REVIEW ARTICLE

A REVIEW OF THE MALIGNANT POTENTIALS OF ORAL PRECANCEROUS LESIONS

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ABSTRACT

In order to improve early detection, it is imperative to increase the health-care providers' depth of knowledge about oral cancer, their risk factors and the most common oral precancerous conditions with malignant potentials. The present study identifies the clinical and histologically relevant parameters of malignancy since it is not every precancerous lesion that has the potentials of turning into cancer. Emphasis was placed on the role of molecular biology in early detection and future management of precancerous lesions with malignant potentials.

Currently, the new innovative visual-based techniques show promising results, but lack strong evidence to support their effectiveness in early detection. Their utilization in clinical practice is still anecdotal. However a combination of molecular investigation techniques, with these visual aids and saliva based diagnostic techniques show a promising future in early detection of oral precancerous lesions.

Key words: Oral cancer, precancerous lesions, risk factors

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Introduction

Oral cancers are often preceded by visible clinical changes in the oral mucosa, usually in the form of white or red patch. Prevention and early detection of such potentially malignant disorders (PMDs) will decrease the incidence and improve survival of those who develop oral cancer.[1-4]

Public awareness about the signs, symptoms and risk factors of PMDs is still very poor. In addition, health-care provider's acquisition of knowledge and necessary skill for management of PMDs is low. Both have resulted in the delay in identifying and early diagnosis of PMDs.[5] The ultimate goal of this review is the reduction of oral cancer mortality through the update of health-care providers about the high-risk PMDs.

1. Clinical factors associated with increased risk of malignancy

Only a small fraction and not all PMDs turn malignant, therefore the challenge has been to identify the high-risk lesions that could turn malignant. Researchers found that greater than normal risk for malignant phenotype are associated with the following clinical parameters:

A. Red and white intermixed lesions.

Two main clinical types of leukoplakia (Figure 1a) are recognized, being homogeneous and non-homogeneous leukoplakia. The distinction of these is purely clinical, based on surface colour and morphological (thickness) characteristics, and do have some bearing on the outcome or prognosis. Homogeneous lesions are uniformly flat, thin and exhibit shallow cracks of the surface keratin. The risk of malignant transformation is

relatively low. On the other hand, non-homogenous lesions transformed more often, consistent with several previous reports one of which found this to be the only factor predictive of malignant transformation.[7] The nonhomogenous appearance of the lesion, amongst other patient factors, has raised clinical suspicion and resulted in the prescription of a more aggressive approach to treatment by surgical excision.[9] Non homogeneous varieties include:

- speckled: mixed, white and red, but retaining predominantly white character;
- nodular: small polypoid outgrowths, rounded red or white excrescences;
- verrucous: wrinkled or corrugated surface appearance.

Oral erythroplakia (Figure 1b) is a fiery red patch that cannot be characterized clinically or pathologically as any other definable disease'. Erythroplakia is often flat with a smooth or granular surface. Numerous other red patches / macules that could arise on the oral mucosa should be excluded before considering erythroplakia as the diagnosis. Erythroplakias is relatively uncommon on their own and often present as mixed red-and-white lesions. These should be considered under the term erythroleukoplakia.[8, 10]

B. Proliferative verrucous leukoplakia (PVL) (Figure 1c).

PVL is a recognized specific type of non-homogeneous leukoplakia with an extremely high propensity for malignant transformation. Verrucous carcinomas exist within the histologic continuum ranging from benign squamous hyperplastic lesions and proliferative verrucous lesions to invasive squamous cell carcinoma.[11] Distinguishing verrucous carcinoma from these similar benign and malignant processes may be difficult. The belief by earlier researchers that verrucous carcinomas may evolve into a conventional invasive squamous cell carcinoma may be due to presence of small foci of squamous cell carcinoma in those lesions with dominant features of verrucous carcinoma. Some investigators consider verrucous squamous carcinomas to be "hybrid" forms of verrucous carcinoma or a squamous cell carcinoma with verrucoid features[12, 13]

C. Idiopathic leukoplakia.

In a study of one hundred and forty five pati with oral epithelia dysplasia, non-smo (idiopathic leukoplakia) were 7.1 times m likely to undergo malignant transformat compared to heavy smokers. Malign transformation occurred after a median of months following diagnosis of dysplasia. higher rate of transformation in idiopa leukoplakia was related to underlying intri factors genetic instability as predisposing facto

D. Duration and number of the lesion by initial diagnosis.

Studies have demonstrated that the longer follow- up period of the persons at risk, the hig the number of malignant transform leukoplakias. A particularly difficult group patients to manage are those with multiple precancerous lesions in whom extensive area mucosa may show signs of dysplastic chan Modern concepts of carcinogenesis that h evolved from the work of Slaughter et al,[14] h emphasized the existence of molecularly alte preneoplastic fields from which multiple les can develop. Widespread leukoplakias have b shown to have higher rates of malign transformation than more localized lesions.

E. Size of lesion.

There is a common agreement by many stu that pathological lesion larger than 200 mm^2 (4 mm^2) appear to have a higher propensity malignant transformation.[6,7]

F. High-risk anatomic sites

Both lateral aspect of tongue and floor of m have been associated with an increased ris malignant transformation. Some studies identified the floor of the mouth as the common site of oral leukoplakia, but man these studies agreed that the lateral border o tongue has the highest malignant transform rate.[17,18,19] A few studies claim that the hig incidence of leukoplakia is in the buccal muco but in a study by Hamadah et al[21] it was obse that single lesions occurred more on the la aspect of the tongue and floor of mouth; w multiple lesions were found more on the bu mucosa.

G. Age at presentation.

Some authors have identified late presentation of leukoplakia at age above 60 years, with a high risk of malignant transformation, especially in conjunction with homogeneous appearance at the lateral aspect of the tongue.[22, 23,24] Recent studies show that 1% to 5% of PMDs affect the younger age group of 30 years. This may be due to the fact that various extrinsic and intrinsic aetiological factors are now more prevalent in today's younger population.[25,26]

H. Gender:

The gender prevalence of oral premalignant lesions is controversial with some studies indicating male preponderance, which was further linked to exposure to high risk habits such as, smoking and alcohol consumption in many instances. However other studies have stated higher prevalence in females. Furthermore the incidence of malignant transformation according to gender is also controversial, as some studies indicate higher malignant transformation in females; while others observed no gender difference in malignant tendency.[28,29]

There are higher transformation rates in erythroplakia, speckled leukoplakia and in proliferative verrucous leuko-plakia. Others with high transformation rates include sublingual leukoplakia, candidal leukoplakia and syphilitic leukoplakia, which is exceptionally rare now. Not. all leukoplakias are potentially malignant; for example, hairy leukoplakia seen mainly in immunosuppressed people has no known malignant potential. The other potentially malignant lesions or conditions may include actinic cheilitis, oral submucous fibrosis and some lichen planus. However, most other oral white lesions, such as homogeneous leukoplakias, have very low potential for malignant transformation.

The clinical dilemma is to determine the malignant potential of an oral PML and, apart from clinical appearance and location, epithelial dysplasia has conventionally been the marker most used.[5,31]

To exclude malignant disease in clinically benign lesions, biopsy should supplement clinical diagnosis. In addition, OSCC, even if clinically visible, can resemble oral PML and some common benign oral lesions. Thus, clinical inspection alone is unreliable for the differentiation of malignant lesions from benign lesions. Also, malignant transformation of potentially malignant lesions cannot be accurately predicted based solely upon clinical characteristics. The only method currently available to reliably determine the diagnosis and give an indication of prognosis is the laboratory histopathology examination of a tissue sample, since it is accepted that dysplasia may precede malignant change. Therefore it is mandatory to biopsy any persistent mucosal lesion where there is no absolute confidence that the diagnosis is that of a benign lesion. There should be a high index of suspicion, especially of a solitary lesion present for over 3 weeks. In practice therefore, all ulcerated, red, white or mixed solitary oral lesions persisting 3 weeks or more require biopsy evaluation.[29,30]

2. Microscopic assessment of malignant potential

The histopathological feature of oral epithelial dysplasia (OED) is characterized by architectural distortion and accompanied by cytological atypia, with loss of normal maturation and stratification of keratinocytes.[32,33]

"Dysplasia" is the term that is used within the context of mucosal pre malignancies. For clarity, there is the need to distinguish between cellular changes and architectural changes.[34]

Cellular Alterations in Dysplasia: In the assessment of epithelial dysplasia, alterations of individual epithelial cells should be evaluated. Features such as dark-staining nuclei, an increased nuclear-to-cytoplasmic ratio, as well as variation in size and shape should be noted. In addition, the nuclei often show marked variation in size and may have enlarged nucleoli. An increase in mitotic activity may also be seen in many reactive lesions, but abnormal mitoses, as well as mitotic figures found in unusual locations above the basal cell layer indicate premalignant cellular alterations.

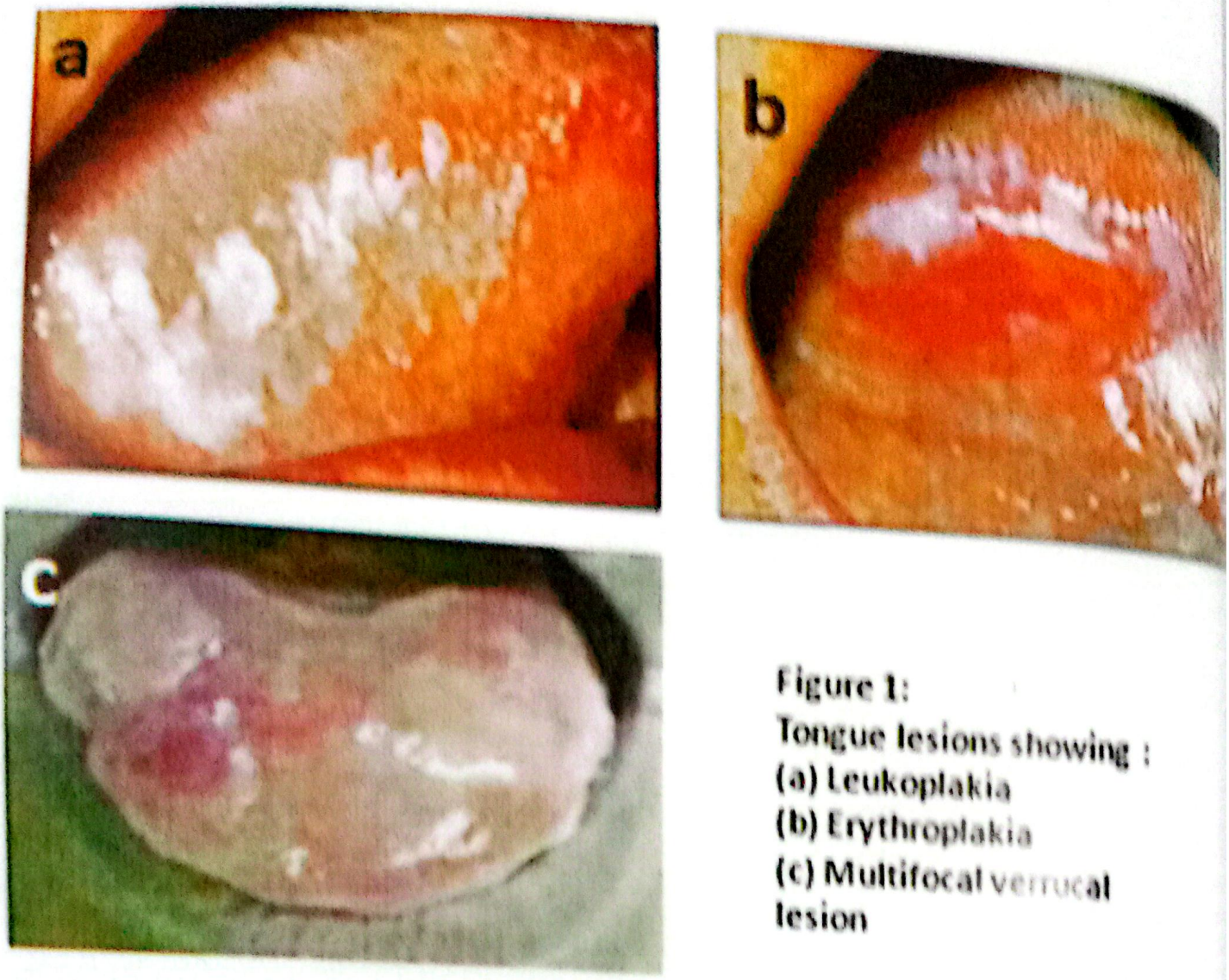

Figure 1:
Tongue lesions showing :
(a) Leukoplakia
(b) Erythroplakia
(c) Multifocal verrucal lesion

Individually keratinized cells or concentric rings of flattened keratinocytes below the surface layer also distinguish epithelial dysplasia from hyperplasia[35]

Tissue (Architectural) Alterations in Dysplasia: Tissue alterations in dysplasia is loss of the orderly cellular maturation from basal cells to flattened superficial cells that may or may not be keratinized, depending on the site in the oral cavity. Thickening of one of these epithelial layers without disturbance of this orderly layered architecture should not be interpreted as dysplastic. A grading system for dysplastic oral epithelium must take into consideration both the degrees of cellular atypia, in addition to the extent of structural tissue alterations as described above.[36]

Traditional light microscopic examination is s
the most reliable method for determining
grade of intraepithelial alterations in spite
shortcomings, such as inter and intra obser
variation and absence of clear-cut prognos
significance. However competing classificati
systems exist, and therefore there are
worldwide generally accepted criteria for
histological grading system in the head and ne
region. Three schemes are currently used
histological grading of head and neck dysplas
they are:

(i) World Health Organization (WH
dysplasia criteria for classifyi
intraepithelial alterations in the o
cavity and larynx,[37]

(ii) the squamous intraepithelial neoplasia system,[35] and
(iii) the Ljubljana classification.[38]

WHO Dysplasia Classification[37]
The WHO dysplasia system includes the following categories:

- Hyperplasia with increased number of cells.

There may be an increased number of cells in the spinous layer (acanthosis) or in the basal and parabasal cell layers (basal cell hyperplasia). The architecture of epithelium is preserved; there is no cellular atypia.

- Dysplasia with Three Grades (Figure 2)

Mild Dysplasia: Architectural disturbance is limited to the lower third of the epithelium and is accompanied by cytological atypia.
Moderate Dysplasia: Architectural disturbance that extends into the middle third of the epithelium is the initial criterion for recognizing this category of dysplasia. However, consideration of the degree of cytological atypia may require upgrading to severe dysplasia.
Severe Dysplasia: Architectural disturbance with associated cytological atypia is greater than two-thirds of the epithelium. However, as noted when discussing moderate dysplasia, architectural disturbance is limited to the lower and middle third of the epithelium, but with sufficient cytological atypia may be included in this category.

- Carcinoma in Situ

Carcinoma in situ is represented by full or almost full thickness architectural abnormalities in the viable cellular layers accompanied by pronounced cytological atypia. Atypical mitotic figures and abnormal superficial mitoses are commonly present. Although the architectural alterations for the various WHO stages are clearly defined, a lack of consensus on the extent of cytological atypia necessitating an upgrade from moderate-to-severe dysplasia makes this latter criterion equivocal.

Squamous Intraepithelial Neoplasia Classification (SIN)[35]
In this classification scheme, oral intraepithelial neoplasia grade 1, 2, and 3 are synonymous with the categories mild dysplasia, moderate dysplasia, and severe dysplasia as defined in the WHO. The most severe intraepithelial alterations are called carcinoma in situ in both classifications. Table 2 shows the inter-relationship between the three classifications of oral epithelial dysplasia.

Ljubljana Classification of squamous intraepithelial lesion (SIL)[38]
Within the Ljubljana grading system, the following categories are distinguished:

- Reactive lesions having a minimal risk of progression to invasive carcinoma

Squamous (Simple) Hyperplasia: This is a benign hyperplastic lesion, normal architecture and cytology of the squamous epithelium is preserved. The epithelium is thickened as a result of an increased prickle cell layer. The cells of the basal and parabasal region, which comprise one to three layers, remain unchanged. There is no cellular atypia; infrequent, regular mitoses are seen in the basal layer.
Basal and Parabasal Cell Hyperplasia (Abnormal Hyperplasia): There is benign augmentation of basal and parabasal cells in the lower part of the epithelial layer, while the upper part, containing regular prickle cells, remains unchanged. The thickened epithelium consists of an increased number of basal and parabasal cells without significant nuclear changes and occupying up to one-half or occasionally slightly more of the entire epithelium. Rare, regular mitoses may be seen, always located in or near the basal layer. Less than 5% of epithelial cells show dyskeratosis, there is abnormal individual cells keratinization and groups of cells that have no prickles and strongly eosinophilic cytoplasm.
Potentially Malignant Lesions: Atypical Hyperplasia (Risky Epithelium) This lesion depicts a definitely increased risk of progressing to invasive carcinoma that is characterized by the following histological appearances; Stratification is still preserved in the epithelium. There is increased number of epithelial cells with atypical features that may occupy the lower half or more of the entire epithelial thickness. Mitoses are moderately increased. They are usually found in the lower two-thirds of the epithelium, although they may occasionally appear at a higher level. Mitoses are rarely, if ever, abnormal. Dyskeratotic cells are frequent within the entire epithelium. Two subdivisions of atypical hyperplasia are recognised: (i) the more frequent "basal cell type" with no intercellular prickles and no cytoplasmic eosinophilia and the cells aligned perpendicularly

acute angle to the basement membrane and the less frequent "spinous cell type" analogous to so-called "keratinizing dysplasia," as defined by Crissman and Zarbo, in this type there are intercellular prickles and increased cytoplasmic eosinophilia.

- Actually Malignant Lesions:

This group comprises carcinoma in situ, a lesion showing the features of carcinoma without invasion. There is loss of stratification or maturation of the epithelium as a whole, although at the surface of the epithelium compressed, horizontally stratified, and sometimes keratinized cells may occur. Moreover, epithelial cells may show all the cytological characteristics of invasive squamous cell carcinoma, and mitotic figures are usually markedly increased throughout the whole epithelium; often more than five in one high-power field. Abnormal mitoses are also frequently seen.

The current histopathological grading of oral dysplasia lesions is notoriously unreliable mainly due to the lack of a validated grading system.[32,39] Many studies show wide variability in the diagnosis and grading of OED with results demonstrating only poor to moderate agreement on grading OED. This is not only a problem for oral dysplasia, but also for grading epithelial dysplasias in other parts of the body such as cervical intraepithelial neoplasia, vulva intraepithelial neoplasia,[41] and Barrett's oesophagus. However, despite the challenges associated with the current histopathological grading systems of oral epithelial dysplasia, histopathological evaluation based on morphology remains the routine method for diagnosis and grading OED.[43]

In an attempt to overcome the challenges associated with the histopathological grading systems of oral epithelial dysplasia, Kujan et al[44] introduced a binary grading system as follows: the cut off point for a "high-risk" lesion (with potential susceptibility for malignant transformation) was based on observing at least four architectural changes and five cytological changes, while the cut-point for a "low-risk" lesion (without potential susceptibility for malignant transformation) is associated with observation of less than four architectural changes or less than five cytological changes. Many studies have alluded to the more reliable performance of the binary grading system of oral epithelial dysplasia as having an increased power in predicting malignant transformation.[45] Whereas, other studies indicate that lesions with slight, moderate, severe, and no dysplasia developed carcinoma with similar frequencies.[46] Such opposing views attempting to correlate the degree of dysplasia with the likelihood of malignant transformation are frustrating and underscore the need for more precise prognosticators. The potential usefulness of molecular methods in the assessment of malignant potentials of OED has been variously stressed by several authors.

3. Genetic alterations and risk of progression to malignancy

There are several histologically distinct lesions of the oral cavity that have malignant potential. These lesions include leukoplakia, erythroplakia, lichen planus and submucous fibrosis, and many other lesions with a spectrum of chromosomal, genetic and molecular alterations. The degree of similarity to OSCC found in premalignant lesions is dependent upon the presence of atypia. However, individual lesions may present molecular genetic alterations similar to OSCC, even in the absence of histologically defined dysplasia.[46]

A series of histological identifiable pre-malignant stages are involved in the development of head and neck squamous cell carcinoma (HNSCC). In particular, oral squamous cell carcinomas (OSCC) may be preceded by the appearance of lesions which have the potential to develop into cancer in the oral cavity. The development of OSCC is generally expected upon the development of multiple, clonal, genetic alterations, which lend a clonal population of cells a growth advantage over others. The distinction between benign and potentially malignant oral lesions is currently based upon the histological examination of biopsy specimens. In the absence of a carcinoma or dysplasia, the ability to quantify the risk associated with malignant transformation is limited. It is for this reason that a molecular and genetic characterization of premalignant lesions may be very important in predicting the malignant potential.[47,48]

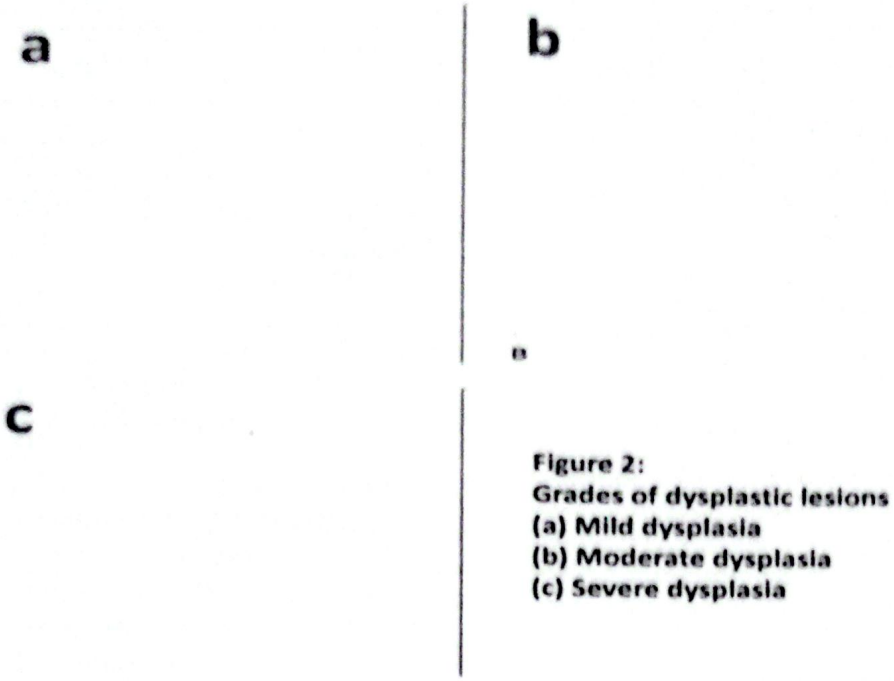

Figure 2:
Grades of dysplastic lesions
(a) Mild dysplasia
(b) Moderate dysplasia
(c) Severe dysplasia

Leukoplakia is the most commonly diagnosed premalignant lesions in the oral cavity. Oral leukoplakia patients with dysplastic features have up to 36% incidence of subsequent OSCC development, in the absence of dysplasia; these lesions still possess a 15% incidence of cancer development.[46,49]

Increased loss of heteroygosity (LOH) was correlated to the histopathological progression in the upper aereodigestive tract. Fifty percent of leukoplastic lesions contain allelic loss of either the 3p or 9p chromosome arms, which are associated with higher risk of malignant transformation. This risk increases further in the presence of additional LOH at the 4q, 8p, 11q, 13q and 17p loci. Loss of heterozygosity or allelic imbalances is due to mutation caused by carcinogenic agents. LOH of tumour suppressor genes on the short arm of chromosome 3, p16 of chromosome 9 and p53 of chromosome 17 are well established. The protein produced by p53 has been well studied. The protein attaches to the transcription starting point of nucleic DNA to block it in any cell involved. Loss of cellular function follows. Other cell-growth control genes like H-ras on chromosome 17 also get damaged. These cell signaling growth control genes when damaged can lead to uncontrolled cell growth. Microsatellite instability (MSI) which is the insertions or deletions of base pairs at microsatellites are another cytogenetic feature shared between premalignant lesions and OSCC. The MSI is present in 55% of leukoplakia and there is increasing MSI prevalence with histological progression of premalignant lesions. In addition, there is increase in polysomy frequency at several loci, such as chromosomes 7 and 17 in the neoplastic progression of the aerodigestive tract, such that lesions with a greater than 3% proportion of cells with trisomy 9 have a significantly higher likelihood of progression to cancer. Also telomerase activity is correlated with the degree of atypia and dysplastic changes.[46,47,50] During ageing and when cancer is developing in any person the tip of the chromosomes (telomeres) shorten as repeated runs of thymidine and guanosine nucleic acids that stabilize the telomere is lost. When telomere shortens genetic materials that can prevent cancer formation are lost.

In response to oxidative damage and stress, mitochondrial genomic mutations may occur in the development of cancer, including HNSCC. It was suggested to occur due to mitochondrial dysfunction in apoptosis or through reactive oxygen species generation.[51,52] Epigenetic changes are also important. Promoter hypermethylation is a mechanism by which tumour suppressor genes are transcriptionally inactivated. Methylation of

Assessment of DNA ploidy status in oral leukoplakia allows the identification of gross genomic alterations and has been shown to be a useful tool in identifying lesions with a high risk of malignant transformation. In a study of DNA content in 150 patients with oral leukoplakia and epithelial dysplasia, thirty six (24%) developed a carcinoma after a mean follow up of 49 months; however, only three of 105 diploid cases (3%) develop invasive disease, as compared with 21 of 25 aneuploid cases (84%) and 12 of 20 tetraploid cases (60%). The negative predictive value for diploid lesions was 97% and the positive predictive value for aneuploid lesions was 84%.[56, 57]

However, DNA content did not show any statistically significant correlation with the grade of dysplasia. In another study of ploidy status of 45 leukoplakic lesions histopathologically negative for dysplasia, in which 35 (78%) lesions were diploid, 5(11%) were tetraploid, and 5(11%) were aneuploid: Four of the five aneuploid cases developed carcinoma (80%), whereas only one of the diploid/tetraploid cases (2%) developed malignancy. Patients with aneuploid leukoplakia have a 98% rate of developing primary oral carcinoma, an 81% rate of recurrent or second primary tumours, and a 76% risk of death from disease despite negative histology of the surgical margins. Thus ploidy status, chromosome polysomy, and loss of heterozygosity (LOH) analysis of oral precancerous lesions may provide important information about the risk of progression to cancer, second cancer development, and tumour specific death.[57,58]

In view of the limited value of histopathological parameters for outcome prediction, it has become necessary to develop novel biomarkers to assess the progression of PMD. One approach has utilized high throughput profiling screening to specify the oncogenic miRNA signature that is associated with the progression of PMD; this approach is able to isolate potential biomarkers that may be useful for risk assessment. Expression of miR-21, miR-181b and miR-345 was found to consistently increase, as the severity of the lesion increases during OSCC progression.[59,60]

Furthermore, expression of miR-21, miR-31, the miR-146 family and the miR-221 family, which are upregulated during HNSCC are also upregulated during oral leukoplakia that undergoes a malignant transformation, compared with their nontransformed counterparts. It is therefore important that the predictive values of these candidates in the progression of PMD are further investigated in more detail.[60-62]

4. Molecular techniques as diagnostic aid for oral premalignant lesions

Leukoplakia and erythroplakia have a well documented risk for transformation into HNSCCs. The risk of malignant transformation increases with the microscopic finding of dysplasia; however, there is significant lack of agreement in the grading of oral dysplasias. One of the promises offered by a better understanding of the molecular basis of preneoplastic lesions of the head and neck is the provision of ancillary tools to provide objective and reproducible grading and estimation of risk of evolving to invasive carcinoma. Oral premalignant tissues frequently show many genomic alterations.[63,64]

Molecular biology is concerned with understanding the interactions between the various systems of a cell including interactions between DNA, RNA, and protein biosynthesis and learning how these interactions are regulated.

Molecular techniques most commonly used in investigative oral pathology are:
Immunohistochemistry
Polymerase chain reaction
Cytomorphometry
DNA image cytometry
Chromosome in situ hybridization
DNA miroarrays
Proteomics
Gene therapy

Immunohistochemistry

Immunohistochemistry or IHC refers to the process of detecting antigens such as proteins in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in

biological tissues. IHC technique is used as a part of histopathological examination in assessing molecular alterations in premalignant and malignant oral lesions. Mutant p53 demonstrates a longer half-life than wild type, and its mutant form is often detectable by molecular biology techniques. p53 protein expression is detected by IHC in 90% of oral leukoplakia, whereas it is absent in normal mucosa. The para-basal detection of p53 by IHC alone or with other markers is associated with greater risk for malignant progression lending credence to the potential application of p53 detection by IHC for stratification of risk of malignant transformation in oral leukoplakia.[5,65]

IHC investigations have also been employed in detection of human papillomavirus (HPV) infection in oral precancerous lesions and oral cancers. HPV type 16 and 18 have close association with the development of oral squamous cell carcinoma and that the infection is maintained even after neoplastic change of the infected cells. IHC is widely accessible and easy to perform at a reasonable cost. However, this semi quantitative procedure is beset by technical artefacts; sensitivity differences between different antibodies, and subjective interpretation, resulting in inter observer variability between pathologists.[66,67]

Polymerase chain reaction

Polymerase chain reaction (PCR) is an extremely versatile technique used for copying of DNA. It allows a single DNA sequence to be copied millions of times or altered in a predetermined way. PCR technique is used for quantitative measurements of DNA or RNA molecules. It has also been used to introduce restriction enzymes sites or to mutate particular bases of DNA.

Polymerase chain reaction technique has been used to amplify DNA in samples from oral precancers/carcinomas and has been analyzed with restriction fragment length polymorphism. It detects the commonly implicated molecular alterations in oral precancers and oral cancers such as loss of heterozygosity, microsatellite instability, and changes in methylation pattern.[68]

One major disadvantage of Q-PCR-based approaches is the requirement for prior sequence data of the specific target gene of interest. Consequently, Q-PCR can only be used for targeting of known genes. Hence, accessing the 'unknown' using Q-PCR or indeed any PCR-based methods is inevitably limited to the analysis of sequences related to those that have already been characterized. Another disadvantage is that PCR does not permit direct visualization of viral transcript in routinely processed tissues.[69,70]

Cytomorphometry

Cytometry is a quantitative technique that evaluates parameters such as nuclear area (NA), cytoplasmic area (CA), and nuclear/cytoplasmic ratio (NA/CA). Papanicolaou smears are prepared, and the NA, CA, and NA/CA are recorded. Cytomorphometric technique is used to assess nuclear diameter and cytoplasmic diameter in dysplastic lesions and oral squamous cell carcinomas. Cytoplasmic diameter was highest in normal mucosa, lower in dysplastic lesions, and lowest in squamous cell carcinomas. By contrast, nuclear diameter was lowest in normal mucosa, higher in dysplastic lesions, and highest in squamous cell carcinoma. Thus, it suggested that increased nuclear size and reduced cytoplasmic size are useful indications of malignant transformation.[68,71]

DNA Image cytometry

DNA image cytometry is a technique that permits quantification of nuclear DNA content, thereby assessing the DNA ploidy status. As a surrogate for individual molecular markers, measurement of gross genomic damage, in the form of aberrant DNA content, could be a valuable method for prognostication of malignant and premalignant lesions.[72,73] Relativity recently, there has been a major advancement in the use of automated image cytometry to measure ploidy in nuclei extracted from routinely processed paraffin sections. This system may be more sensitive in the evaluation of oral potentially malignant lesions into 'low' and 'high' risk. Evaluation of ploidy status in oral leukoplakia and oral lichen planus allows the identification of gross genomic alterations and has been shown to be a useful tool in identifying lesions with high risk of malignant transformation to oral cancers as anueploid lesions has the maximum incidence of malignant transformation.[74,75]

DNA image cytometry has also been used to detect cancer cells with abnormal DNA content at

the invasive tumour front, which is associated with poor prognosis of the patients with oral carcinomas. Thus, it could help to find the appropriate treatment option for the patients. The sensitivity of cytological diagnosis combined with DNA cytometry is 98% and specificity 100% when compared with gold standards of histology. Routine use of these techniques is, however, hampered by the complexity of the tests, the lack of facilities in many routine laboratories, and the high cost involved.[76,77]

Chromosome in situ hybridization

Chromosome in situ hybridization (CISH) is a cytogenic technique that is used to detect and localize the presence or absence of specific DNA sequence or chromosome. It uses fluorescent probes that only bind to those parts of the chromosomes with which they show a high degree of sequence similarity. Fluorescence microscopy show where the fluorescent probe is bound to the chromosome, thus providing an ability to directly visualize the genetic change in tissue sections or exfoliated cells.[78]

Analyses of normal and premalignant lesions adjacent to tumours have demonstrated that chromosome instability can be detected in the field of the tumour (i.e. in normal and premalignant cells in a tissue at 100% risk of tumour development), and the degree of chromosome instability increases with the degree of histological progression toward cancer.

Analyses of premalignant lesions such as oral leukoplakia and erythroplakia from individuals at risk for cancers by CISH have uncovered varying degrees of chromosome instability [4] ; most leukoplakia lesions contain an abnormal number of chromosomes 7 and 17, and lesions with greater than 3% proportion of cells with trisomy 9 have a significant higher likelihood of progression to cancer.[4,78]

DNA microarrays

DNA microarray is a high throughput technology used in molecular biology and helps in the study of sequence of genes. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing a specific DNA sequence. This is a short section of gene or other DNA element that are used as probes to hybridize DNA or RNA samples. Because an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel.[79]

A number of issues must be addressed before establishing a microarray platform and beginning expression profiling studies, in particular, the overall cost. For a cDNA microarray platform, one must purchase a clone set, robot, printing pins, and reagents needed for DNA amplification and purification. The cost of these materials can vary significantly, but one can expect to need at least $100,000 to establish such a platform. However, once the process of printing and hybridizing microarrays has been optimized, the cost per experiment will fall dramatically.[80]

A decision must be made whether the number of planned experiments is enough to warrant the time and cost of establishing a microarray platform. Another challenge is the efficient management and analysis of the large volume of data generated by microarray approaches. However, increasingly sophisticated computational methods continue to be developed that are amenable to large data sets generated from microarray experiments. As key disease pathways are identified, custom arrays containing relevant subsets of genes will eventually be integrated into clinical settings for more widespread use.[81]

Proteomics

The term "proteomics" indicates proteins expressed by a genome, and is the systematic analysis of protein profiles of tissues, paralleling the related field of genomics.[81] This system facilitates protein capture, purification, analysis, and processing from complex biological mixtures directly onto protein chip array surfaces and the detection of the purified proteins is performed by time-of-flight mass spectrometry.[82] A reproducible correlation was found between the expression patterns of multiple proteins within epithelial cells and the progression of oral cavity tumour. A comparison of the protein maps of normal and malignant prostate were used to identify 20 proteins lost in malignant transformation, including PSA, a-1 antichymotrypsin, haptoglobin, and lactylglutathione lyase.[82]

Proteomic technologies have the potential to greatly aid the development of molecular diagnostics and serve as markers for the early detection of cancer. These technologies will also accelerate the anticancer drug target discovery

and validation. Furthermore, a proteomic technology is used to design rational drugs according to the molecular profile of the cancer cell, and thus facilitate the development of personalized cancer therapy.[81]

5. Early detection of the malignant potentials of oral premalignant lesions

Oral CDx

Oral CDx brush biopsy uses the concept of exfoliative cytology to provide a cytological evaluation of cellular dysplastic changes. Although exfoliative cytology and brush biopsy techniques are helpful in establishing a more definitive diagnosis of already visible lesions, they are of no value in detecting mucosal changes that are not readily visible to the naked eye. Advances in the development of automated cytomorphometric methods combined with genetic and proteomic profiling may provide the required tools to refine screening strategies in the future. A scalpel biopsy is still suggested if there is clinical suspicion of a lesion regardless of the Oral CDx result.[83,84]

Chemiluminescence: Vizilite

It involves the use of a hand-held, single-use, disposable chemiluminescent light stick that emits light at 430, 540 and 580 nm wavelengths. The use of the light stick is intended to improve the visual distinction between normal mucosa and oral white lesions. Normal epithelium will absorb light and appear dark, whereas hyperkeratinized or dysplastic lesions appear white. The difference in colour could be related to altered epithelial thickness, or to the higher density of nuclear content and mitochondrial matrix that preferentially reflect light in the pathological tissues.[85] Lately, a combination of both TB and ViziLite systems (ViziLite Plus with T Blue System; Zila, Batesville, AR, USA), received Food and Drug Administration clearance as an adjunct to visual examination of the oral cavity. A recent study of high risk patients showed that the majority of lesions with a histological diagnosis of dysplasia or carcinoma in situ were detected and mapped using ViziLite with TB.[86]

Veloscope system

Normal oral mucosa appears pale green due to the tissue auto fluorescence resulting from stimulation with intense blue light excitation at 400–460 nm wavelength. In contrast, dysplastic and malignant lesions will appear darker than the surrounding healthy tissues as they have decreased autofluorescence.[87] Two recent studies emphasized the controversial use of this system for early diagnosis. One study, demonstrated that Veloscope examination did not provide a definitive diagnosis regarding the presence of epithelial dysplasia, and that loss of auto fluorescence is not useful in diagnosing epithelial dysplasia without relevant clinical interpretation.[88] While the other study showed that the Veloscope was useful in confirming the presence of oral leukoplakia and erythroplakia and other oral mucosal disorders, but the device was unable to discriminate high-risk from low-risk lesions.[89]

Saliva as diagnostic tool

Discovery of analytes in saliva of normal and diseased subjects suggests a very promising function of saliva as a local and systematic diagnostic tool. However, due to lack of knowledge of disease markers and an overall low concentration of these markers in saliva when compared to serum, the diagnostic value of saliva has not been fully realized. However, nowadays, highly sensitive and high-throughput assays such as DNA microarray, mass spectrometry and nanoscale sensors can measure protein and RNA markers at low concentrations in saliva, thus expanding the utility of saliva as a diagnostic tool.[90,91]

Targeted therapies for oral cancer and oral premalignant lesions

Although considerable advances have been made in understanding of the molecular biology of oral cancer and new approaches are in development, chemotherapy and radiotherapy still remain the back bone of treatment for cancer. Head and neck cancer patients can benefit from specific targeted therapeutic agents such as the family of tyrosine kinase inhibitors—in particular, the epidermal growth factor-receptor antagonists and cyclin-dependent kinase inhibitors. The chimeric IgG anibody C225, which has binding affinity equal to that of the natural ligand, can effectively block the effect of epidermal growth factor and transforming growth factor in head and neck cancer patients.[92,93]

Preclinical tests has shown that the CDK inhibitor flavopiridol repressed the transcription of cyclin D1, induced cell-cycle arrest at the transitions

between the G2 and M phases and between the G1 and S phases, and induces p53-independent apoptosis.[93,94]

Various tyrosine kinases are also crucial targets for pharmacological intervention. ZD1839 ("Irressa") is an orally-active selective epidermal growth factor receptor-tyrosine kinase inhibitor showing anti-tumour activity in SCCHN in combination with radiation treatment.[94]

Gene therapy strategies was used to deliver therapeutic genes to advanced cases of HNSCC with the aid of highly efficient adenovirus vectors ONYX-015, an adenovirus with the EIB 55-kDa gene deleted, which was engineered to selectively replicate in and to lyse p53-deficient cancer cells while sparing normal cells. A Phase II trial of a combination of intra tumoural ONYX-015 injection with cisplatin and 5-fluorouracil in patients with recurrent disease suggest that it is effective, and that the response at injected tumour sites is durable.[95,96]

In vitro experiments using Ad5-p16 treatment (inactivates tumour suppressor gene p16INK4a) significantly inhibited cell growth with 96% efficiency. Introducing wild type p53 via a recombinant adenoviral vector, Ad5CMV-p53 in SCCHN cell lines successfully attenuated their replication and tumour growth.[97]

The direct oncolytic effect of bacterial strains on tumour and the immunodulating effect provide a useful means of tumour therapy and genetically modified bacteria also form a part of cancer gene therapy. A Lipid mutant Salmonella provides selective target for solid tumours and the advantages include: (1) Target multiple of tumours from a distant inoculation site, (2) Salmonella can grow under aerobic as well as anaerobic conditions such as those found in tumours, (3) Suicide genes such as herpes simplex virus thymidine kinase (HSV-tk) are expressed by the Salmonella specie.[98,99]

Gene therapy

Gene therapy is the insertion of gene into an individual's cells and tissues to treat a disease in which a definitive mutant allele is replaced with a functional one. Gene therapy uses an adenovirus vector to introduce modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein. Gene therapy approaches to oral cancers and pre cancers include the following:

- Addition gene therapy: Aim of approach is to regulate the tumour growth by introducing tumour suppressor gene that inactivates the carcinogenic cells.
- Gene therapy using oncolytic viruses: This approach uses viruses that replicate only in the tumour cells and thus kills them.
- Suicide gene therapy: In this therapy, enzyme encoding gene is introduced into the tumour cell that stimulate the generation of products that are toxic for the cells.
- Immunotherapy: The aim of immunotherapy is to increase the patient's immune response to the tumour.
- Introduction of genes to inhibit tumour angiogenesis:

This technique uses microencapsulated cells for the release of therapeutic proteins to encapsulate recombinant cells. Use of molecular biology for management of oral cancers is still at its infancy. In the future, it may be the treatment of choice that will overcome all the complications associated with the present trend of management of oral cancers, thus proving to be a blessing to the patients.[100-102]

Future direction of molecular biology of oral cancer and premalignant lesions

The war against cancer remains a top priority in biomedical research and new therapies are being sort, therefore, concerted efforts are needed to pin-point key growth factors, growth factor receptors, tyrosine kinases, cyclins, cyclin dependent kinases, protein phosphatases and other factors that are involved in oral carcinogenesis. Promoters of angiogenesis such as VEGF and matrix metalloproteinase (MMP) family members responsible for neo-vascularization should be identified.[103,104]

Apoptotic pathway involved in squamous epithelial cells of oral cavity should be delineated, and at which step it is blocked during the process of carcinogenesis. Various anti-cancer drugs then can be screened, which either remove this block or pushes the tumour cells through an alternative route to apoptosis. The anti-sense technology can also be useful in shutting off the oncogenes like Stat-3, playing a critical role in oral carcinogenesis.[105]

Conclusion

Oral premalignant lesions represent a unique

opportunity for the early interception of oral cancer progression. It is imperative to increase the health-care providers' depth of knowledge in the recognition of predisposing factors and clinical presentation in the early stages of development, and appropriate microscopic determination of malignant potentials of oral premalignant lesions, which are essential steps in oral cancer prevention. Attempt is further made at identification of the subtle premalignant stages that are not detectable by clinical and microscopic examination, through a review of the role of molecular biology in early detection. Furthermore, molecular biology is expected to play a prominent role in the future management of oral premalignant lesions.

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