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REVIEW

Demystifying oral epithelial dysplasia: a histological guide

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Summary

Oral epithelial dysplasia is a histologically diagnosed potentially premalignant disorder of the oral mucosa, which carries a risk of malignant transformation to squamous cell carcinoma. The diagnosis and grading of oral epithelial dysplasia is challenging, with cases often referred to specialist oral and maxillofacial pathology centres for second opinion. Even still there is poor inter-examiner and intra-examiner agreement in a diagnosis. There are a total of 28 features of oral epithelial dysplasia listed in the 5th edition of World Health Organization classification of tumours of the head and neck. Each of these features is poorly defined and subjective in its interpretation. Moreover, how these features contribute to dysplasia grading and risk stratification is even less well defined. This article discusses each of the features of oral epithelial dysplasia with examples and provides an overview of the common mimics, including the normal histological features of the oral mucosa which may mimic atypia. This article also highlights the paucity of evidence defining these features while offering suggested definitions. Ideally, these definitions will be refined, and the most important features identified to simplify the diagnosis of oral epithelial dysplasia. Digital whole slide images of the figures in this paper can be found at: https://www.pathogenesis.co.uk/r/demystifying-dysplasia-histology-dataset.

Key words: Oral epithelial dysplasia; oral pre-cancer; malignant transformation; oral cancer; oral squamous cell carcinoma; histological grading.

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INTRODUCTION

Oral epithelial dysplasia (OED) is a disorder of oral mucosa that is diagnosed by histological identification of architectural and cytological abnormalities of the oral epithelium.[1] Some of these abnormalities are long established,[2,3] whereas others are described in more recent diagnostic criteria.[1,4,5] OED carries a risk of malignant transformation to oral squamous cell carcinoma (OSCC), though reported transformation rates vary.[6,7] OED develops as a result of genomic alterations which are often shared with the later carcinoma if transformation occurs.[8] However, most OED do not transform and many regress without intervention.[7] OED is usually initiated by chemical carcinogens such as tobacco smoke and alcohol,[3] with a smaller number driven by high-risk human papilloma virus (HPV) infection.[9] This is in contrast to cervical dysplasia where HPV is the most common cause,[10] or epidermal dysplasia where ultraviolet light exposure plays a significant role.[11]

OED manifests clinically as white, red or mixed lesions, though other presentations can be seen. Attempts have been made to correlate clinical appearances of OED to malignant transformation;[12] however, the supportive evidence is weak.[7]

Diagnosis of OED is complex and there are many mimics, due to the phenomenon of reactive atypia in many inflammatory diseases of the oral cavity. A diagnosis is reached by identification of a range of architectural and cytological features, though in some cases an individual feature, if extensive enough, may qualify a diagnosis. Some cases of OED are straightforward with obvious cytological abnormalities. However, the so called 'differentiated' or 'architectural dysplasias' tend to lack frank cytological atypia with architectural changes being the predominant feature making these cases more challenging to diagnose.[4] In the latest World Health Organization (WHO) classification of head and neck tumours[1] there are 28 histological features listed, most of which are poorly defined and with limited evidence of correlation to clinical outcomes.[4] OED is graded to assist with prognostication and treatment planning, with higher graded lesions believed to have a higher risk of malignancy.[6] However, OED grading is an unreliable predictor of cancer risk, complicated by the multiple proposed grading systems,[1,13] and wide inter- and intra-examiner variability.[14–17] Complicating matters further, there is no minimum agreed number or 'extent' of features required for a diagnosis in the WHO classification, and no consideration to the importance of individual features is given. The binary grading system proposed to simplify this by suggesting two categories with a minimum number of features for grading. However, the 'extent or abundance' of a feature, correlation of individual features and analysis of verrucous and differentiated dysplasia are still not considered, hence robust evidence to support its routine use is lacking.[13] The complexity of OED diagnosis often leads to referrals to specialist centres by pathologists inexperienced in examining oral mucosa.[18] However, variabilities and inconsistencies exist even amongst these specialists.[17]

The advent of digital pathology, artificial intelligence (AI) and machine learning (ML) has increased opportunities to explore tissue sections through automated and quantitative means, allowing more objective analysis of histological

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features of disease. These methods are increasingly used to study OED and may help with defining the histological criteria. At present, there remain many questions with regards to a diagnosis: how many basal cells constitute basal cell crowding; how superficial does a high mitotic figure need to be, and how much variation in nuclear size constitutes pleomorphism? Without strict definitions, there will continue to be subjectivity for the diagnosis and grading of OED. In Table 1 we have proposed working definitions for individual OED features listed in the 5th edition WHO classification, as these features are considered the 'gold standard' at present.[1] We believe that the list of proposed features in this classification is somewhat academic and excessive with poor scientific evidence and prognostic correlation. Though many of

Table 1 Proposed definitions for the architectural and cytological features of oral epithelial dysplasia presented in the upcoming World Health Organization 5th edition classification of head and neck tumours[1]

Features	Proposed definition
Architectural features (Fig. 4/5)	
1. Irregular stratification (Fig. 4A)	Disturbance of the stratified layers of the epithelium, with haphazardly organised and difficult to distinguish layers.
2. Loss of basal cell polarity (Fig. 4E)	Abnormal nuclear location (away from the basement membrane) and abnormal nucleus orientation (no longer parallel with other basal cell nuclei).
3. Drop shaped rete processes (Fig. 5C)	The rete process is broader at the base than at the apex.
4. Basal cell clustering/nesting (Fig. 5E)	This feature is better defined in skin with crowding of atypical basal cells, followed by budding into the lamina propria, eventually taking on an irregular outline and exceeding the thickness of the epithelium.[26,27]
5. Expanded proliferative compartment	Thickening of the basal cell layer with evidence of mitotic activity.
6. Mitoses high in the epithelium (Fig. 5B)	Mitotic figures present outside the basal cell layer. No consensus or evidence about the minimum number for dysplasia. There may be value in combining this feature with mitoses in maturing cells.
7. Mitoses in maturing cells (Fig. 5B)	Mitotic figure present in the prickle cell or granular cell layers. No consensus or evidence about the minimum number for dysplasia.
8. Generalised premature keratinisation (Fig. 4B/5C)	Increased prickle cell cytoplasmic eosinophilia due to keratinisation in excess of what is normally expected at that oral cavity site.
9. Keratin pearls in rete processes (Fig. 4D)	The formation of an intra-epithelial collection of keratin with no surface connection. It is better to consider any keratin pearl formation as dysplastic.
10. Reduced keratinocyte cohesion (Fig. 4E)	A spectrum of changes; begins with widening intercellular junctions and ends with acantholysis.
11. Altered keratin pattern for oral sub-site	An increase in the amount or change in the type of keratin from the normal at that subsite, in the absence of features of trauma.
12. Verrucous/papillary architecture (Fig. 5A/5C)	Verrucous: hyperkeratinised surface composed of sharp or blunt epithelial projections with keratin filled invaginations without fibrovascular cores. Papillary: exophytic projections of epithelium with fibrovascular cores.
13. Extension along salivary ducts (Fig. 4F)	Features of dysplasia observed within salivary ducts adjacent the dysplastic surface epithelium.
14. Sharply defined lesion (Fig. 4C)	Abrupt transition between the normal epithelium and one with the features of dysplasia.
15. Multiple patterns of dysplasia (Fig. 5F)	Multiple regions of dysplasia each with a distinct collection of dysplastic features, either adjacent each other or separated by areas of normal epithelium.
16. Multifocal or skip lesions	Multiple epithelial lesions with features of dysplasia clearly separated by areas of normal epithelium.
Cytological features (Fig. 6)	
17. Abnormal variation in nuclear size	Variation in the size of keratinocyte nuclei beyond that expected in a normal epithelium, there is no current definition of normal oral epithelial nuclear size variation.
18. Abnormal variation in nuclear shape	Variation in the shape of keratinocyte nuclei beyond that expected in a normal epithelium, usually deviation from a circular to oval or an irregular shaped nucleus.
19. Abnormal variation in cell size	Variation in the size of keratinocytes beyond that expected in a normal epithelium, there is no current definition of normal oral keratinocyte size variation.
20. Abnormal variation in cell shape	Variation in the shape of keratinocytes beyond that expected within that epithelial layer, basal cells are usually more cuboidal, prickle cells are usually more polygonal and granular cells are usually the most squamoid.
21. Increased mitotic activity	Readily identifiable, numerous, normal mitotic figures throughout the thickness of the epithelium.
22. Increased nuclear size	Larger nuclear size beyond what would be normal for that epithelial location, the normal nucleus size has not been defined. It may be best to combine increased nuclear size with increased nucleus to cytoplasmic ratio.
23. Increased nucleus: cytoplasm (N:C) ratio	Increase in the nuclear size leading to reduction of the cytoplasmic area normal for the epithelial location. The normal nuclear to cytoplasmic ratio has not been defined.
24. Atypical mitotic figures	Readily identifiable mitoses which do not have a normal morphology.
25. Increased number and size of nucleoli	A greater number of nucleoli or a larger size of at least one nucleolus, beyond what is normal in the oral epithelium. The normal number and size of nucleoli is not known.
26. Single cell keratinisation	Individual cells with keratinisation giving the cytoplasm a strongly eosinophilic appearance with retraction from neighbouring keratinocytes.
27. Nuclear hyperchromasia	Keratinocyte nuclei with greater basophilia than would be normal for the epithelial location, the normal degree of basophilia has not been defined.
28. Apoptotic mitoses	This relates to the observation of a mitotic catastrophe, though the histological appearance is not well defined in the literature.

these features can be seen in OED, future research should focus on determining which features are most common, the threshold of these features for diagnosis, and which of these are most important prognostically. By reducing the number in this list to only the most important, merging some of the overlapping cytological and architectural features and assessing the quantification/weighting of features with prognostic importance, some clarity may be gained not only for reporting pathologists but also for the surgical teams involved in treatment.

The main aim of this paper is to review the currently known and suggested histological features of OED and offer definitions which may be less opaque to pathologists, students, trainees and patient-facing clinicians. In this context, the authors also report on the histology of healthy oral tissue and other histological mimics to highlight the complexity of OED diagnosis. Digital whole slide images of all the figures provided in this paper can be found at: https://www.pathogenesis.co.uk/r/demystifying-dysplasia-histology-dataset. Finally, the authors comment on the challenges and limitations of the current diagnostic criteria and existing grading systems for prediction of malignant transformation risk.

NORMAL ORAL MUCOSA

The first step in OED diagnosis is recognising variations of the normal oral epithelium (Fig. 1–3). Each subsite has unique features that may be misinterpreted as OED. Oral mucosa can be placed into three broad categories: (1) masticatory mucosa (gingivae, hard palate), (2) lining mucosa (labial, buccal, ventral tongue, floor of mouth, soft palate), and (3) specialised mucosa (dorsal tongue, vermillion lip border)[19] (Fig. 1–3). Although oral stratified squamous epithelium has the same layers as the epidermis (basal cell layer, prickle cell layer, granular cell layer and keratinised layer) these vary in extent, and may not always be apparent.[19] The specialist mucosa of the tongue is highly variable and most likely to be confused with OED, though OED accounts for less than 5% of cases at this site.[20] In the anterior tongue the filiform papillae form sharp projections of parakeratin and the filiform papillae of the posterior tongue form broader projections with fibrovascular cores. These structures must not be mistaken for verrucous or papillary changes associated with some OED lesions.[21]

ARCHITECTURAL FEATURES OF OED

Irregular stratification

Irregular stratification is the first architectural feature listed in the recent WHO criteria.[1] This feature is long established,[2,3] though poorly defined. Keratinocytes mature as they approach the surface of the normal oral epithelium, having a distinct morphology in each of the layers. The thickness of each epithelial layer varies by site, but each layer should be easily identified. Irregular stratification describes disorder of this maturation, giving the epithelium a haphazard appearance. Cells from each layer become admixed making the layers harder to distinguish (Fig. 4A).[22] There is no definition for how much disorder is required to count this feature, though the epithelial thickness can be used to inform OED grading.[1,4] The tastebuds of the posterior tongue may give a disordered appearance to the epithelium, but this should not be mistaken for irregular stratification of OED (Fig. 3D,E). Irregular stratification is best identified at low power to allow comparison with adjacent normal epithelial layers.

Abnormalities of the basal compartment

Architectural abnormalities of the basal cell layer are common in OED and account for three of the listed features mentioned in the WHO criteria.[1] In the normal epithelium, basal keratinocytes are cuboidal with round to oval nucleus positioned adjacent to the basement membrane (Fig. 2B).[23] In OED, this regular arrangement is lost, and the nuclei of basal cells become abnormally located away from the basement membrane (known as loss of basal cell polarity) (Fig. 4E).[1–3] This feature gives a disordered appearance to the basal layer as the basal cell nuclei no longer occupy a consistent location. Unfortunately, basal cell palisading is variable in the oral cavity and may not be fully appreciated in crosscut sections, making interpretation difficult. Additionally, a lichenoid pattern of inflammation, often present in OED and inflammatory oral diseases, causes disruption of the basal cell layer, further complicating interpretation.[1,3,4,24]

Bulbous (or tear drop) rete processes are a long-described feature of OED (Fig. 4B, 5C).[1–4] These can be defined as an increase in the width of the rete processes, leading to a broader base with a narrow isthmus where the rete process joins the superficial epithelium. Normal rete processes

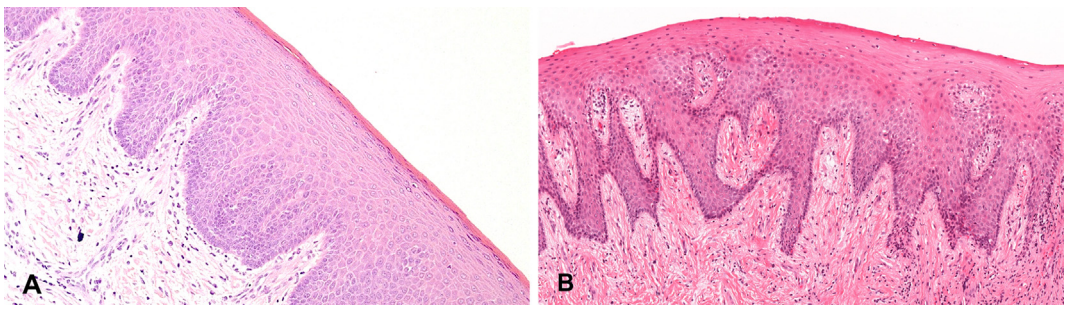

Fig. 1 Examples of the masticatory mucosa of the oral cavity. The masticatory mucosa is exposed to friction during mastication and possesses a parakeratinised or orthokeratinised surface, has a thicker epithelium than lining mucosa and a denser lamina propria. As a result of the keratinisation, masticatory mucosa is usually more eosinophilic than lining mucosa. If eosinophilia is seen in the lining mucosa, however, it may represent the premature keratinisation of OED. This is one example where variation in normal oral mucosa may be mistaken for OED or a potential OED missed. (A) Hard palate: the hard palate is a site subjected to high masticatory forces and comprises orthokeratinised stratified squamous epithelium. There is a visible granular cell layer in this epithelium, which is often not present in the oral epithelium. (B) Gingivae: this is a form of masticatory mucosa comprising parakeratinised stratified squamous epithelium with elongated and branching rete ridges. Note the lamina propria is more densely collagenous at this site.

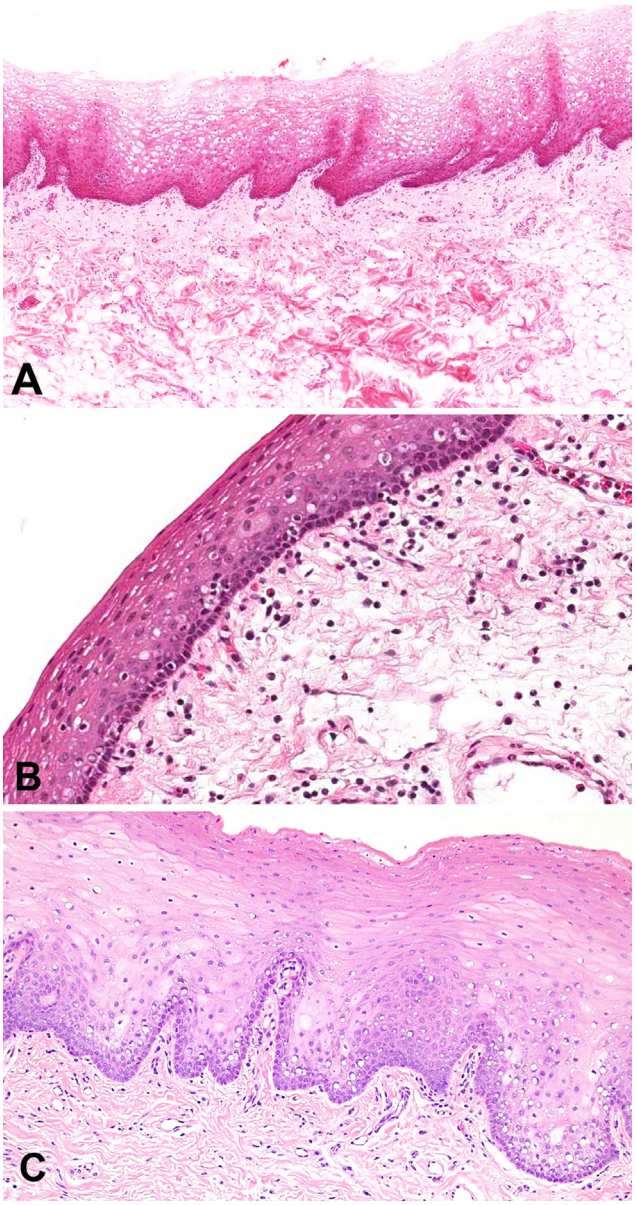

Fig. 2 Examples of lining mucosa of the oral cavity. The lamina propria of the lining mucosa is looser and the submucosa often contain abundant minor salivary gland lobules. The epithelium is non-keratinised, lacks a granular layer and the keratinocytes may contain significant cytoplasmic glycogen. The thickness of the epithelium and length of rete processes is variable with the labial and buccal mucosa being thicker (with longer rete processes) than the floor of mouth and ventral tongue which generally have a flat interface with the lamina propria. The buccal mucosa is also more prone to keratinisation because of trauma than other lining mucosa sites. This must not be mistaken for abnormal keratinisation in OED. (A) Buccal mucosa: this is a form of lining mucosa from an area not usually subjected to highly abrasive forces, therefore the epithelium is non-keratinised with mildly branching rete ridges. (B) Ventral tongue: similar to the buccal mucosa the ventral tongue is a type of lining mucosa which is non-keratinised and is approximately 10–14 cells in thickness with a flat basal compartment lacking rete ridges. The basal keratinocytes can be seen to have a regular arrangement and orientation towards the basement membrane. The underlying submucosa is loose and when combined with the epithelial features makes it a useful site for rapid absorption of various medications. (C) Soft palate: the soft palate is a form of lining mucosa composed of non-keratinised stratified squamous epithelium with small rete ridges and looser submucosa.

maintain an even width or become narrower with depth. Even in reactive or inflammatory lesions, rete processes may increase in length but do not significantly increase in width. At some oral sites (floor of mouth, ventral tongue) the normal basal layer is essentially flat with few or small rete processes (Fig. 2B).[25] As such, any degree of bulbosity should raise concern at these sites. There is no definition of how broad a rete process must be, or how narrow its isthmus, to consider it bulbous.

Basal cell clustering and nesting (Fig. 5E) is a recently proposed feature of OED.[1] This feature has been described in epidermal dysplasia, with three defined stages.[26] First, crowding of atypical basal cells within the epithelium, followed by slight budding of basal cell nests into the papillary dermis, and finally rounded nest of atypical basal cells extending beyond the deepest epidermis without features of frank invasion.[26,27] The presence of this feature in skin has shown to be more important for malignant transformation than upward spread of dysplasia throughout the epidermis.[26] This budding may be suggestive of the keratinocytes moving towards an invasive phenotype;[4] however, it has not been reported in OED to date, nor has its association with malignant transformation been proven in the oral cavity. Another feature common in epidermal dysplasia is extension along adnexal structures.[26,27] Rarely, OED can be seen extending downwards into and along salivary ducts[28] (Fig. 4F). This must not be confused with invasion; useful clues include identification of duct lumen and a lack of stromal reaction around the dysplastic duct.

Tangentially sectioned bulbous rete processes or nested basal cells may simulate invasion. It may be particularly difficult in some cases to distinguish between OED and early invasive OSCC. Further tissue levels are often of benefit as it may reveal that the apparently invasive islands are crosscut rete processes which join up with the epithelium, or alternatively that true invasion exists. In some cases, it is impossible to completely rule out invasion and so a report expressing this uncertainty must be issued. Communication with the surgical team can allow an appropriately conservative excision on which interpretation is often easier.

Number, type and location of mitoses

The next three architectural features of OED are related to mitoses. The first is expansion of the proliferative compartment.[1] In the normal epithelium, the basal cells are the only mitotically active keratinocytes. The basal cell layer is normally only a few cells thick but is often thicker in OED, leading to an increased thickness of the epithelium occupied by mitotically active cells. Some authors have suggested the use of adjuncts such as Ki-67 staining to highlight the altered distribution of keratinocytes in the cell cycle.[4] Positive Ki-67 staining can be seen in S, G2 and M phases of the cell cycle, with variable staining in the G1 phase.[29] Some evidence suggests suprabasal expression can be indicative of dysplasia.[30] However, Ki-67 as an adjunct to dysplasia diagnosis is not widely used due to lack of robust validation. Specific mitosis markers have been investigated in many other tumours and may be more valuable in highlighting

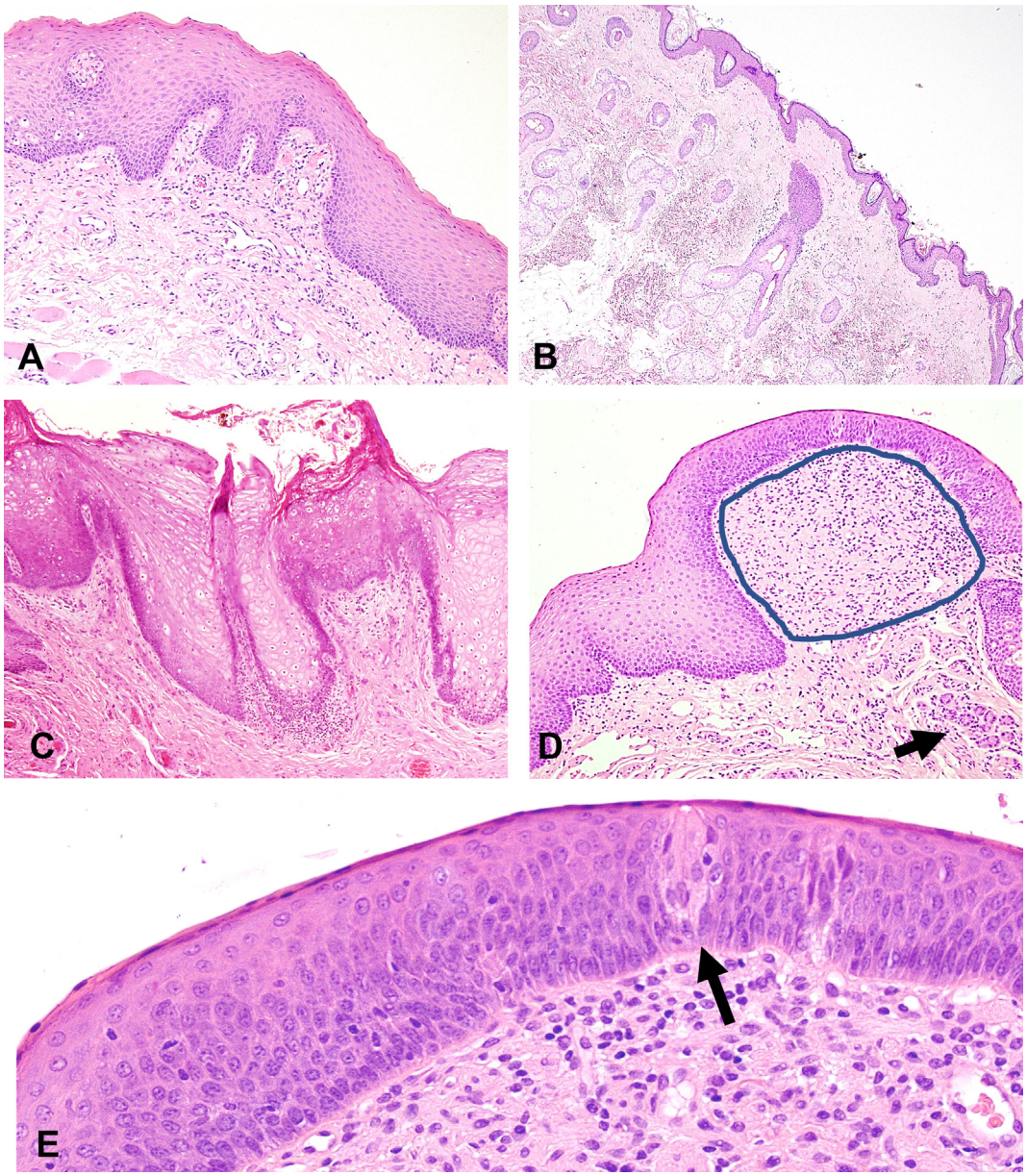

Fig. 3 Examples of specialised mucosa of the lip and tongue. The dorsal tongue epithelium is parakeratinised, thicker than at other sites with long rete processes and forms several types of papillae depending on location. In the anterior tongue, there are filiform papillae with sharp parakeratin projections. The more posterior fungiform papillae have broader projections with an associated specialised lamina propria rich in blood vessels. The epithelium of the fungiform papillae tends to be thinner, and taste buds may be present. Where taste buds are present, there is often disruption of the epithelium, giving it a somewhat disordered appearance, which may be mistaken for dysplasia. The identification of taste buds, however, helps rule out OED. (A) Lip vermillion border, (B) lip skin: the lip is a unique site of the oral cavity as on one aspect it is lined by non-keratinised lining mucosa and on the other aspect is lined by orthokeratinised skin. The transition between these two types of epithelium is called the vermillion border. The vermillion border comprises parakeratinised stratified squamous epithelium with branching rete ridges. The underlying lamina propria is thin and tightly bound to the underlying muscle. (C) Dorsum tongue: showing filiform papillae; the dorsum tongue is subject to masticatory forces and thus comprises parakeratinised stratified squamous epithelium overlying variably dense fibrous connective tissue. The dorsum tongue is also where structures such as filiform papillae are identified. Filiform papillae are heavily parakeratinised with a narrow core of connective tissue. (D) Posterior tongue: showing several structures of the normal posterior tongue; blue ring (subgemmal neurogenous plaque, arrow) serous glands of von Ebner. The subgemmal neurogenous plaque is a neural plexus that serves the overlying taste buds. The polypoid appearance of the mucosa is due to the presence of fungiform papillae. (E) Posterior tongue: showing the surface epithelium of the posterior tongue (taste bud, arrow). The adjacent epithelium is disrupted by the taste bud mimicking disordered stratification.

abnormally distributed mitoses in the oral epithelium.[31] Finally, it should be noted that an increased number of normal mitotic figures limited to the basal layer does not qualify a diagnosis of OED as this can be seen in reactive and inflammatory conditions.

Mitoses high in the epithelium (Fig. 5B), which are referred to as superficial mitotic figures are another feature of OED.[1–3] Any mitotic figure observed superficial to the basal layer can be considered aberrant and the location may range from immediately adjacent to the basal layer through to the surface. These superficial mitoses may be in maturing keratinocytes (as discussed below) or immature cells which have retained a basal cell morphology.

Finally, mitoses in maturing cells can be considered distinct from mitoses high in the epithelium.[1] For example, a mitosis immediately adjacent to the basal cells, and therefore not high in the epithelium, but occurring in a mature cell (such as a prickle cell keratinocyte) is abnormal and can be a

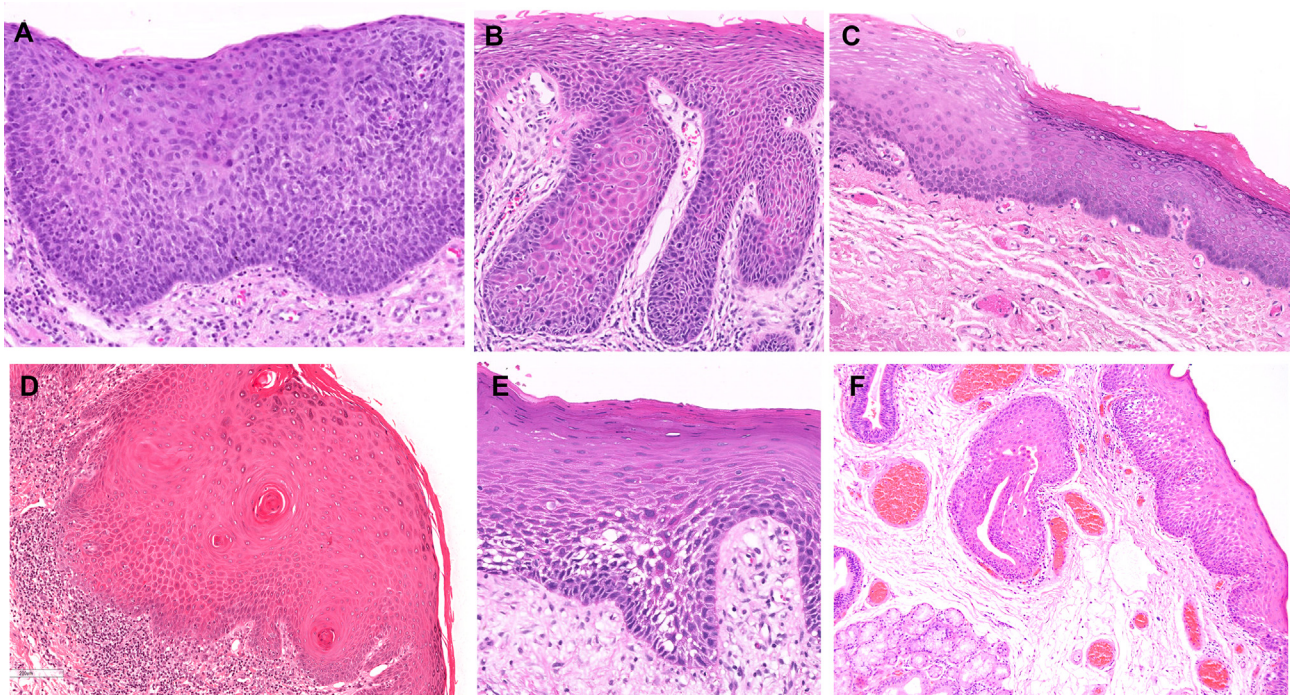

Fig. 4 Examples of the range of architectural abnormalities seen in OED. (A) Irregular stratification: in this example the regular stratification of the epithelium is disordered, and it is difficult to identify and distinguish the basal cell and prickle cell layers. (B) Premature keratinisation: a group of cells in the prickle cell layer show bright eosinophilic cytoplasm and retraction from neighbouring keratinocytes, highlighting premature keratinisation. Only cells within the superficial keratin layer should exhibit such an eosinophilic appearance. (C) Abrupt transition: a sharp and defined change from normal epithelium to dysplastic epithelium is seen. The dysplastic epithelium shows a change in keratin pattern (from parakeratinised to orthokeratinised), and shows bulbous rete ridge morphology alongside basal cell hyperplasia and nuclear and cellular pleomorphism in the basal compartment. (D) Keratin pearl formation: intra-epithelial spherical collections of keratin without extension to the overlying epithelium are identified within the spinous layer of the epithelium. (E) Loss cell epithelial cohesion and basal cell polarity: retraction between adjacent keratinocytes and subsequent loss of cohesion is identified in the prickle cell layer; in addition the nuclei in the basal compartment are seen in an abnormal location (i.e., away from the basement membrane). (F) Extension of OED along a salivary duct: OED is observed in the epithelium in this section with similar features identified in a salivary gland duct (such as loss of cell cohesion) in the underlying connective tissue. In deeper tissue sections the salivary gland duct would show communication with the surface epithelium.

feature of OED.[4] This is also the case for mitotic figures present in the granular layer. However, in order to provide clarity and reduce the extensive number of OED criteria it may be valuable to group superficial mitoses and mitoses in maturing cells as one feature (abnormally located mitotic figures).

It may be difficult to interpret these features in practice. Assessment of the location of mitotic figures may be hampered by cross cutting of the epithelium, giving mitotic figures a more superficial appearance. There is also no definition for the minimum number of abnormally located mitotic figures to qualify an OED diagnosis, therefore this feature needs to be taken in the context of other features. Finally, reactive and inflammatory conditions often lead to increased mitoses and basal cell hyperplasia which may mimic the expanded proliferative compartment of OED.

Abnormal keratinisation

Abnormal keratinisation is another important feature of OED that bridges both cytological and architectural changes. A change in the thickness or type of keratin abnormal for that oral mucosal site, for example, from parakeratin to orthokeratin or non-keratinised to keratinised, may be part of the spectrum of abnormal keratinisation. This feature is often present in differentiated dysplasias, where an abrupt 'clonal' change between parakeratin and orthokeratin is seen with limited or no atypia.[4,32,33] This feature must be interpreted with caution as it can appear similar in reactive processes such as traumatic (frictional) keratosis or inflammatory conditions such as lichen planus, although the change in keratinisation pattern in these reactive lesions is usually more subtle and not abrupt. Anecdotally, excessive keratinisation (in particular presence of orthokeratinisation) of the floor of the mouth is worrying, particularly when paired with atrophic epithelium.[5] Another useful clue for OED is generalised premature keratinisation within the prickle cell layer.[1,2,4] Where early keratinisation is present, cells in the prickle cell or even the basal cell layer will become larger with a more eosinophilic cytoplasm (Fig. 4B,D, 6C). This feature is relative, as some sites in the oral cavity are more keratinised than others, making comparison with the adjacent normal epithelium crucial. Keratin pearl formation in the rete processes (Fig. 4D) is a particularly alarming feature of OED[1,3,4] as it closely mimics the appearance of the invasive squamous cell carcinoma.[34] Keratin pearls appear as an eosinophilic acellular collection of keratin with no connection to the surface. Some authors have suggested that requiring keratin pearls to be within the rete processes is inappropriate and keratin pearls anywhere in the epithelium should be considered concerning.[3] Abnormal keratinisation may also take the form of single cell keratinisation (also referred to as dyskeratosis) (Fig. 6C), though it is listed as a cytological feature.[1] It is usually easily identified, as dyskeratotic keratinocytes have

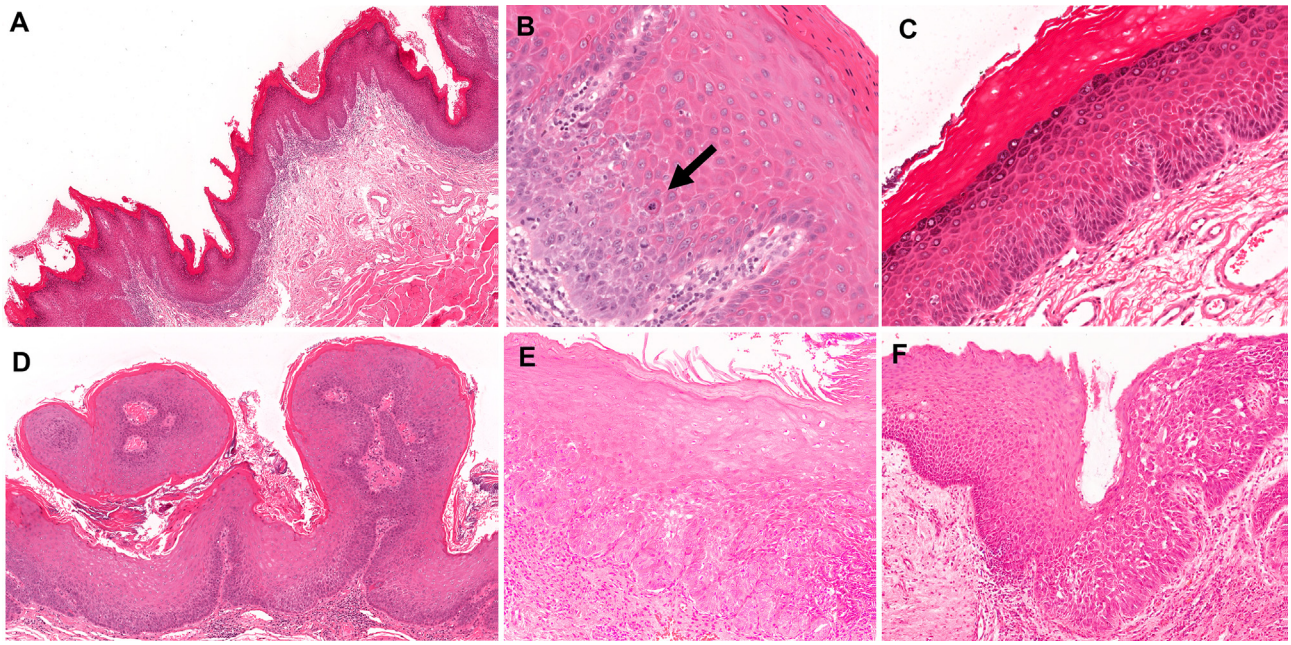

Fig. 5 Further examples of the range of architectural abnormalities seen in OED. (A) Verrucous surface architecture: in this example the epithelium is hyperplastic and orthokeratinised with sharp and pointed epithelial projections. (B) Superficial mitosis (black arrow): a mitotic figure is identified outside of basal compartment. There is no consensus on the number required to arrive at a diagnosis of dysplasia. (C) Bulbous rete processes and generalised premature keratinisation: this example demonstrates rete processes that are wider at the base than at the apex giving a bulbous and drop-shaped architecture. Cells within the spinous layer also show premature keratinisation with deeply eosinophilic cytoplasm, a feature that should only be seen in the surface keratin layer. (D) Papillary architecture: the epithelium is folded into exophytic projections with central fibrovascular connective tissue cores. Basal cell hyperplasia and nuclear and cellular atypia can be seen in the basal compartment of this example. (E) Basal cell nesting and clustering: the basal compartment of the epithelium shows the pleomorphic cells to be tightly packed together in small nests. This feature is better described and more frequently seen in skin biopsies. (F) Multiple patterns of dysplasia: within one specimen it is possible to see more than one pattern of dysplasia. Here we can see more 'conventional' dysplastic changes in the epithelium on the left side of the image with basal cell crowding, hyperchromatism and nuclear and cellular pleomorphism observed. Towards the right side of the image the epithelium shows marked loss of keratinocyte adhesion (acantholysis) alongside basal cell hyperplasia and nuclear and cellular pleomorphism.

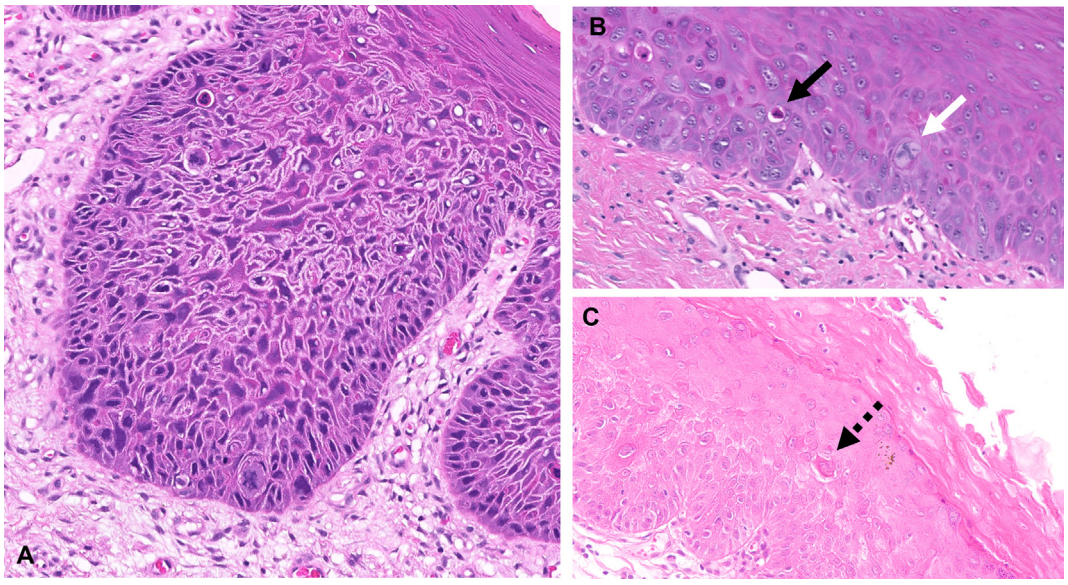

Fig. 6 Cytological abnormalities seen in OED. (A) Severe OED: an abundance of cytological features of OED are seen in this bulbous rete process including abnormal variation in nuclear size and shape, abnormal variation in cell size and shape, increased mitotic activity, increased nucleus:cytoplasm ratio, atypical mitotic figures, increased number and size of nucleoli, nuclear hyperchromasia and abnormal mitoses. (B) Abnormal mitosis and apoptosis: an apoptotic cell with pyknotic nucleus, brightly eosinophilic cytoplasm and retraction from neighbouring keratinocytes (black arrow); an abnormal mitotic figure with asymmetrical chromatin (white arrow). (C) Single cell keratinisation: there is generalised premature keratinisation seen in this example of OED giving the epithelium a strongly eosinophilic appearance. Black dotted arrow highlights single cell keratinisation with even more eosinophilic cytoplasm and retraction from adjacent keratinocytes.

a strongly eosinophilic cytoplasm and a clear halo around the cell, due to retraction from the neighbouring keratinocytes. However, if only a single cell is independently keratinising it is difficult to ascribe this to dysplasia; therefore, multiple instances of single cell keratinisation usually need to be identified alongside other features of OED.

Loss of epithelial cell cohesion

A loss of cohesion between keratinocytes (Fig. 4E) appears to be important in the prediction of malignant transformation risk and recurrence,[22] and has been described in OED for a long time.[1–3,5] This feature initially appears as a widening of the space between keratinocytes before leading to complete separation (acantholysis) of epithelial cells (Fig. 4E). Acantholysis is also seen in vesiculobullous diseases (i.e., pemphigus vulgaris). However, acantholysis is much more pronounced in vesiculobullous disease than OED and does not tend to show atypia. Contact inhibition of epithelial cell locomotion and proliferation through intercellular adhesion complexes such as E-cadherin is well described.[35] Loss of cohesion may give OED a proliferative advantage through loss of contact inhibition.

Verrucous and papillary architecture

A verrucous or papillary surface architecture on its own may be enough to make a diagnosis of dysplasia as these changes are highly abnormal for oral mucosa. Often these patterns, though dysplastic, may have only subtle cytological atypia.[3,5] A verrucous architecture is characterised by a hyperkeratinised surface composed of sharp or blunt epithelial projections with keratin-filled invaginations without fibrovascular cores[36,37] (Fig. 5A). A verrucous pattern may be seen in the keratin alone with the epithelial rete processes maintaining their normal shape. However, in some cases the epithelial morphology may also be affected, leading to an undulated appearance with broad and somewhat 'pushing' rete processes. These changes may be seen in proliferative verrucous leukoplakia (PVL) which will be discussed later.[1,38] A papillary pattern comprises projections of the epithelium supported by fibrovascular connective tissue cores (Fig. 5D). Though both of these patterns are features of dysplasia they may be present in other benign diseases of the oral cavity.[21] Attention must also be paid to the site of the specimen as the filiform papillae of the dorsal tongue may superficially resemble a verrucous surface architecture, and the fungiform papillae show a papillary pattern (Fig. 3C,D,E).[21] Similarly, it is important to be mindful of squamous papilloma like lesions in unusual locations (i.e., gingivae) and clinicopathological correlation is needed to rule out a verrucous lesion.

Abrupt transition, multifocal OED and multiple patterns of OED

The way that the architectural and cytological features manifest can be suggestive of OED. An abrupt transition from normal epithelium to abnormal is very telling of a clonal population and a feature highly suggestive of OED (Fig. 4C).[5,38] It is a recently added feature to the WHO classification.[1] Reactive atypia tends to taper off with increasing distance from the insult, rather than having an abrupt border.

Another feature recently included in the WHO classification is the presence of several different patterns of OED in one lesion (Fig. 5F).[1,4] This is suggestive of several competing clonal keratinocyte populations. Histologically, this is seen as regions of dysplasia each with a distinct collection of atypical features. For example, one area may be basaloid with obvious cytological atypia, whereas another area may show abnormal keratinisation and bulbous rete processes with limited cytological atypia. These regions may be adjacent to each other or separated by areas of normal epithelium. Another recently proposed feature is the presence of multiple epithelial lesions with a consistent pattern of OED separated by regions of normal oral epithelium.[1,4,5]

CYTOLOGICAL FEATURES OF OED

Most OED will display cytological changes, alongside architectural changes (Table 1). In normal oral epithelium, there is little variation in the appearance of the keratinocytes within each epithelial layer as each basal cell will be a similar size, shape, and colour to another basal cell. A well reported cytological feature of OED is pleomorphism.[1,2] This appears as variation in the shape and size of the keratinocytes and their nuclei[1] (Fig. 6A). It is useful to compare the suspected dysplastic keratinocytes to normal keratinocytes within the same epithelial layer when assessing pleomorphism. This accounts for variation in cell morphology over the maturing layers of the epithelium, where basal cells tend to be cuboidal, prickle cells polygonal, and granular cells flattened.[23] There is no defined degree of acceptable variation in normal epithelium, making assessment of pleomorphism highly subjective.

Nuclear variation accounts for several of the cytological features of OED and has many forms[1] (Table 1, Fig. 6). The nucleus may be hyperchromatic, appearing darker, due to increased chromatin content. Variation in nuclear haematoxylin staining within and between laboratories makes hyperchromatism difficult to interpret. Amongst all OED features, hyperchromasia has the lowest interobserver agreement,[22] but comparison with adjacent normal epithelium is useful to account for staining variation. There may be an increase in the nucleus size, either as an absolute increase or an increase compared to the whole cell size (increased N:C ratio).[1] However, both animal and human pathological studies have questioned the use of nuclear to cytoplasmic ratio as a feature of dysplasia.[39,40] Usually, an increased N:C ratio is due to an increase in nuclear size, so having both as separate criteria is likely superfluous.[3] Pleomorphism of nuclei is also an important feature, but similar to cellular pleomorphism is poorly defined. Both hyperchromatism and nuclear pleomorphism are features which have recently been correlated with risk of malignant transformation and recurrence of OED.[22] An increase in the number and size of nucleoli can also be seen in OED, though these changes may also manifest in reactive epithelium and are again poorly defined.

Atypical mitoses, regardless of location, are a feature of OED.[1,4,5] There are many forms of abnormal mitoses including: aneuploid mitoses (tripolar mitoses), asymmetrical mitosis, chromosome bridging and chromosome lagging[41] (Fig. 6; Supplementary Fig. 1, Appendix A). An abnormal mitosis suggests a cell has abnormal chromosome numbers, a failure of chromosome segregation or a general failure of DNA replication. Abnormal mitosis likely leads to further acquisition of oncogenic mutations.[42] Unfortunately there is no evidence to suggest how many abnormal mitoses are required before being ascribed as a diagnostic feature. A recent addition to the diagnostic criteria is the presence of apoptotic mitoses.[1,4] These are also known as mitotic

catastrophes, are generally recognised within the spectrum of abnormal mitoses, and are thought to be indicative of chromosomal instability.[4] To standardise the diagnosis and keep the list of OED features concise and clear, it would be best for this to be considered under the broader category of abnormal mitotic figures.

HPV-RELATED ORAL DYSPLASIA

It is estimated that 27% of OED harbour HPV infection with the majority having high-risk HPV (HPV16, 18).[9] These lesions have distinct histological features when compared with OED caused by traditional risk factors such as tobacco and alcohol consumption. The discussion so far has been focused on OED caused by traditional risk factors and the features discussed here (though not all are specific to HPV-related OED) are clues that further testing is warranted. HPV-related OEDs often have extensive architectural and cytological changes.[43] The keratinocytes are basaloid due to a high nuclear to cytoplasmic ratio and the epithelium is often parakeratinised, with the keratin being densely eosinophilic (Supplementary Fig. 2, Appendix A).[1,4,43] Karyorrhectic keratinocytes known as mitosoid bodies and apoptotic keratinocytes are characteristic of HPV-related OED.[1,4,43] A mitosoid body has condensed nuclear chromatin and a pericellular halo, giving the appearance of a mitotic figure.[43] Apoptotic keratinocytes will have a strongly eosinophilic cytoplasm with a pyknotic nucleus[43] (Supplementary Fig. 2, Appendix A). Though apoptotic cells may be seen in OED, the above-mentioned features are not common in conventional OED and can be helpful in distinguishing HPV-related OED from other causes.[43] In summary, if OED is generally basophilic with bright parakeratin, mitosoid bodies and apoptosis, HPV should be suspected as the causative factor rather than the traditional risk factors.

Ultimately, as HPV infection in the oral cavity is uncommon, it requires confirmation by a combination of p16 immunohistochemistry which is positive in 62% of HPV-infected cases,[9] and the detection of high-risk HPV by polymerase chain reaction (PCR) or *in situ* hybridisation (Supplementary Fig. 2D, Appendix A).[1] Almost 20% of HPV-negative OED can show p16 staining and up to 40% of HPV-positive OED are p16 negative, making p16 staining alone an unreliable surrogate for HPV infection in OED.[9] At present there is limited research into the outcomes of these lesions; as such, they should be graded and managed the same as conventional OED lesions.[1] The malignant transformation rate of these lesions has been reported to be approximately 15%.[43]

DIFFERENTIATED DYSPLASIA

Differentiated dysplasias present an area of diagnostic difficulty, as they tend to lack cytological atypia.[4,33] Despite this lack of cytological change, some suggest they account for a significant portion of OED which transforms to OSCC.[33] They have also been referred to as architectural dysplasias[4] and are often considered a subtype of dysplasia rather than a distinct entity.[4,33] Although there is little consensus on what features constitute a differentiated dysplasia, they can include hyperkeratosis in the presence of an atrophic epithelium, abrupt change when compared with the adjacent mucosa, multiple skip lesions,[32] premature keratinisation and loss of epithelial cell cohesion.[33,44] The requirement for cytological atypia to be present is debated and most classify lesions with similar features but a verrucous architecture as distinct.[4,32–44] Practically, the presence of an unusual pattern of keratin for the oral subsite being examined and an abrupt transition are incredibly useful clues for the presence of differentiated OED, even in the absence of any cytological atypia (Supplementary Fig. 3, Appendix A). However, clinicopathological correlation is important as similar changes are seen in the early stages of PVL.[1,32] Grading of these lesions is difficult as the thickness of the epithelium affected by atypia is often limited, with very few lesions having full thickness dysplasia despite undergoing malignant transformation.[33] As such, a good 'rule of thumb' is to assign the lesion one grade higher than it would be given based on traditional 'thirds' assessment, and clinical follow-up should also be recommended.

PROLIFERATIVE VERRUCOUS LEUKOPLAKIA

PVLs can only be diagnosed by careful clinicopathological correlation.[45] They are distinct entities from OED with a high risk of transformation to OSCC.[1] PVL is characterised clinically by flat or verrucous leucoplakias, a long clinical history, multiple lesions and a predilection for the gingivae (Supplementary Fig. 4A,B, Appendix A).[1,45,46] The histological features are variable and broadly separated into the categories 'hyperkeratotic lesions' and 'lesions with epithelial proliferation' (Supplementary Fig. 4C,D, Appendix A).[1,45] Although these two categories are often considered as early and late phases of the disease,[1] there is no evidence to suggest one category progresses to the other.[45] The hyperkeratotic lesions show hyperkeratosis possibly with a corrugated, or verrucous surface.[1,45] These changes may be accompanied by a verrucous morphology in the epithelium.[1,45] Alternatively, the verrucous pattern might be absent and there is hyperkeratosis with epithelial atrophy and an abrupt transition[45] in a similar pattern to differentiated dysplasia.[4,32] The 'proliferative epithelium' category often shows less significant keratinisation, but has bulky epithelial hyperplasia, often with both exophytic and endophytic expansion.[1,45] The rete processes are usually broad and may coalesce, and a lichenoid pattern of inflammation is also commonly encountered.[45] In both categories cytological atypia is often limited.[45,46] Distinguishing the proliferative epithelium category from verrucous OSCC is challenging. Extension of the lesion deeper than the adjacent epithelium is suggestive of transformation to OSCC,[1] but this may be difficult to assess or may not be always present.[45] Due to the histological similarities between OED and PVL, communication between the pathologist and the clinician managing the patient is essential where PVL is suspected by either party.

MIMICS OF OED

The features of OED can overlap with several other entities, primarily inflammatory diseases where the epithelium displays so called 'reactive atypia'.[38] In these instances, there is limited or no risk of malignant transformation, and the atypia will resolve with removal of the cause. A diagnosis of OED given to these mimics will lead to over-treatment, whereas failure to diagnose true OED in a lesion mimicking a reactive process will lead to under-treatment. It is often difficult in these contexts to distinguish mild OED from these reactive changes.

Reactive atypia is commonly seen in oral candida infections;[5] however, there are usually features of candida infection as well, and fungal hyphae visible with special stains such as periodic acid–Schiff (Supplementary Fig. 5, Appendix A). Testing on multiple tissue levels may be useful. However, it is important to be mindful of the fact that candida can get trapped on the surface of dysplastic lesions and the atypia present may not always be reactive. There is also a correlation between a diagnosis of OED and the presence of candida,[47,48] though this complex relationship and how one impacts the other is not fully understood. In difficult cases where the atypia is deemed too prominent to confidently call it reactive, clinicopathological correlation becomes vital. A useful tool is to defer the decision to a second later biopsy soon after treatment with antifungal therapy.[4,5,38] Such treatment will resolve or reduce fungal-related reactive atypia, whereas changes related to OED will persist.

Oral lichen planus (OLP) and oral lichenoid tissue reactions (LTR) are inflammatory diseases of the oral cavity. Both can display features which overlap with OED clinically and histologically[49] (Supplementary Fig. 6, Appendix A). OLP and LTR are characterised by a lichenoid pattern of inflammation. Unfortunately, OED and OSCC often have lichenoid inflammation and other features of OLP/LTR in up to 29% of cases, particularly in mild and moderate OED lesions.[1,4,24,50] Lichenoid dysplasia (OED with features of OLP) has been suggested as a distinct entity,[51] although this is not widely accepted, with recent molecular studies demonstrating significant transcriptional overlap between OLP and lichenoid dysplasia.[52] Ultimately, despite the presence of a lichenoid infiltrate or other features of OLP or LTR, if any features of dysplasia are seen, a diagnosis of OED should be given, as OLP/LTR should not show any 'true' features of dysplasia.[50]

Ulcers are common in the oral cavity and are frequently biopsied to rule out the possibility of OED or OSCC. Supplementary Fig. 7 (Appendix A) shows an example of reactive atypia in an ulcer, while Supplementary Fig. 8 (Appendix A) shows true OED with ulceration. Although there are limited studies which aim to distinguish reactive atypia from true atypia,[38] a useful clue is resolution of atypia with distance from the area of ulceration. This is a feature usually seen in reactive atypia; conversely, OED atypia will persist beyond the epithelium immediately adjacent to the ulcer.

Other mimics of OED include hyperkeratosis and epithelial hyperplasia (HK+EH) (Supplementary Fig. 9, Appendix A) which may have overlapping architectural features with OED such as altered keratinisation,[5,53] oral hairy leukoplakia (OHL) (Supplementary Fig. 10, Appendix A) which is driven by Epstein–Barr virus (EBV) infection[54] and multifocal epithelial hyperplasia which is cause by HPV13 and HPV32[1,55] (Supplementary Fig. 11, Appendix A).

HK+EH may be particularly difficult to distinguish from mild OED. Both usually present clinically as a leukoplakia and they may show similar histological architectural changes.[5] HK+EH should show hyperkeratosis associated with acanthosis, basal cell hyperplasia, and intracellular oedema[53] with an absence of disordered stratification and basal cell pleomorphism, though some basal cell hyperchromatism is acceptable.[38] It may be challenging to distinguish the basal cell hyperplasia seen in HK+EH from mild cytological atypia. The context of the lesion and other features suggestive of trauma are helpful in this scenario. In HK+EH the underlying lamina propria is often more densely collagenous in reaction to the traumatic causes of these lesions, whereas this may be absent in OED. Another consideration is the site of lesion, for example buccal mucosa and lateral tongue are often exposed to trauma, making them frequent sites for HK+EH. However, if thick keratin is seen, particularly in the absence of other traumatic features (epithelial acanthosis and oedema, denser extracellular matrix in the lamina propria) in a site not prone to trauma such as the floor of mouth or ventral tongue, dysplasia must be highly suspected, even where limited cytological atypia is seen.

GRADING ORAL EPITHELIAL DYSPLASIA

Grading of OED was first introduced in 1969[56] with the purpose of stratifying lesions based on risk of malignant transformation. Ironically, the value of histological grading in prediction of cancer risk has been somewhat limited due to subjectivity and lack of reproducibility.[57,58] Numerous grading classifications have been proposed, amongst which the WHO criteria remains the most widely accepted system.[59] This system originally had five tiers but now has three (mild, moderate and severe dysplasia). This simplification aimed to reduce bias and simplify management by grouping 'severe dysplasia' and 'carcinoma *in situ*' into the 'severe' category, and hyperplasia and mild OED into the 'mild' category.[59] This method is in part based on analysis of epithelial 'thirds' i.e., ascribing a grade based on the collective appearances of a wide range of features and their location (or height) within the epithelium. In mild dysplasia, the dysplastic changes are confined to the basal and parabasal layers, whereas in moderate dysplasia, the changes extend to the middle third of the epithelium, and in severe dysplasia, the changes extend through the entire thickness (or more than half) of the epithelium. However, this method is unreliable, and arguably over-simplistic. Furthermore, the presence of a single feature in abundance, irrespective of its location in the epithelium, may be sufficient to upgrade a lesion. The WHO grading system historically has also not been fit for grading verrucous lesions or differentiated dysplasia where marked architectural changes may arise without significant cytological atypia. This ambiguity and subjectivity results in wide inter- and intra-observer variability,[14–17] and consequently may lead to inaccurate diagnosis and inadequate management. However, this problem is not specific to oral dysplasia, and extends to other parts of the body including cervical intraepithelial neoplasia,[60] vulvar intraepithelial neoplasia[61] and Barrett's oesophagus.[62]

An alternative binary grading system was proposed in 2006 with the aim of increasing diagnostic reproducibility.[13] This classification graded lesions based on the total number of histological features ('low' risk: <four architectural features, < five cytological features; 'high' risk: ≥ four architectural features, ≥ five cytological features). A recent systematic review and meta-analysis, however, comparing this system with the WHO classification showed inconclusive results with regards to its prognostic value.[63] This system does not consider verrucous lesions or the extent of features present. As such, the binary system is not deemed robust enough for routine clinical use at present.

Historically, no prognostic weight had been ascribed to one feature over another. However, a recent study has indicated

that certain features may be associated with an increased risk of progression to OSCC, including bulbous rete processes, hyperchromatism, loss of epithelial cohesion, loss of stratification, suprabasal mitoses and nuclear pleomorphism.[22] These six features were also statistically associated with OED recurrence, in addition to dyskeratosis. In this study, two prognostic scoring models were developed and tested. The first, a 'six-point' model allocated one point for the presence of each of the six OED features which were associated with a greater incidence of transformation and recurrence. Using this model, a score of '4–6 points' produced the highest risk of malignant transformation and recurrence at five years, estimated at 38% and 49%, respectively. This model demonstrated greater prognostic performance than that achieved by the WHO (2017) grading system for both transformation and recurrence, but only a marginal improvement over binary grading.[22] The second 'two-point' model allocated a point for each of the two features that had the highest inter-rater agreement and were also associated with transformation and recurrence (loss of epithelial cohesion and bulbous/drop shaped rete pegs). The presence of both features was associated with an increased risk of malignant transformation at 5 years, in comparison to each single feature in isolation.[22] This study also evaluated the individual prognostic relationships of less conventional but commonly observed features of OED, including verrucous architecture, lymphocytic band (lichenoid-like inflammatory infiltrate) and abrupt orthokeratosis. Whilst the inter-observer agreement for these features was better (Cohen's kappa 0.60–0.73) than other conventional features, interestingly, none of these were associated with malignant transformation or OED recurrence.

Several suggestions have been proposed to overcome the reliance on grading, such as the use of molecular markers,[64–67] morphological descriptors[68] and computer-aided analyses.[69] The latter has seen a surge of interest, particularly with the increasing ubiquity of digital slide scanners in pathology laboratories. Various image analysis platforms have been developed which allow for automated cell nuclei detection, extensive feature evaluation and quantitative approaches for more objective histological and morphometrical feature analysis. Machine learning, a branch of AI, has been shown to reduce variability in classification of precancerous and cancerous lesions by ensuring standardisation and providing quantifiable outputs for risk stratification.[70,71] However, further research is needed to correlate histological features with OED progression to malignancy and to discover novel digital markers important in prognostication. This may support the development of new and improved prognostic models to assist with clinical decision making. However, no molecular, digital or histological features (singly or combined) have been well correlated with malignant transformation in prospective studies. Due to this lack of evidence, though the features of dysplasia have been extensively described, listed and here defined, OED is still poorly understood.

CONCLUSION

The diagnosis of OED is complicated by the great variety of features, most of which are poorly described and have limited support by good quality evidence. Therefore, it is not surprising that OED diagnosis and grading can show such significant inter- and intra-observer variations. The many features of OED must be interpreted in the context of other factors including the extent of any one feature, the presence of inflammatory disease which may mask or enhance the changes seen, and the clinical scenario in which the lesions have arisen. Strict definitions do not exist for most of the features listed in the WHO criteria, but it is hoped the descriptions provided in this paper will help improve the understanding of these features. Digital whole slide images of all figures have been provided to aid the reader in their understanding of the range of features which may be seen in OED (https://www.pathogenesis.co.uk/r/demystifying-dysplasia-histology-dataset). Efforts should be made to create clear definitions for all features to aid diagnosis, training and future research. There is great potential for automation and objective quantitative assessment of histological features using digital and computational methods. When properly assessed in real-world clinical settings, such approaches may assist decision making and improve patient management by yielding more reliable prognostic information to aid risk stratification. Future directions should be to work closely with the WHO and stakeholders to simplify the current criteria, unify understanding, and study the possibility of quantitatively using OED features as prognostic indicators.

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Data availability: All data generated or analysed during this study are included in this published article. The whole slide images examples have also been shared as a cloud-based open access dataset. https://www.pathogenesis.co.uk/r/demystifying-dysplasia-histology-dataset.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2023.10.002.

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