found that mitotic index, apoptotic index and Ki-67 expression were increased significantly in parallel with the severity of dysplasia and also with the clinical stage (homogenous, nodular and erythroleukoplakia). Farrar M (2004) applied a panel of monoclonal antibodies (AE1/AE3, cytokeratin [CK] 14, Ki-67 and p53) to 10 cases of human oral tissue in each of six categories to establish staining patterns indicative of its likely progression to malignancy. The six tissue categories were the normal tissue, abnormal benign lesions, mild, moderate and severe dysplasias and SCC. The results showed that AE1/AE3 and CK 14 expression was reduced particularly in poorly differentiated SCC presuming it to be a late event in oral carcinogenesis. Expression of Ki-67 and p53 proved to be statistically significant predictor of malignant progression in oral tissue.

Increased expression of K1/K10 expression is a feature of oral hyperkeratotic lesions. It is believed that the histologically apparent keratosis can be due to increase in K1/K10 expression. Anand Lalli et al., (2008) in their immunohistochemical study found an increase of K1 and K10 in the suprabasal layers, induction of K6 in the basal layer and complete loss of K19 in the epithelium. Furthermore, there was increased K17 expression in
the suprabasal layers, which correlated with disease severity. In a subset of the most severe OSF cases, K17 expression was completely lost in the basal layer. They proposed that keratin K17 may be most susceptible keratin involved in malignant transformation. They concluded that the altered keratin profiles could be useful as histological diagnostic markers and these may provide important insights into the pathogenesis of the disease and its predisposition to malignancy.

High cytoplasmic OPN staining was observed in various potentially malignant disorders in various studies. Several studies have reported little or no osteopontin in normal oral tissues. Devoll et al. (1999) detected osteopontin (OPN) in significant percentage of premalignant and malignant lesions while normal epithelium expressed no change. In their study, osteopontin was detected in a significant percentage of premalignant and malignant oral lesions, but not normal oral epithelium. Localization of osteopontin was seen in a high percentage of hyperplasias (75%). This, according to them, is suggestive of fact that osteopontin expression may be associated with benign changes not directly related to malignancy. They inferred that osteopontin expression may represent an early event in tumor progression. The expression of osteopontin was found to be consistently associated with the middle epithelial layers in premalignant lesions and early malignant disorders. Zhu et al., (2008) found serum concentrations of OPN and TNF-a were significantly higher in OLP patients than the normal control group. Osteopontin is known to have a potential prognostic role in evaluation of epithelial disorders.

IV. Conclusion

Besides epithelial markers, non-collagenous bone proteins have been used in evaluation of potentially malignant disorders. In future, biological markers will play an important role in diagnosis and prognosis of potentially malignant disorders.

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References


