

HISTOLOGICAL GRADING SYSTEMS OF EPITHELIAL DYSPLASIA & SQUAMOUS CELL CARCINOMA

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Abstract:

Oral precancer lesions or conditions are primary indication to be alert about oral health. As there are high chances of oral precancer to turn into malignant lesion. It is difficult to assess that which precancer lesion is highly malignant and which is not. There are lots of classification and grading systems about the grading of oral precancerous lesion, which shows great variability and inter observer bias. This review gets importance as we reviewed all grading systems for oral precancer and cancer lesions with their post and cones.

Key words: Oral Precancer, Cancer, Tobacco, Dysplasia, Classification

INTRODUCTION

The term 'dysplasia' was introduced by Reagon in 1958 in relation to the cells exfoliated from lesions of the uterine cervix. Dysplasia means abnormal, atypical proliferation, encountered principally in the epithelium. In past epithelial dysplasia, epithelial atypia and dyskeratosis were used synonymously. Pindborg (1977) defined epithelial dysplasia as the term used for "A lesion in which part of the thickness of the epithelium is replaced by the cells showing varying degrees of cellular atypia." Burkhart and Maerker (1981) stated that the degree of dysplasia is determined "As a measure of tissue and cellular deviation from the normal." Kumar et al (1992) defined dysplasia "as disturbance in the maturational sequence of the stratified squamous epithelium and disturbance in cell kinetics of the proliferative compartment with cytological changes." Exposure of a cell to carcinogen leads to cytological changes, depending on the extent and duration of stimuli. An increase in cell proliferation, diminishing the cytosolic volume and the associated organelle load, could be an attempt in this direction. In the context of oral epithelium, an accelerated growth phase depicted by broadening of the progenitor compartment (hyperplasia) is the earlier sequel of exposure to an irritant. When the irritant persists, the epithelium shows features of cellular atrophy, again a well characterized feature of adaptation. At a later stage when the stages of adaptation and reversible cell damage surpasses, the cells progressively slips into a stage of irreversible cell damage, manifest either as cell death or neoplastic transformation. The changes of dysplasia, are in many cases the earliest microscopic evidence

of the subsequent development of carcinoma. But dysplasia can be found in association with a variety of non-neoplastic conditions, such as in the neighbourhood of chronic inflammatory ulceration or burns. Furthermore, dysplasia may regress, as has been shown in the case of the cervix.^{1,2}

Oral precancer lesions can be defined as altered epithelial lesions which have an increased likelihood of progressing to squamous cell carcinoma. The nomenclature, natural history and predictive value of this group of lesions was reviewed at an expert workshop held in London in 2005, and has been reported in a series of recent papers^{3,4}. The group recommended that the distinction between potentially malignant lesions and conditions should be abandoned in favour of a common terminology of Oral potentially malignant disorders^{3,5}. This recognises the fact that even in patients with lesions such as leukoplakia, malignancy may arise elsewhere as a result of field change. The most common disorders recognised as potentially malignant are leukoplakia and erythroplakia. The WHO definition of these lesions is generally regarded as unsatisfactory, since it largely a definition by exclusion. The Working group recommended a new definition for Leukoplakia which recognises the lack of evidence about risk and the nature of the lesions 'The term leukoplakia should be used to recognise white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer' However even this remains unsatisfactory and a clear definition of precursor lesions may have to wait for further diagnostic criteria based on molecular or genetic markers. For the present time, the prognostic significance of an individual lesion is difficult to determine, and none of

the currently available molecular markers have proved to be prognostically significant and none have yet been evaluated in large prospective studies. The gold standard for the assessment of oral potentially malignant disorders remains the microscopic evaluation of haematoxylin and eosin stained sections for the presence of epithelial dysplasia. Some texts use the terms squamous intraepithelial neoplasia (SIN) or squamous intraepithelial lesions (SIL)⁶. The categories under each scheme are similar, but the terminology is different. In the oral cavity, use of the SIL terminology of 'atypical hyperplasia' may lead to confusion because of the large number of common

benign hyperplastic lesions which may be encountered. In oral and maxillofacial pathology therefore, oral epithelial dysplasia is regarded as the standard terminology^{4,7}.

Criteria for the diagnosis of oral epithelial dysplasia

The diagnosis and grading of oral epithelial dysplasia is based on a combination of architectural and cytological changes⁶, but evaluation of these is subjective and has been subject to considerable inter- and intra-observer variations in the grading of lesions, with Kappa values showing only fair to moderate agreement between observers^{8,9,10}.

Table 1: Cytological and architectural features of oral epithelial dysplasia¹³.

Cellular changes:	Architectural (Tissue) changes:
<ul style="list-style-type: none"> Abnormal variation in nuclear size and shape (anisonucleosis and pleomorphism) Abnormal variation in cell size and shape (anisocytosis and pleomorphism) Increased nuclear/cytoplasmic ratio Enlarged nuclei and cells Hyperchromatic nuclei Increased mitotic figures Abnormal mitotic figures (abnormal in shape or location) Increased number and size of nucleoli. 	<ul style="list-style-type: none"> Loss of polarity Disordered maturation from basal to squamous cells Includes top-to-bottom change of carcinoma in situ Increased cellular density Basal cell hyperplasia Dyskeratosis (premature keratinization and keratin pearls deep in epithelium) Bulbous drop shaped rete pegs Secondary extensions (nodules) on rete tips.

Table 2: Observer variability in head and neck lesions.

Studies/ references	localisation	Number of slides	Histopathological classification	Number of examiners	agreement	Kappa value
Abbey et al. 1995	oral cavity/ oropharynx	120	WHO ^o	6	35.8-57.5%	0.15-0.41
Fischer et al. 20041	oral cavity/ oropharynx	87	WHO ^o	24	-	0.59 (95% CI: 0.45-0.72) 0.70 (95% CI: 0.56-0.84) ²
Karabulut et al. 1995	oral cavity/ oropharynx	100	WHO ^o	4	49-69%	27-45% ³
Tabor et al. 2003	oral cavity/ oropharynx	43	WHO ^o	3	53%	0.58
Abbey et al. 1998	oral cavity/ oropharynx	120	WHO ^o	6	38.5%	0.17 ⁴
Brothwell et al. 2003	oral cavity/ oropharynx	64	WHO ^o	3	51%	0.37
Kujan et al. 2006.	oral cavity/ oropharynx	68	WHO and binary system ("low-risk" or "high-risk")	4	WHO: 37.7% (unweighted) 92.8% (weighted) Binary system: 74.3%	WHO: 0.22 (95% CI: 0.11-0.35 unweighted) 0.63 (95% CI: 0.42-0.78 weighted) Binary system: 0.50
Mclaren et al. 2000	larynx	100	WHO and two grade (low and high Grade)	13	-	WHO: 0.32 Two-grade: 0.52

^o = WHO is not explicitly stated, but terms are in agreement with this system.

- 1 = The unweighted kappa considers all disagreements to be equally important, while the weighted kappa (Kw) yields a higher reliability when disagreements between raters are small compared with when they are large.
- 2 = the pathologic diagnoses are restricted to three categories ('no abnormality/hyperkeratosis', 'mild, moderate, or severe dysplasia', 'carcinoma in situ/carcinoma').
- 3 = when comparing the kappa values between the two pairs of pathologists with the same education, these values did not diverge from the general level of kappa values, indicating that the interobserver variability was due to individual differences rather than to educational background.
- 4 = Clinical information submitted with biopsy.

More recently there has been an attempt to more carefully define the criteria for grading of epithelial dysplasia^{7,8}. Largely this has involved an adaptation of the scheme used in cervical pathology where it has been traditional to grade cervical intraepithelial neoplasia (CIN) according to the thickness or levels of involved epithelium. It should be noted however that full thickness change analogous to CIN3 (carcinoma-in-situ) is rarely seen in the mouth. Nevertheless, the latest WHO classification⁶ now recommends a more objective grading which does account of levels of involvement up to some extent. The criteria for grading of oral epithelial dysplasia are summarised as follows: Mild dysplasia (grade I): demonstrates proliferation or hyperplasia of cells of the basal and para-basal layers which does not extend beyond the lower third of the epithelium. Cytological atypia is generally slight with only mild pleomorphism of cells or nuclei. Mitoses are not prominent, and when present are usually basally located and normal. Architectural changes are minimal. Moderate dysplasia (grade II): demonstrates a proliferation of atypical cells extending into the middle one-third of the epithelium. The cytological changes are more severe than in mild dysplasia and changes such as hyperchromatism, and prominent cell and nuclear pleomorphism may be seen. Increased and abnormal mitoses may be present, but these are usually located in the basal layers. Architectural changes may be seen in the lower half of the epithelium where there may be loss of basal polarity and hyperplasia leading to bulbous rete pegs. However stratification and maturation are relatively normal, often with hyperkeratosis

Severe dysplasia (grade III): there is abnormal proliferation from the basal layer into the upper third of the epithelium. Cytological and architectural changes can be very prominent. All the changes seen in mild and moderate dysplasia are seen but in addition there is marked pleomorphism often with abnormally large nuclei with prominent or even multiple nucleoli. Prominent and suprabasal mitoses are usually evident and abnormal tripolar or star-shaped forms may be seen. Apoptotic bodies may also be prominent. Architectural changes are severe, often with complete loss of stratification and with deep abnormal keratinisation and even formation of keratin pearls. Abnormal forms of rete pegs are usual and we regard bulbous rete pegs as particularly

significant in the diagnosis of severe dysplasia. Abnormal shaped rete pegs may also be seen, with lateral extensions or small branches. These are quite abnormal and may be the earliest signs of invasion. Occasional lesions may show prominent acantholysis with severe disruption of the architecture. Although the epithelium may be thickened, severe dysplasia is sometimes accompanied by marked epithelial atrophy. This is especially prominent in lesion from the floor of mouth, ventral tongue or soft palate and may be a feature of lesions which have presented clinically as erythroplakia. In these cases there may be minimal evidence of stratification or keratinisation, and atypical cells may extend to the surface. Carcinoma in situ: It is the most severe form of epithelial dysplasia and is characterised by full thickness cytological and architectural changes. In the oral cavity such changes are rare, and often, even in the presence of the most severe atypia, there is still an intact keratinised surface layer. Carcinoma in situ is thought by some to be a premalignancy but others regard it as evidence of actual malignant change but without invasion.

When grading epithelial dysplasia the pathologist should take into account both the cytological and architectural changes. Changes regarded as particularly significant include marked cell and nuclear pleomorphism, drop shaped rete pegs and abnormal mitoses. When the cytological changes are very marked this may indicate that a lesion should be upgraded. The challenge is therefore to identify the lesions that have a potential to develop into carcinoma by accurately grading the dysplastic features and which, accordingly demand particular attention.

Grading of epithelial dysplasia and its applications

The severity of dysplastic features is designated as Grade of epithelial dysplasia. Many dysplastic features in varying combinations have been used for grading. However difficulties have been encountered in assessing and standardizing the different degrees of epithelial dysplasia. Many systems of grading epithelial dysplasia have been proposed in order to standardize the severity of dysplastic features. Any grading system is said to be clinically useful if they are reproducible between separate observers. In addition, the parameters considered in the histological assessment should be

biologically meaningful, reflecting the malignant potential of the lesion.¹¹ The various grading systems put forth by different authors are as follows:

1. Smith and Pindborgs' photographic method (1969)
2. Banoczy and Csiba (1976)
3. WHO (1978)
4. Kramer (1980)
5. Burkhardt and Maerker (1981)
6. Lumermann H. et al (1995)
7. Neville et al (1995)
8. Speight P M et al (1996)
9. Kuffer and Lombardi (2002)
10. Ljubljana (2003)
11. Brothwell DJ (2003)
12. Binary system (2005)

1. Smith and Pindborg (1969): They attempted to standardize the grading of dysplasia by photographic method. They placed the diagnosis of epithelial dysplasia on an objective and semi quantitative level by

1. Concentrating the observers attention on one photographically standardized microscopic feature at a time
2. Enabling the observer to assess each feature individually and allocate a weighed score to each one.

Katz et al (1985)¹² after evaluation of 214 cases of epithelial dysplasia using Smith-Pindborg (1969) method of standardization, found the system to be of considerable value for purposes of standardization and eliminated observer bias by the use of standardized photographs. But they questioned the accuracy of the weightage given to each of the histological characteristics. They suggested to testing it further, as to which histological criteria was of greatest value in predicting the potential for malignant change. Thus this system despite providing more objective data, could not find general favour among pathologists and in routine diagnostic pathology, quantification was tedious. Warnakulasuria (2001)¹³ commented on this system and noted that even inflammatory or reactive lesions which are considered non-neoplastic may show some features of dysplasia. The system was subjective involving the comparison of histological sections with a series of standardized photographs. They used 13 histologic features which were standardized by a set of photographs. Each feature was graded Absent, Slight and marked as follows:

2. Banoczy and Sciba (1976)¹⁴: Epithelial dysplasia was diagnosed using the following criteria suggested by Mehta et al (1971). Irregular epithelial stratification, Increased density of the basal cell layer or prickle cell layer or both, Increased number of mitotic figures (a new abnormal mitoses may be present), Increased nuclear cytoplasmic ratio, Loss of polarity of cells, Nuclear pleomorphism, Hyperchromatism, Keratinization of single cells or cell groups in the prickle cell layer, and loss of intercellular adherence.

They graded epithelial dysplasia as: Mild- When 2 of the above listed histological changes was present. Moderate – When 2 to 4 changes were present. Severe – When 5 or more of the changes were present.

3. W.H.O. SYSTEM (1978): In an attempt to standardize the criteria for oral precancer, WHO established a collaborating reference centre in 1967. The centre aimed to characterize and define those lesions that should be considered as oral precancer and to determine, if possible their relative risks of becoming malignant. In its report in 1978, it defined and listed out the 12 histologic characteristics that characterized the epithelial dysplasia: Loss of polarity of basal cells, The presence of more than one layer of cells having basaloid appearance, An increased nuclear-cytoplasmic ratio, Drop shaped rete-pegs, Irregular epithelial stratification, Increased number of mitotic figures, The presence of mitotic figures in the superficial half of the epithelium, Cellular polymorphism, Nuclear hyperchromatism, Enlarged nucleoli, Reduction of cellular cohesion, Keratinization of single cells or cell groups in the prickle cell layer (Kramer IRH et al, 1978).¹⁵ It graded epithelial dysplasia as, Mild dysplasia: slight nuclear abnormalities, most marked in the basal third of the epithelial thickness and minimal in the upper layers, where the cells show maturation and stratification. A few, but no abnormal mitoses may be present, usually accompanied by keratosis and chronic inflammation. Moderate dysplasia: More marked nuclear abnormalities and nucleoli tend to be present, with changes most marked in the basal 2/3rd of the epithelium, nuclear abnormalities may persist upto the surface, but cell maturation and stratification are evident in the upper layers. Mitoses are present in the parabasal and intermediate layers, but none is abnormal. Severe dysplasia: Marked nuclear abnormalities and loss of maturation involve more than 2/3rds of the epithelium, with some stratification of the most superficial layers. Mitoses some of which are abnormal may be present in the upper layers.

Table 3: Smith and Pindborg Grading system

	Type of change	Severity of dysplasia		
		None	Slight	Marked
1	Drop shaped retepegs	None	Slight	Marked
2	Irregular epithelial stratification	None	Slight	Marked
3	Keratinization of cells below keratinized layer	None	Slight	Marked
4	Basal cell hyperplasia	None	Slight	Marked
5	Loss of intercellular adherence	None	Slight	Marked
6	Loss of polarity	None	Slight	Marked
7	Hyperchromatic nuclei	None	Slight	Marked
8	Increased nucleo-cytoplasmic ratio in basal and prickle cell layers	None	Slight increase	Marked increase
9	Anisocytosis and anisonucleosis	None	Slight	Marked
10	Pleomorphic cells and nuclei	None	Slight	Marked
11	Mitotic activity	Normal	Slight increase	Marked increase
12	Level of mitotic activity	Normal	Slight	Marked
13	Presence of bizarre mitoses	None	Slight	Marked

Severe grades of dysplasia may merge into the lesion customarily designated as carcinoma in situ, in which the whole or almost the whole thickness of epithelium is involved however, whether the histologic convention of distinguishing between severe dysplasia and carcinoma in situ was of practical value remained to be seen. In the present knowledge, it was not possible to say whether the presence of severe dysplasia carried a different degree of risk of subsequent development of invasive carcinoma than the presence of carcinoma in situ. It was generally believed that mild degrees of epithelial dysplasia did not indicate any great danger for the patient, although, special reference had to be made to certain high risk sites such as the floor of the mouth and the ventral surface of the tongue. Moderate dysplasia, however called for a more cautious approach and severe dysplasia indicated that there was a very considerable risk of the development of cancer.

4. KRAMER (1980)^{1,16,17}: This grading system suggests that an epithelium shows dysplasia if it has any two or more of the following features:

- Drop shaped retepegs: retepegs that are wider in the deeper portions than they are more superficially.
- Disturbed polarity of the basal cells: There is disturbed polarity when the basal cells are not perpendicular to the epithelial connective tissue junction, but are at an angle to the junction.
- Basal cell hyperplasia: The development of basal layer that is several cells thick.
- Irregular epithelial stratification or Disturbed maturational sequence: This denotes disturbance in the arrangement of cells as they pass, from the basal cell layer to the surface, thus affecting the regular stratification pattern.
- Cellular pleomorphism /anisocytosis: Variation in size and shape of the cells.

- Nuclear hyperchromatism: The nuclei in the cells are darkly stained due to increase DNA synthesis.
- Prominent nucleoli: In some dysplastic epithelia and in some carcinomas, the nuclei become larger and denser.
- Increase in nuclear cytoplasmic ratio: The nucleus enlarges and occupies a greater part of the cell as compared to the cytoplasm.
- Cell crowding: There is increase in the number of cells per unit area due to hyperplasia of the basal cell layer.
- Increased mitosis: It is the increase in frequency of mitotic figures.
- Mitosis in upper layers: It is the spread of mitotic activity to the higher levels of the epithelium.
- Abnormal mitosis: It is the appearance of mitotic figures in various forms other than normal in any one layer of epithelium e.g. tripolar mitotic figures.
- Loss of cellular adhesion or cohesion: The cells lose their attachment to the neighbouring cells, because of faulty or reduced attachment of their desmosomes.
- Intraepithelial keratinization: There is abnormal keratin formation within the cytoplasm of individual cells or group of cells.

5. Burkhardt and Maerker (1981)^{18,19,20}: Listed 6 relevant histological and cytological parameters, based on which diagnosis and classification of epithelial dysplasia could be made: Basal cell hyperplasia, Loss of basal cell polarity, Cellular pleomorphism, An increase in mitotic figures, Dyskeratosis, Abnormal and absent epithelial stratification.

Additional indicators for dysplasia are increase in subepithelial lymphocytes, plasma cells and interepithelial cells (stroma reaction), Presence of candida organisms. They graded dysplastic criteria for classification according to degree of dysplasia and characteristics of carcinoma in situ.

Table 4: Burkhardt and Maerkar grading system

Degree	Characteristics
Low	-Basal cell hyperplasia, Basal cell polarity disrupted.
Medium	-Basal cell hyperplasia, Loss of basal cell polarity, Moderate degree of cellular polymorphism, Slight increase in rate of mitosis, Occasional dyskeratosis.
High	-Basal cell hyperplasia, Basal cell polarity lost, Marked cellular pleomorphism, Increase in ratio of mitosis, Numerous dyskeratoses, Abnormal epithelial stratification.
Ca in situ	Characteristics of high degree dysplasia more marked, Epithelial stratification lost, Stroma not yet invaded.

6. Lumermann H. et al (1995) considered:

-Basal cell hyperplasia.

-Nuclear enlargement and hyperchromaticity.

-Drop shaped retepegs as 'minimal' criteria for the diagnosis of oral epithelial dysplasia.

The dysplastic changes were graded as:

1. Mild epithelial dysplasia: 'Minimal' dysplastic alterations confined to the lower third of the epithelium.

2. Moderate epithelial dysplasia: Dysplastic changes seen in up to 2/3rds of the thickness of the epithelium.

3. Severe epithelial dysplasia: Dysplastic cells fill more than 2/3rds but less than the entire thickness of the epithelium.

4. Carcinoma in situ: The entire thickness of the epithelium contains less differentiated basaloid or squamous epithelial cell with enlarged, hyperchromatic nuclei and a variable number of typical and atypical mitotic figures with no invasion into the sub-mucosa.

5. Verrucous hyperplasia with dysplasia: The epithelium exhibits considerable thickening with surface papillations, hyper-parakeratosis and parakeratin plugging and occasional dysplastic cells confined to the lower 1/3rd of the epithelium.^{17, 20}

7. Neville et al (1995) graded dysplasia as:

Mild: Hyperchromatic and slightly pleomorphic nuclei are noted in the basal and supra-basal cell layers of stratified squamous epithelium.

Moderate: Dysplastic changes extend from the basal layer to the mid portion of the spinous layer and are characterized by nuclear hyperchromatism, pleomorphism and cellular crowding. Hyperkeratosis on the epithelial cell layer along with prominent granular cell layer.

Severe: Cellular crowding and disordered arrangement throughout most of the epithelial thickness, although slight maturation and flattening of the cells appears to be present at the epithelial surface. Epithelial cells are seen to mature very little as they progress toward the hyperparakeratotic surface.

Carcinoma in situ: When the entire thickness of the epithelium is involved, the term carcinoma in situ is used. Dysplastic cells extend from the basal layer to the surface of the mucosa (Top to bottom change)

with no invasion into the underlying connective tissue.²¹

8. Speight P M et al (1996)

Considered the thickness (height) to which the cellular and tissue changes may extend, as important in grading dysplasia.^{20,22,23} According to them, Mild forms of dysplasia represented recognizable changes limited to the Parabasal layers (lower third) Moderate dysplasia represented recognizable changes extending to middle third. Severe dysplasia represented as recognizable changes extending to the upper layers. However, Warnakulasuria 2001 commented that there was wide variation in the thickness of the covering epithelium in the oral cavity, with much undulation which lead to practical difficulties in using this grading system.

9. Kuffer and Lombardi (2002)²⁴: Felt that the choice of clinical rather than histological criteria in the diagnosis and terminology of precancer is the cause of a disorderly mixture of dysplastic and non-dysplastic lesions. Therefore, they proposed to dismember the classical "oral precancerous lesions" to classify all cases which histologically do not show dysplasia into the category of "risk lesions" (eg. Simple tobacco keratosis) and to place lesions with dysplasia (i.e. already engaged in the process of malignant transformation) into the category of "precursors" of squamous cell carcinoma (e.g: tobacco keratosis with dysplasia) This "precursor" term seems to be the most accurate to characterize the limited but already malignant intraepithelial alterations of dysplasia and carcinoma in situ, which herald the onset of an invasive squamous cell carcinoma

Drawbacks: As there was considerable difference in potential for transformation between lesions without dysplasia or with mild/moderate dysplasia and those with severe dysplasia, the application of the term "risk lesion" to lesions without dysplasia which have a "zero risk" of transformation (ex. Frictional keratosis) was inappropriate. It could lead dentist with less expertise in this area to exaggerate the risks posed by the lesions with little or no possibility of developing into cancer. The use of the term "precursor of oral squamous cell carcinoma" to denominate dysplastic lesions suggested that they were unequivocally associated with the future development of cancer.

This had no scientific evidence. On the contrary, as demonstrated by Mincer et al²⁵, 20 % of oral dysplasia's regressed and 40% showed no modification in severity. According to Gupta et al²⁶, 13% of cases regressed and 40 % showed no modification in severity [Moles M.A.G. 2002].²⁷ The fact that "dysplasia" considered as "pre-malignant" was usually treated conservatively and "carcinoma in situ" considered as "malignant" was surgically treated, was criticized by Richart (1967)²⁸ and he demonstrated that dysplasia and carcinoma in situ were different aspects of the same disease "cervical intraepithelial neoplasia (CIN)" and treatment should be same for both. This concept of CIN (one or more clones of transformed cells slowly replacing normal keratocytes starting from basal and parabasal layers to progressively invade the whole epithelial height) has now replaced almost completely that of cervical dysplasia. It has been extended with some modification to oral mucosa as "oral intra epithelial neoplasia" OIN and in general as squamous intraepithelial neoplasia (SIN)²⁰

As for CIN, there are 3 grades of OIN. OIN 1: Mild dysplasia- less than 1/3rd involvement of the epithelium

OIN 2: Moderate dysplasia- 1/3rd to 2/3rd involvement, OIN 3: Severe dysplasia – full thickness involvement or equivalent to carcinoma in situ.

This system is largely based on subjective interpretation and lacks consistency in diagnosis among pathologists. The "Bethesda classification"¹⁹ for cervical cytopathology, includes only 2 grades

1. "Low grade squamous intraepithelial lesion (LSIL)" corresponding to CIN 2. "High grade squamous intraepithelial lesion (HSIL)" corresponding to CIN2, CIN3.

Based on this "Bethesda classification" the former system with 3 grades for OIN was replaced by a 2 grade system, which helped in better stratifying patients for clinical protocols. Accordingly they chose to report the diagnosis of oral dysplastic lesions as: Low grade oral intraepithelial neoplasia (Loin) - including OIN 1 (mild dysplasia) or as High grade oral intraepithelial neoplasia (Hoin)- including both OIN2(moderate) and OIN3 (severe dysplasia).

10. Ljubljana grading system.

Zerdoner DJ²⁹ (2003) evaluated the applicability of the Ljubljana grading system, a classification proposed for grading of epithelial hyperplastic lesions of the larynx, to hyperplastic epithelial lesions arising in the oral cavity^{14, 27}. A total of 135 epithelial lesions were categorized according to Ljubljana classification as

Simple hyperplasia: A benign hyperplastic process with retention of the normal pattern of the epithelium

which is thickened because of an increased pickle cell layer. The cellular components of the basal and parabasal region remain unchanged. There is no cellular atypia.

Abnormal hyperplasia: A benign augmentation of basal and parabasal layers. They are augmented to a degree which constitutes upto one- half of the total epithelial thickness. Stratification is fully retained. Occasionally, more than this proportion of the epithelium may be involved by the hyperplastic cells without significant atypical nuclear changes. Nuclei in the cells of the augmented basal and parabasal layers may be moderately enlarged but still maintain a uniform distribution of nuclear chromatin. Occasional typical mitoses may be found in or near the basal layer. Small numbers of epithelial cells, less than 5% are dyskeratotic.

Atypical hyperplasia: or "risky" epithelium demonstrates a recognizable alteration of epithelial cells towards malignancy, but not to such a degree as is seen in carcinomatous cells. Stratification is still preserved in the general epithelial structure. The nuclei are enlarged and nuclear contour may be irregular with marked variations in staining intensity. The nuclear cytoplasmic ratio is increased. Mitotic figures are increased but not numerous, and they are found within 2\3rd of the epithelium above the basement membrane. They are, rarely, if even abnormal Dyskeratotic cells are frequent. Civatte bodies (apoptotic cells) may be present.

Carcinoma in situ: shows features of carcinoma without invasion. Stratification of the epithelium as a whole is lost. Marked cellular alteration of the type found in atypical hyperplasia are present to a considerable greater degree. Many mitotic figures present throughout the epithelium, including its upper 1/3rd and abnormal mitoses are frequently found.

11. Brothwell D J et al (2003):

In an attempt to determine the extent of observer agreement in diagnosis of oral epithelium dysplasia, Brothwell D J et al (2003)³⁰ graded 64 sections of epithelial dysplastic lesions according to 5 point scale routinely utilized at their institution (Faculty of dentistry, University of Toronto)

The criteria were:

0 = No dysplasia

1 = Mild dysplasia: Increased number of cells in the basal and parabasal epithelial regions showing nuclear hyperchromatic and pleomorphism

2 = Moderate dysplasia: Bulbous rete-pegs with increased numbers cells showing nuclear hyperchromatism and pleomorphism, extending to and including the basal, parabasal and prickle cell layer.

3 = severe dysplasia: Bulbous retepegs with increased numbers of cells showing nuclear hyperchromatism

and pleomorphism through the entire thickness of epithelium.

4 = Carcinoma in situ: Markedly atypical changes showing nuclear hyperchromatism and pleomorphism and encompassing the entire thickness of the epithelium, with the suggestion of early superficial connective tissue invasion, but without convincing evidence.

Using this system, and a different method of statistical analysis, the authors proved that intra and inter-observer agreement in grading the dysplastic lesions were consistent and had almost perfect conformity.

12. Binary system (2005):

Proposed by Omar Kujan et al³¹, considered the lesions under:

- High –risk lesions (with potential susceptibility for malignant transformation): was based on observing at least four architectural changes and five cytological changes. (W.H.O criteria 2005).
- Low risk lesions (does not have the potential susceptibility for malignant transformation): was associated with observation of less than four architectural changes or less than five cytological changes. (W.H.O criteria)

Oral potentially malignant lesions are characterized most frequently by the appearance of white patches on the oral mucosa. Overall malignant progression in these lesions is only of the order of 5% and there are no currently accepted markers to distinguish those that may progress from those or not.^{32, 33} The diagnosis of epithelial dysplasia is a subjective assessment of the discrepancy of epithelial maturation patterns and a variety of cellular changes. It implies an increased risk of malignant transformation that is relative to the grade of dysplasia. However, accurate diagnosis and grading of epithelial dysplasia presents an enormous challenge to the histopathologists and is essentially subjective. Accordingly many systems of grading epithelial dysplasia have been proposed. Thus on reviewing the different grading systems, it is observed that the photographic method by Smith and Pindborg (1969), which was based on scoring of 13 features of epithelial dysplasia except thickness of epithelium involved, did not find much favor among pathologists. Warnakulasuriya (2001) noted that even inflammatory or reactive lesions may demonstrate some features which were considered dysplastic.

The grading by Banoczy and Csiba (1972) was based on subjective interpretation of the features and didn't

take into account which factor was important in determining the malignant potential. However, WHO in 1978 defined histologic changes that contributed to the diagnosis of oral epithelial dysplasia and classified epithelial changes as mild, moderate, severe and carcinoma in situ. Most authors like Neville et al (1995), Speight P M et al (1996) have taken into consideration a combination of microscopic features enlisted by the WHO and graded dysplasia indifferent ways. To these features, Burkhardt and Maerker (1981) added the presence of Candida organisms and so also Lumermann H et al (1995) added verrucous hyperplasia with dysplasia and considered them in their grading systems. The recent classification by Kuffer and Lombardi (2002) which was based on gynacecological model of grading cervical dysplasia (CIN) and Bethesda classification for cervical cytopathology had major drawbacks and failed to overcome the subjectivity in assessing dysplasia.

The Ljubljana (2003) grading system for laryngeal hyperplastic lesions, applied to the hyperplastic lesions of oral cavity appears to be a better one as it divides the hyperplastic lesions into benign, risky, and carcinoma in situ which require separate treatment options. These system further needs to be defined for oral dysplastic lesions in predicting malignant transformation. Regarding the different classification systems, data concerning the WHO classification system are the most available in current literature. There is no simple relationship or overlapping between the classifications systems. Further studies should be done to see whether other systems have advantages above the current WHO system and to discover indications that could lead to a universal classification system for intraepithelial lesions of the head and neck.³⁴

Grading of dysplasia continues to be a hotly debated subject. It is subjective and lacks intra and inter-observer reproducibility due to the insufficiency of validated morphological criteria and the biological nature of dysplasia. Moreover, due to the absence of a consensus, several systems are currently employed. The search for alterations in molecular and genetic characteristics has so far not yielded predictive risk markers to assess the malignant potential of oral dysplastic lesions. Despite many alternative approaches conventional histopathological evaluation based on light microscopic examination of haematoxylin and eosin stained slides is still, the gold standard for assessing the malignant potential of preneoplastic head and neck lesions.¹¹

Table 5: Classification schemes for epithelial dysplasia

Oral epithelial Dysplasia	Squamous Intraepithelial Neoplasia (SIN)	Squamous Intraepithelial Lesions ('Ljubljana System')	Classic Laryngeal System
Epithelial hyperplasia	n/a	Simple hyperplasia	Laryngeal Keratosis
Mild dysplasia	SIN 1	Basal/Parabasal Hyperplasia	Hyperplasia
Moderate dysplasia	SIN 2	Atypical	Keratosis with
Severe dysplasia	SIN 3	Hyperplasia	Dysplasia
Carcinoma in situ		Carcinoma in situ	Carcinoma in situ

Histopathological Grading Systems for Oral Squamous Cell Carcinoma:

1. BRODER'S SYSTEM (1927)
2. JAKOBSSON ET AL (1973)
3. FISHER (1975)
4. LUND ET AL (1975)
5. WILLEN ET AL (1975)
6. CRISSMAN ET AL (1980)
7. ANNEROTH ET AL (1987)
8. BRYNE'S INVASIVE FRONT GRADING (1989, 1992)

I. BRODER'S SYSTEM (1927)³⁵: Broder's suggested a system of grading tumors in which a grade I lesion was highly differentiated (its cell were producing much keratin) while grade IV was poorly differentiated (the cells were highly anaplastic and showed practically no keratin formation) ¹¹⁹. Broder's initiated quantitative grading in cancer. His classification has been used for many years in squamous cell carcinoma and based on proportion of neoplasm resembling normal squamous epithelium. A lack of correlation between Broder's degree of differentiation and prognosis has been reported. One of main reason being that squamous cell carcinoma usually exhibits a heterogenous cell population with difference in degree of differentiation. Thus in study of squamous cell carcinoma they found that the histologic grade reflected the aggressiveness of the individual neoplasm and that there was a clear relationship between grade and cure rate, stage of disease and metastatic involvement.

II. JAKOBSSON ET AL. (1973)^{35,36}: This system not only includes the morphologic parameters "structure", "tendency to keratinization", "nuclear aberrations", and "number of mitosis", but also an evaluation of tumor-host relationship as estimated by parameters such as "mode," "stage of invasion", "vascular invasion" and "degree of lymphoplasmocytic infiltration"

Table 6: Histological malignancy grading system developed by Jakobsson et al.

Histological grading of malignancy based on tumor cell population				
Tumor cell Population	Points			
	1	2	3	4
Structure	Papillary and solid Strands		Small cords and groups of cells	Marked cellular Dissociation
Differentiation	Highly: keratinization	Moderately: Some Keratinisation	Poorly: Minimum keratinization	Poorly: No-keratinization
Nuclear polymorphism	Few enlarged nuclei	Moderate number of enlarged nuclei	Numerous irregular enlarged nuclei	Anaplastic immature enlarged nuclei
Mitoses	Single	Moderate number	Great number	Numerous
Histologic grading of malignancy based on tumor-host relationship				
Tumor -host Relationship	points			
	1	2	3	4
Mode of invasion	Well-defined borderline	Cords less marked borderline	Groups of cells, no distinct borderline	Diffuse growth
Stage of invasion	Possibly	Micro-carcinoma (few cords)	Nodular into connective tissue	Massive
Vascular invasion	None	Possibly	Few	Numerous
Cellular response (plasma-lymphocytic Infiltration)	Marked	Moderate	Slight	None

III. FISHER (1975)^{35,36}: They modified slightly, the grading system developed by Jakobsson et al. and indicated the malignancy grade of biopsy tissue tended to be lower than the grade of definitive section obtained from surgical specimen.

Table 7: Histologic Malignancy Grading System Developed By FISHER

	Tumor score			
	1	2	3	4
Differentiation	Much keratin	Some keratin	Squamous	Anaplastic
Nuclear polymorphism	Few aniso	Moderate aniso	Many aniso	Bizarre
Mitoses	Occasional	Few	Moderate	Many
Stroma	Abundant	Dense	Delicate	None
Mode	Pushing	Bands	Cords	Diffuse
Stage	No invasion	Micro-invasion	In connective tissue	Deep
Vascular	None	Possible	Few	Many
Inflammatory response	Marked	Moderate	Slight	None

IV. LUND et al (1975)^{35,36}: They also modified, grading system of Jakobsson et al. by presenting a more exact definition of each parameter and grade and by introducing a histologic score, defined a total sum of points divided by the number of parameters evaluated. They found a statistically significant correlation between microscopic score and death rate as well as the frequency of local recurrence and regional lymph node metastases in a series of 438 patients with squamous cell carcinoma of the tongue.

Table 8: Histologic malignancy grading system developed by LUND:

Microscopic grading				
POINTS				
	1	2	3	4
Appearance	Exophytic papillomatous	Inverted papillomatous	Small cords and group of cells	Marked cellular dissociation
Cytoplasmic differentiation (keratinization)	High > 50% keratinized	Moderate 20-50% keratinized	Poor 5-20% keratinized	None 0-5%
Nuclear differentiation (Broder's)	High more than 75% mature	Moderate 50-75% mature	Poor 25-50% mature	None 0-25% mature
Mitosis*	Single 0-1	Moderate number 0-3	Great number 0-5	Numerous more than 5
Mode of invasion (modus)	Well defined borderline	Cords, less marked borderline	Group of cells. No distinct borderline	Diffuse growth
Stage of invasion (depth) †	Possible invasion	Micro-invasion (few cords)	Nodular into Sub mucosa	Invasion deeper than sub mucosa
Vascular invasion	None	possible	Lymph, vessels	Blood vessels
Cellular response (plasma lymphocytic)	Marked (continuous rim)	Moderate (many large patches)	Slight (a few small patches)	None

*Minimum evaluation of five fields x 250, † No invasion may constitute preinvasive lesion.

V. WILLEN et al (1975)^{35,36}: They also used modified system of Jakobsson et al. They consisted of the deletion of two morphological parameter "structure" and "vascular invasion". The results showed no definitive correlation between the clinical stage and histologic grading of malignancy. In the group with no metastases the neoplasm were highly differentiated and mitotic rates were low, but nuclear polymorphism was sometime prominent. In the group with metastases the neoplasm were less differentiated and advanced nuclear aberrations with increase mitotic rates.

Table 9: Histologic malignancy grading system developed by Willen et al.

Histologic grading of malignancy				
1. Tumor cell population				
	1	2	3	4
Differentiation	Highly keratinized	Moderately, some keratinization	Poorly, minimal keratinization	Poorly, no keratinization
Nuclear Polymorphism	Few enlarged nuclei	Moderate number of enlarged nuclei	Numerous irregular enlarged nuclei	Anaplastic immature enlarged nuclei

Mitoses	Single	Moderate number	Great number	Numerous
Histologic grading of malignancy				
2. Tumor-host relationship				
	1	2	3	4
Mode of invasion	Well-defined borderline	Cords, less marked borderline	Groups of cells, no distinct borderline	Diffuse invasion
Stage of invasion	Suspicious	Micro-carcinoma few cords	Nodular invasion in connective tissue	Massive invasion
Cellular response	Marked	moderate	slight	None

VI. CRISSMAN et al (1980)^{35,36}: They modified the criteria outlined by Jakobsson et al. in two steps. They included a different point scale for vascular invasion and structure and mode of invasion into a single parameter “pattern of invasion”. The new parameter was considered to reflect the capacity of the tumor cells cohesiveness to keep the tumor cell population together as well as the association of the invading tumor cell and host stroma. “Differentiated” cohesive neoplasm infiltrated with well delineated pushing margins, whereas “less differentiated” non-cohesive neoplasm infiltrated as small, irregular neoplastic cell aggregates or single cells. This modified system applied on 73 oral squamous cell carcinoma patients. This result shows only the “frequency of mitosis.”

Table 10: Histologic malignancy grading system developed by Crissman et al.

HISTOLOGIC CRITERIA	TUMOUR SCORE			
	1	2	3	4
Tumor cytology				
Cytoplasmic Keratinization	High degree (> 50% of cell), Well-formed keratin pearls	Moderate degree (20%-50% of cells), attempts at pearl formation	Low degree (5%-20% of cells)	None identified
Nuclear differentiation	Few enlarged nuclei, 75% mature	Moderate number enlarged, variable sized nuclei 50-70% mature	Numerous enlarged pleomorphic nuclei, 25-50% mature	Anaplastic nuclei, 0-25% mature
Frequency of mitosis*	0-1	2-3	4-5	>5
Stroma Of Tumour –Host Interface				
Inflammatory Cells Response Tumour Growth Pattern	Marked continuous rim	Moderate, patchy	Slight, few small patches	None
Stage Of Invasion	CIS,† probable invasion	early or micro invasion	nodular infiltration into sub mucosa	invasion through sub mucosa
Pattern Of Invasion	Verrucous or Exophytic pushing border	Exophytic with infiltrating cords	Sessile with infiltrating cords	Infiltrating in small groups and dissociated cells
Vascular Invasion	Not identified			Identified

*HPF=high power field (average count/HPF, as many microscopic fields counted as possible), † CIS = carcinoma in situ.

ANNEROTH et al (1987)^{35,36}: They also use Jakobsson et al. system for application to squamous cell carcinoma in the tongue and floor of mouth. One of the parameters, “vascular invasion” was omitted. Statistical analysis revealed that the reproducibility of the system was good for all morphologic variables. Mean total malignancy, tumor population and tumor-host relationship scores showed statistically significant correlation with mean rating for all the different morphologic parameters with certain specified exceptions. The clinical validity of the system was tested in a comprehensive study was tested in 89 patient of squamous cell carcinoma in the floor of mouth. A statically significant correlation was found between mean total malignancy scores and clinical staging, frequency of recurrence, and death from first oral primary carcinoma.

Table 11: Anneroth et al (1987) histologic grading

Histologic grading of malignancy of tumor cell population				
Morphologic Parameters	1	2	3	4
Degree of keratinisation	Highly keratinized (>50% of the cells)	Moderately keratinized (50-20% of the cells)	Minimal keratinization (5-20% of the cells)	No keratinization (0-5%)
Nuclear polymorphism	Little nuclear polymorphism (>75% mature cells)	Moderately abundant nuclear polymorphism (50-75% mature cells)	Abundant nuclear polymorphism (25-50% mature cells)	Extreme nuclear polymorphism (0-25% mature cells)
Number of mitoses/HPF*	0-1	2-3	4-5	>5
Histologic grading of malignancy of tumor-host relationship				
Morphologic parameters	1	2	3	4
Pattern of invasion	Pushing, well delineated infiltrating borders	Infiltrating, solid cords, bands and or strands	Small groups or cords of infiltrating cells (n>15)	Marked and widespread cellular dissociation in small groups of cells (n<15) and/or in single cells
Stage of invasion (Depth)	Carcinoma in situ /or Questionable invasion	Distinct invasion, involving lamina propria only	Invasion below lamina propria adjacent to muscles, salivary gland tissues and periosteum	Extensive and deep invasion replacing most of the stromal tissue and infiltrating jaw bone
Lymphoplasmocytic infiltrate	Marked	Moderate	Slight	None

VII. BRYNE'S (1989, 1992) (ITF) INVASIVE TUMOR FRONT GRADING SYSTEM^{35,36}: Bryne M. (1998) presented a hypothesis suggesting that molecular and morphological characteristics at the invasive front area of various squamous cell carcinomas may reflect tumor prognosis better than other parts of the tumor. He further states that several molecular events of importance for tumor spread like gains and losses of adhesion molecules, secretion of proteolytic enzymes, increased cell proliferation and initiation of angiogenesis occur at the tumor host interface; consequently they have developed a simple morphological malignancy grading system that restricts the evaluation to the deep invasive front of the tumor. Several studies have shown that this system is a significantly better predictor of prognosis. All studies performed so far show that invasive front grading is a valuable supplement to clinical staging, suggesting that it should be introduced into the clinic.

Table 12: BRYNE'S (1989, 1992) (ITF) Invasive Tumor Front Grading System

Morphologic Feature	1	2	3	4
Degree of keratinisation	Highly keratinized (>50% of the cells)	Moderately keratinized (50-20% of the cells)	Minimal keratinization (5-20% of the cells)	No keratinization (0-5%)
Nuclear polymorphism	Little nuclear polymorphism (>75% mature cells)	Moderately abundant nuclear polymorphism (50-75% mature cells)	Abundant nuclear polymorphism (25-50% mature cells)	Extreme nuclear polymorphism (0-25% mature cells)
Number of mitoses (high power field)	0-1	2-3	4-5	>5
Pattern of invasion	Pushing, well delineated infiltrating borders	Infiltrating, solid cords, bands and or strands	Small groups or cords of infiltrating cells (n > 15)	Marked and widespread Cellular dissociation in small groups of cells(n<15) and or in single cells
Host response (lympho-plasmacytic infiltrate)	Marked	Moderate	Slight	None

DISCUSSION

As outlined before, a histological dysplasia system ideally should meet two basic requirements. At first, it should be easily applicable in daily routine practice with low inter- and intra-observer variability. Secondly, it should allow a clear separation between patients who need treatment to prevent progression towards malignancy and those for whom no treatment is needed. Regarding inter- and intra-observer variability, evaluation of the WHO classification for oral lesions, its prognostic significance is 12–67%, as can be inferred from the data mentioned in some studies. When looking at the SIN classification it has to be noted, that with respect to reproducibility, no data of head and neck lesions are available in current literature. Concerning prognostic significance of laryngeal lesions the following data are available: SIN I 5%, SIN II 25%, SIN III 11–25%. Data concerning the SIN classification in relation to predictive value of oral lesions are not available in current literature.

Regarding the Ljubljana classification, its use for the larynx has been documented extensively. Its relevance for prognosis has been amply demonstrated by the pathologists and clinicians who developed the system. However, its usefulness has not yet resulted in widespread acceptance. For the oral cavity, there is only one study that reports its use in this anatomic location, Further studies should be done to see whether it has an advantage above the current WHO dysplasia system. Although the histological assessment of the WHO dysplasia system and the Ljubljana system are based on the same architectural and cytological changes, there is no simple relationship or overlapping between the classifications systems. According to Gale et al., comparing the three discussed classification systems, it is unlikely that they will come together in the very near future. On the other hand, future discoveries mainly in molecular biology could be the basis for a single, universal classification system for intraepithelial lesions of the Upper Aero-digestive tract.

CONCLUSION

Regarding the different classification systems, data concerning the WHO classification system are the most available in current literature. There is no simple relationship or overlapping between the classification systems. Further studies should be done to see whether other systems have advantages above the current WHO system and to discover indications that could lead to a universal classification system for intraepithelial lesions of the head and neck.

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