Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 1260-1271 | ISSN:2251-6727

Reframing Oral Epithelial Dysplasia as a Public Health Priority: Diagnostic Advances, Molecular Insights, and Preventive Strategies

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(Received: 27 September 2025 Revised: 05 October 2025 Accepted: 18 November 2025)

KEYWORDS

Oral Potentially Malignant Disorders (OPMDs), Oral Epithelial Dysplasia (OED), Squamous Intraepithelial Neoplasia (SIN), Grading Systems, Biomarkers, Next-Generation Sequencing (NGS), Artificial Intelligence (AI), Deep Learning

ABSTRACT:

Oral potentially malignant disorders (OPMDs) represent a significant public health concern due to their measurable risk of malignant transformation into oral squamous cell carcinoma (OSCC), one of the most prevalent cancers in low- and middle-income countries. The global burden of oral cancer, largely driven by tobacco, alcohol, and areca nut consumption, underscores the necessity for early detection, effective screening, and evidence-based management of precursor lesions. Histopathological assessment for the presence and grading of oral epithelial dysplasia (OED) remains the current gold standard for predicting malignant transformation; however, this approach faces challenges of subjectivity, variability, and limited reproducibility across observers and regions. Several grading systems such as those proposed by Smith and Pindborg (1969), the Ljubljana classification, and successive World Health Organization (WHO) iterations (1978, 2005, 2017, 2022) have attempted to standardize diagnosis, yet inconsistencies persist. These limitations can directly affect early detection programs, delay intervention, and increase the disease burden at the community level. Hence, improving diagnostic objectivity is not merely a laboratory refinement but a critical public health goal.

Recent advances in molecular markers including p53, p63, Ki-67, β-catenin, and E-cadherin, along with genomic profiling through next-generation sequencing (NGS), have enhanced understanding of dysplasia progression and its molecular drivers. Incorporating such biomarkers into community screening models could significantly strengthen early cancer risk stratification and surveillance programs. Furthermore, artificial intelligence (AI) and deep learning (DL)-based systems show promise for large-scale, low-cost, and

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JCHR (2025) 15(6), 1260-1271 | ISSN:2251-6727

(DL), and Molecular Diagnostics.

automated detection of dysplastic changes, particularly in resource-limited settings.

This review examines the evolution of grading systems for OED, emphasizing their implications for public health policy, screening efficiency, and preventive oncology. By integrating histopathological evaluation with molecular diagnostics and AI-driven technologies, healthcare systems can move toward a more standardized, objective, and scalable diagnostic framework. Strengthening these integrative diagnostic pathways is essential not only for clinical precision but also for reducing the incidence, mortality, and socioeconomic impact of oral cancer at the population level.

According to the GLOBOCAN 2020, oral cancer accounts for 0.46% of all cancers worldwide,while in India, it constitutes 10.3% of all cancer cases. Alarmingly, India accounts for nearly one-third of the world's oral cancer cases, highlighting a serious public health concern [1]. Oral potentially malignant disorders (OPMDs) are defined as a heterogeneous group of clinically defined conditions associated with a variable risk of progression to oral SCC. Most produce clinically visible lesions[2].

The sequence of malignant transformation of OPMDs depends on the progression of epithelial dysplasia and alterations in the connective tissue tumor microenvironment. This transformation does not necessarily follow a predictable sequence from mild to moderate to severe dysplasia. Moreover,even when the underlying etiological stimulus is removed, the dysplastic alterations in the epithelium may revert to normal [3]. The malignant transformation of OPMDs depends upon a range of patient and/or lesion-related factors, including sex, type of lesion, habits, and the presence of epithelial dysplasia [4]. A systematic review by Warnakulasuriya and Ariyawardana stated that the overall malignant transformation rate of leukoplakia is 1.5% to 34%, which is 3% in homogenous lesions and 14.5% in non-homogeneous lesions [5]. The overall malignant transformation rate and annual transformation rate of Oral Submucous Fibrosis (OSMF) have been reported as 4.2% and 0.73%, respectively [6]. The malignant transformation rate of oral lichen planus and lichenoid lesions is 2.28% and 1.95%, respectively [7].

The significant rates of malignant transformation rates in different OPMDs stress the importance of early diagnosis and proper management. Early screening and detection of OPMDs not only alleviate symptoms but also prevent their progression to oral squamous cell carcinoma [8]. Despite the implementation of mass screening programs, increasing public awareness about OPMDs and their potential for malignant transformation remains essential for promoting timely diagnosis and effective treatment.

Presence of OED is currentlyregarded as the gold standard for predicting malignant transformation of OPMDs[9].OED is characterized by cytological and architectural alterations reflecting the disruption in the normal maturation and stratification patterns of surface epithelium. [10]. Transition from normal oral epithelium to epithelial dysplasia and ultimately to tumour development, is a progressive and cumulative process of genetic mutations. Simple epithelial hyperplasia progresses through mild epithelial dysplasia, and culminates in more severe dysplastic changes as genetic aberrations accumulate. The presence and severity of OED correlate with an increased risk of progression to OSCC, with higher grades of OED corresponding to a greater malignant potential. Hence, histological grading of OED can be used to assess the probability of malignant transformation of OPMDs [10]. The WHO three-tier grading system categorizing dysplasia as mild, moderate, and severe is the most widely adopted approach for the histopathological evaluation of OED. [11]. In 2006, the WHO Working Group introduced a two-tier (binary)system for classifying OED, categorizing lesions as either low risk or high risk for malignant transformation.However, this binary system requires further validation to establish its reliability in predicting malignant transformation before it can be incorporated intoroutine clinical practice[12,13].

The main objective of a standardizedOED grading system is to facilitateconsistent reporting and management, concurrently serving as a valuable tool in epidemiological studies. Most importantly a clinically useful grading system for OED, should be reproducible,

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JCHR (2025) 15(6), 1260-1271 | ISSN:2251-6727

clinically relevant and accurately reflect the malignant potential of the lesion based on histological features.

To date, many grading systems for OEDhave been proposed, each with inherent limitations that challenge their predictiveaccuracy and clinical utility. These shortcomings highlight the ongoing need for a more precise and standardized system to assess the malignant transformation risk. In response, several refinements have been introduced over time; however, further large-scale research is essential to establish an optimal grading framework.

This review aims to summarize the key features of the main OED grading systems proposed to date, and to discuss their limitations and prognostic value. It also explores the applicability of recent advancements in molecular diagnostics and AI-driven approaches. This integrated strategy holds promise for enhancing early detection and intervention strategies for OPMDs, ultimately improving patient outcomes.

Main OED grading systems proposed to date

1. Smith and Pindborg (1969)

Smith and Pindborgproposed the first system for grading OED in 1969, using a set of photographs as visual references [14]. Two major factors were considered inscoring:

a) A single histopathological feature was focused at a time, paying full attention to its details.

b) Individual assessment of each character was done by the observer allotting a particular score to each one.

Thirteen histologic features were assessed and graded as absent, slight, or marked, with eachassigned specific scores [Table 1]

Criteria	Scoring
1. Drop-shaped rete ridges	Criteria were categorized as absent, slight, and marked. Scoring: absent = zero, slight or marked = 1 to 10. The scores were cumulated = eai *varied from 0-75.
2. Loss of stratification regularity.	
3. Keratin formation by individual cells.	
4. Hyperplasia of the basal layer	
5. Loss of intercellular adherence.	
6. Loss of basal cell polarity	
7. Nuclear hyperchromatism.	Total score: 0-10 (no dysplasia) 11-25 (mild dysplasia) 26-45 (moderate dysplasia) 45-75 (severe dysplasia)
8. Increased n:c ratio.	
9. Anisocytosis, anisonucleosis.	
10. Cellular and nuclear pleomorphism	
11. Increased mitotic activity.	
12.Presence of mitotic figures in the superficial half of epithelium.	
13. Abnormal mitosis	

Table 1 –Main histologic features

Merits and demerits:
An objective and semi-quantitative diagnosis of dysplasia was provided by this system.Numerical values added in this system could help to provide statistical analysis, which was an outstanding merit in current research studies.
However, it does not address why some non-neoplastic lesions also show evidence of dysplasia, and many researchers found this monograph to be complex and time-consuming to apply.

2. Ljubljana classification of SIL (squamous intraepithelial lesion)

The Ljubljana system was primarily developed to address the clinical and histological problems of laryngeal lesions [15]. It categorizes hyperplastic epithelial lesions into four grades: simple hyperplasia, abnormal hyperplasia, atypical hyperplasia and carcinoma in situ (CIS). The term simple hyperplasia is used to indicate an increase in the thickness of stratum spinosum and abnormal hyperplasia for delineating basal cell hyperplasia. In atypical hyperplasia, synonymously called as risky hyperplasia, epithelial stratification was retained, while atypia was evident. In CIS loss of stratification was evident along the entire epithelium, although three to five layers of compressed cells could be found on the surface. Severe degrees of atypia and mitotic abnormalities were characteristic.

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Laryngeal pathologists Kambic and Lenart proposed this system of classifying dysplasia in laryngeal hyperplastic lesions in 1971 [16]. Zerdoner in 2003, suggested its use for grading hyperplastic epithelial lesions of the oral cavity [17].

Merits and demerits

It provides comprehensive information to clinicians for the accurate diagnosis and management of OED.It also focuses on important factors essential for identifying patients with benign lesions, mild to moderate grade, and severe grade lesions, aiding in decisions regardingthe need for follow-up and treatment.

However, this classification does not effectively categorize some oral lesions such as OSMF and OLP, which often show atrophy of epithelium and minimal cytological atypia. It is complicated and more time consuming, and its applicability for oral lesions needs further validation. Incorporating numerical values into the grading system would enhance its utility by enabling the statistical tests for significance.

3. WHO classification (1978)

In an effort to introduce standardized criteria for gradingOED, WHO established a collaborating reference center in 1967,with the objective of identifying precancerous lesions and their relative risk of malignant transformation [18]. Using specific parameters, they graded OED into three: mild, moderate, and severe in 1978 [Table 2].

Criteria	Grades
1. Loss of basal cell polarity.	Mild: dysplastic features in the lower 1/3rd of epithelium. Moderate: dysplastic features in the lower 2/3rd of epithelium. Severe: dysplastic features involving > 2/3rds of epithelium.
2. Multiple layers of cells having basaloid appearance	
3. Increased n:c ratio	
4. Drop-shaped epithelial ridges.	
5. Loss of stratification.	
6. More number of mitotic figures	
7. Mitotic figures in the superficial layers	
8. Cellular polymorphism	
9. Nuclear hyperchromatism.	
10. Enlarged nucleoli.	
11. Reduction of cellular cohesion	
12. Keratin pearls in the spinous cell layer.	

Mild dysplasia indicated a low-risk potential for malignant transformation; however, high-risk sites such as the floor of the mouth and the ventral surface of the tongue needed greater concern. Moderate dysplasia signified the forewarning sign, and severe dysplasia indicated a considerable risk of malignant changes [19].

Merits and demerits

This system was user-friendly and more efficient and the classification focused on both cellular and architectural features.

However, it failed to explain which factor was important in determining the malignant potential. Practical values to distinguish between severe dysplasia and cis were not elucidated and in different cases, the agreement may vary depending upon the observer.

4. WHO classification (2005)

WHO classification in 2005 used a combination of cytological and architectural changes, based on which the epithelium was divided into “thirds” andthe lesions were classified into five categories as follows;

- Hyperplasia: Increase in number of cells in basal/parabasal cell layers without cellular atypia.
- Mild dysplasia: Architectural changes limited to the lower third of the epithelium accompanied by cytological atypia.
- Moderate dysplasia: Architectural changes limited to the middle third of the epithelium accompanied by cytological atypia.
- Severe dysplasia: Architectural disturbances extending above 2/3rds of the epithelium accompanied by cytological atypia.
- Cis: Architectural abnormalities in full thickness accompanied by cytological atypia.

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Both cytological and architectural criteria were used for diagnosing OED.

Architectural criteria: 1. Irregular epithelial stratification, 2. Loss of polarity of basal cells, 3. Drop-shaped rete ridges, 4. Increased number of mitotic figures, 5. Abnormal superficial mitoses, 6. Premature keratinization in single cells (dyskeratosis), 7. Keratin pearls within rete ridges,

Cytological criteria: 1. Abnormal variation in nuclear size (anisonucleosis), 2. Abnormal variation in nuclear shape (nuclear pleomorphism), 3. Abnormal variation in cell size (anisocytosis), 4. Abnormal variation in cell shape (cellular pleomorphism), 5. Increased nuclear cytoplasmic ratio, 6. Increased nuclear size, 7. Atypical mitotic figures, 8. Increased number and size of nucleoli, 9. Hyperchromatism [20].

Merits and demerits:

The classification considered both cellular and architectural changes in the epithelium and demonstrated good inter- and intra-observer concordance. In addition, it introduced the term 'squamous hyperplasia' to describe specific non dysplastic changes.

However, the classification had several limitations, such asthe lack of numerical values, which made statistical analysis challenging. It also failed to account forrisk factors associated with malignant transformation. Furthermore, variability in the thickness of the oral epithelium thickness could lead to inconsistencies in grading.

5. Squamous Intraepithelial Neoplasia / Dysplasia Classification (2005)

It represents the modified WHO classification 2005 as "Oral Intraepithelial Neoplasia" (OIN) and the broader term SIN was adopted to encompass in multiple sites of the upper aerodigestive tract [21].According to this classification, dysplasia is viewed as a spectrum, with hyperplastic keratinizing SIN/dysplasia referred to as keratinized dysplasia at one end, and atrophic SIN/dysplasia resemblingWHO-type dysplasia at the other [22]. In this grading system, lesions are classified as SIN 1, SIN 2, and SIN 3 and it follows the same architectural criteria and cytological criteria as WHO classification 2005.

SIN 1: Architectural changes limited to the lower third of the epithelium accompanied by cytological atypia.

SIN 2: Architectural changesaccompanied by cytological atypiaare limited to the middle third of the epithelium.

SIN 3: Combination of architectural disturbances extending above 2/3rds of the epithelium or full thickness accompanied by cytological atypia.

Merits and demerits:

This classification is simple and easy to apply. It also considers the other regions of the upper aerodigestive tract,allowing for a broader assessment of the severity of dysplasia.

However, it fails to explain as to why many potentially malignant lesions of the oral mucosa do not transform into carcinoma.Additionally, overlapping features between the two ends of the spectrum can lead to inaccurate grades. This system is mainly based on subjective interpretation, which may limit its reliability and reproducibility.

6. WHOclassification (2017)

In the 2017 classification for grading oral epithelial dysplasia (OED), specific modifications have been made to the criteria originally established by the WHO in 2005. [23,24].

The classification recognizes 08 architectural and 08 cytological changes for diagnosing oral epithelial dysplasia (Table 3). It describes a grading system based on "thirds" and oral epithelial dysplasia is graded as mild, moderate, and severe depending on whether dysplastic features extend into the lower third, middle third, or the upper third of the epithelium, respectively. The term "carcinoma in situ" is used synonymously with severe dysplasia. [13]

Architecture	Cytology
Irregular epithelial stratification	Abnormal variation in nuclear size (anisonucleosis)
Loss of polarity of basal cells	Abnormal variation in nuclear shape (nuclear pleomorphism)

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Drop-shaped rete ridges	Abnormal variation in cell size (anisocytosis)
Increased number of mitotic figures	Abnormal variation in cell shape (cellular pleomorphism)
Abnormally superficial mitotic figures	Increased nuclear-cytoplasmic ratio
Premature keratinization in single cells (dyskeratosis)	Atypical mitotic figures
Keratin pearls within rete ridges	Increased number and size of nucleoli
Loss of epithelial cell cohesion	Hyperchromasia

Table 3: Criteria used for diagnosing oral epithelial dysplasia

The terms “squamous hyperplasia” and “CIS” have been excluded from the WHO 2005 system,with “CIS” corresponding to severe dysplasia. Also, the feature “increase in nuclear size” has been removed, while “loss of epithelial cell cohesion” has been incorporated into this classification.

In general, the initial criterion for categorizing dysplasia in this three-tier WHO system is the level of extension of architectural disturbance. However, sometimes the dysplasia in a particular lesion may require upgrading or downgrading, considering the degree of cytologic atypia. For example, while a lesion with marked atypia extending only into the middle third of the epithelium is upgraded from moderate to severe dysplasia, another lesion with mild atypia extending into the middle third of the epithelium may be downgraded as mild dysplasia.

This system is simple and easy to use and has improved the previously used classification by WHO in 2005. However, its limitations included a lack of numerical values, leading to a failure to provide statistical analysis. It was also based on subjective interpretations, and it does not imply the continuous progression of dysplasia. Moreover, it can't predict its malignant potential.

However, several studies have shown great inter and intra observer variability and poor reproducibility in the assessment of the grade of oral epithelial dysplasia with this three-tier WHO system.

7. WHO classification 2022 version

This classification has further expanded the architectural and cytological features for diagnosing OED, while retaining the 3-tiered grading system [25].

A distinction has been made between single-cell keratinization and generalized premature keratinization, withsingle-cell keratinization now recognized as a cytological feature.

Compared to the 2017 iteration, the 2022 version includes significant updates in pathological features, increasing the total number of features from 16 to 27 [25–27]. The newly added features are as follows [25];

Architectural features	Cytological features
Altered keratin pattern for oral sub-site Verrucous or papillary architecture Extension of changes along minor gland ducts A sharply defined margin for changes Multiple different patterns of dysplasia Multifocal or skip lesions Expanded proliferative compartment Basal cell clustering/nesting	Single cell keratinization Apoptotic mitoses Increased nuclear size

Despite these advances, the longstanding concern regarding the oversimplification inherent in the three-tiered grading system remains unresolved.

The efficacy and usefulness of histopathology in predicting malignant transformation of oral potentially malignant disorders has been a constant debate, as malignant transformation of oral potentially malignant disorders can even occur in the absence of oral epithelial dysplasia. Moreover, wide intra- and inter-observer variability have been recognized in

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histopathological diagnosis and grading of oral epithelial dysplasia, raising concerns over its reproducibility and clinical utility.

Recommendations to subdue these limitations include the use of clinical determinants and molecular markers to supplement the grading system.

Molecularmarkers

Molecular testing may provide valuable insights into the risk of malignant transformation in dysplastic lesions

DNA mutations accumulate as dysplasia progresses towards OSCC.Alterations such as loss of heterozygosity and microsatellite instability have been observed, which may serve asindicators for assessing the risk of malignant transformation [28].

Interestingly, researchers have identified several biomarkers that are associated withthe grading of dysplasia. De vicente et al. [29] observed an association between Nanog (a key regulator of pluripotency and self-renewal in embryonic and adult stem cells) and the grade of dysplasia. It was noted that the expression of Nanog is increased with the grade of dysplasia. Grubelnik et al. concluded that Nanog is helpful in differentiating the gradeof dysplasia[30]. Detection of Nanog protein leads towards the diagnosis of oral high-grade dysplasia, distinguishing it from low-grade dysplasia and non-neoplastic reactive lesions.p63 and cd31 are the markers explored primarily in many studies. In normal cells p63 protein is found in the basal layer of squamous epithelium [31]. Bavle et al. [32] found that p63 expression increases with the severity of dysplasia and is primarily in suprabasal cells. p63 is necessary to maintain cell proliferation. As the severity of dysplasia increases, the proliferation rate increases while the cell differentiation is disturbed [33].cd31 protein is a marker of angiogenesis, andthe correlation between p63 with cd31 is helpful in categorizing leukoplakic lesions as low or moderate dysplasia [32].

P53, also known as *tp53*, is a tumour suppressor gene that encodes a protein that regulates the cell cycle [34].Pandya et al. [35] demonstrated that, thereisa statistically significant difference between tp53 expression in mild and severe dysplasia.According to Patil et al. [36] the expression of p53 increases with the grade of OED.

Several researchers, including Kamala et al. [37], and Dash et al. [38]observed an increase in Ki-67 expression with the severity of dysplasia. Mondal et al. [39], stated that there is astatistically significant difference betweenKi-67 expression in normal mucosa and mild dysplasia, as well as between mild, moderate, and severe dysplasia. The Ki-67 antigen can be used as a marker for histological evaluation of OED and as a prognosticator as well.

Intercellular junctions determine the cell polarity and enable tissue integrity, growth, and maturation.β -catenin is a component of intercellular junctions that forms a complex with E-cadherin [40,41]. According to a study by Chowdhury et al. [42] expression of β-catenin increases with the dysplasia grade andhelpful in differentiating the respective grade of dysplasia.Sharma et al. [43] found that E-cadherin expression decreases significantly with increasing dysplasia grade.

Role of next-generation sequencing, artificial intelligence, and neural networking in oral epithelial dysplasia grading

The NGS method is advantageous as it helps in routine DNA extraction, and enable large-scale sequence data acquisition within 24 hours [44]. Thereby it provides valuable insight regarding the genomic pathways and thus contribute to our understanding of disease development and subsequent progression.

Oral squamous cell carcinoma is a common epithelial malignancy known for its heterogeneous nature. The complexity of the lesion challenges accurate diagnose and management, resulting in poor prognosis. The use of NGS has enabled researchers to identify the genomic alterations evident in oral squamous cell carcinoma. Whole exome sequencing studies have identified alterations with tp53, cdkn2a, pik3ca, and hras genes[45]. Another important alteration that was identified using NGS is the notch1 gene which is involved in regulating squamous differentiation[46]. According to some studies, pik3ca mutations may be associated with the later stages of OSCC, as pik3ca is frequently mutated in stage IVOSCC [47].

Useof AI-based tools in analysing histopathology whole slide images, points towards the potential for automated pathology diagnosis. These tools also enable more effectiveinterrogation and quantification of parameters

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JCHR (2025) 15(6), 1260-1271 | ISSN:2251-6727

within the tumor microenvironment. AI algorithms developed through deep learning methods rely on artificial neural network trained on large datasets of digital images,allowing them to classify previously unseen images [48]. Accordingly, Das et al., developed a segmentation method that could identify keratin pearls and quantify the keratinization in the oral mucosa of patients with OSCC. The study utilized a keratinization index (cki) measure for the automated diagnosis and grading of OSCC. The results were promising, demonstrating the potential of using AI to diagnose OSCC through quantitative analysis of microscopic images of oral tissues at lower magnification [49]. Similarly, using deep learning, Tanriver et al. [50] proposed a two-stage model to detect and classify oral lesions into three classes (benign, OPMD, and cancer).This study identified a correlation between the degree of dysplasia and the time taken for malignant transformation. They concluded that severe dysplasia was a significant predictor of malignant transformation [51].

Fu et al. proposed a deep learning (DL) approach to detect OSCC from photographic images with an accuracy that is comparable to a specialist panel that classified the images manually [52]. Further, Adel et al. proposed a computer-aided diagnostic approach to detect and classify OED using basic machine learning (ML) techniques with an accuracy of 92.8%[53]. In contrast, Gupta et al. used a DL model to classify dysplastic tissue images with an accuracy of 89.3%.[54], whereas few authors proposed a convolutional neural network (CNN)-based approach to classify, segment, and visualize cancer from microscopic biopsy images[55-57]. By using convolutional neural network-based clinical predictors, oral dysplasia in an image can be classified accurately in early stages [58].

Exfoliative cytology-the basic tool

Oral exfoliative cytology is a simple and non-invasive diagnostic technique that could be used for early detection of OPMDs and malignant lesions [59]. The procedure is painless, bloodless, non-invasive, quick, and simple. Hence, it can be safely performed in patients who are contraindicated for biopsy, and eliminates post-biopsy complications [60].

Exfoliative cytology is based on epithelial physiology; loss of cohesion between cells enables collection of exfoliated cells for microscopic examination[61]. When diseased, the cells in the epithelium show certain cellular and nuclear changes and these changes are prominentin the dysplastic epithelium involved with OPMDs and malignancy. The cytological features evident include increased nuclear-cytoplasmic ratio, variations in size and shape of the cell and its nucleus, nuclear discontinuity, mitotic figures, texture of the nucleus, and micronuclei. These cytological alterations are studied under a microscope to aid indiagnosis. It is also possible to analyse these cells both quantitatively and qualitatively [62].

Depending on the cellular and nuclear features, the cytologic smears of OPMDs and oral cancer are categorised into 5 classes. [63,64,65]. Class i - normal, class ii - atypical, classiii -intermediate, classiv - suggestive of cancer, and class v -presence of cancer.The cytosmear can also be reported as negative for cancer (class i and class ii), suggestive or intermediate (class iii and class iv), and positive for cancer (class v) and inadequate sample [64,65].

Cytomorphometry is an objective quantitative tool, which has demonstrated a promising role in the detection of dysplasia in OPMDs. It was observed that alterations in cellular and nuclear morphology in exfoliative cells from OPMDs and OSCCs compared to normal mucosa are indicative of dysplastic changes [66].

Dysplastic/malignant cells usually show an increase in nuclear size, sometimes with irregular shapes [67]. Increase in nuclear size is possibly due increase in the synthesis of DNA [68]. It has been observed that abnormal DNA content increases from mild to severe degrees of dysplasia [69] and suggested as a predictor of oral epithelial dysplasia [69,70]. OPMDs with epithelial dysplasia have higher nuclear density and nuclear area as compared to normal mucosa. A significant increase in the mean NA/CA ratio(nuclear-to-cytoplasmic area ratio) was reported in the studies by Cowpe et al. [71] and Khandelwal and Solomon [72].

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Journal of Chemical Health Risks
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JCHR (2025) 15(6), 1260-1271 | ISSN:2251-6727

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