CLINICAL PRACTICE GUIDELINE

Recommendations for Research to Develop a Patient-Centered Clinical Follow-Up Protocol for Oral Epithelial Dysplasia

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ABSTRACT

Oral epithelial dysplasia (OED) is the primary histological marker for assessing the progression of oral potentially malignant disorders (OPMDs) to cancer. Despite challenges in grading and low inter-pathologist reproducibility, OED severity remains the key predictor of malignant transformation. However, globally accepted guidelines for OED monitoring are lacking, despite calls for individualized management based on host and lesion characteristics. The proposed research protocol involves acquiring high-quality intraoral images, assessing oral hygiene and periodontal status, eliminating chronic mechanical irritation and Candida infections, and applying adjunctive diagnostic methods like toluidine blue staining, optical evaluation, and brush cytology. Tailored follow-up regimens based on individual risk assessments are emphasized, with frequent monitoring for high-grade dysplasia or patients at higher risk of progression. Therefore, effective OED management should consider the patient's immune status, dietary habits, and oral microbiota, aiming to develop personalized treatment strategies that optimize patient-centered care.

1 | Introduction

To date, despite the complexity in detecting, and the low reproducibility of grading of dysplasia among pathologists [1–5], the severity of oral epithelial dysplasia (OED) has been regarded currently as the major predictor of malignant transformation in oral potentially malignant disorders (OPMDs) [6], which present a high risk of progression to oral cancer than normal oral mucosa [1, 2, 7, 8]. OED has dynamic behavior and may progress either to malignancy, remain stable, or may regress over time [7, 9, 10]. In this context, rather than conceptualizing the association between the severity of OED and the subsequent onset of malignancy as a linear and absolute pathway, the progression of OED into malignancy may be examined through a more comprehensive approach assessing the other factors that may contribute to the development of cancer via different mechanisms. Beyond lesion-specific characteristics such as the severity of OED and the anatomical location and size of the lesion [6, 7, 11–13], a multitude of host-dependent and behavioral determinants such as age and gender, genetic predispositions, tobacco and alcohol consumption, local or systemic inflammatory conditions, systemic pathologies including autoimmune disorders, pharmacological interventions, and oral microbial constituents can modulate both cellular metabolism and the immune system of the host [7, 12, 14], collectively impacting the patient's immunosurveillance mechanisms and contributing to

the disease process. Additionally, oral dysbiosis is regarded as a significant component contributing to chronic inflammation in the oral cavity that fosters tumor progression [15–17]. Tumor microenvironment immune cells also have been incorporated into prognosis prediction models as well [18].

1.1 | Lesions Requiring Close Monitoring

There are numerous studies and meta-analyses on the management of OED [9, 13, 19–21], but consistent results to formulate a globally accepted guideline for OED management have not yet been achieved. Recently, more distinct profiles of "high-risk" and "low-risk" patients were delineated based on both host and clinico-pathological lesion-related characteristics in each case. A patient with a history of familial malignancy, tobacco and alcohol consumption, poor oral hygiene, multiple systemic diseases, high-stress levels, and inadequate nutrition may be considered as a "high-risk patient" [12]. Consequently, a tailored treatment regimen may be tested to devise the most suitable management approach for individual OED patients.

Oral leukoplakia and erythroplakia are OPMDs that present with some potential to progress to malignancy among others [19]. Despite the numerous treatment modalities for leukoplakia and erythroplakia which include excision with scalpel or laser surgery, as well as observational management [9, 19, 22–24], they may recur or progress to malignancy [20, 25]. Recent systematic reviews suggest a malignancy progression rate of 9.8% (95% CI: 7.9–11.7) for oral leukoplakia [26] and 33.1% for erythroplakia (13.6%–56.2%) [27]. Considering the increased risk for oral leukoplakia to progress to malignancy within the first 2 years [28, 29], close follow-up of patients for at least 5-year post-treatment is crucial for proper patient care [12, 30]. According to the European Association of Oral Medicine, follow-up is the preferred management strategy over treatment for non-homogeneous leukoplakias without dysplasia or with mild dysplasia [31].

1.2 | Procedures for Detecting Lesion Changes and New Mucosal Aberrations

At each recall appointment during follow-up, the aim is to detect changes in the lesion area, or emergence of new primary or secondary mucosal aberrations; however, the primary challenge lies in determining the specific procedures to be administered. In addition to a comprehensive clinical extra- and intra-oral examination, administration of adjunctive methods such as toluidine blue (TB) staining, optical tests, diffused white light, or loss of tissue autofluorescence alongside brush cytology can effectively enhance the mucosal evaluation of whole oral cavity [32–34]. These adjunctive methods are valuable for their ability to assist the practitioner to notice the areas with potential epithelial derangement. Moreover, real-time assessment enables early diagnosis and treatment, leading to improved health outcomes and reduced treatment costs for patients. In high-risk patients, Cromwell et al. [21] advocated a comparable management strategy, proposing the utilization of a loss of heterozygosity assay to assess the potential of progression to malignancy in OED and adjusting the follow-up schedule based on the patient's risk status. The authors stated that this approach has the potential to reduce oral cancer rate by an average of 51.1%, and individuals identified as high-risk may experience a decrease in mortality rate by 12.7%. The proposed approach was promoted as "less costly and more effective than the current standard of practice," with the additional advantage of reducing the economic burden of the disease on the healthcare system [21]. Recently, RNA-Seq has significantly advanced the understanding of carcinogenic pathways by identifying novel genes linked to OPMD transformation, and gene enrichment analysis highlighted DLX2 and CD46 as key regulators of the Notch signaling pathway [35]. Transcriptomic analysis revealed more transcript changes in SCCs than OPMDs, with both conditions showing alterations in pathways related to cell migration, basement membrane disruption, and metastasis, but with greater intensity in SCCs [36]. A logistic regression model using "RPTN" and "IGSF10" effectively distinguished OPMD from SCC and predicted OPMD progression [36].

Among adjuncts, in vivo staining with TB is the most commonly used and extensively studied non-invasive method [37]. TB selectively targets and binds to nuclear material rich in DNA and RNA, and stains the abnormally developing tissue in royal blue coloration. Consequently, it enhances the visibility for clinicians, facilitates the identification of suspicious areas, and enables a more detailed assessment [38]. TB staining studies report a broad range sensitivity varying from 38% to 98% (median 85%) and specificity from 9% to 93% (median 67%). Positive predictive values span from 33% to 93% (median 85%), whereas negative predictive values range from 22% to 92% (median 83%) [39]. In this regard, the variability in the sensitivity and specificity of TB staining has underscored the need for additional adjunctive methods. Oral brush cytology which allows minimally invasive collection of cells is emerging as a promising research area with high potential [40]. By examination of cellular samples under a light microscope and utilizing nuclear morphometric parameters with standard evaluation criteria (Figure 1), the presence and severity of dysplasia can be assessed [40–42]. This may be further enhanced by incorporation of molecular techniques in cells collected for cytologic evaluation. Artificial intelligence can also be employed to determine the risk of malignancy by analyzing the pathology slides of cytological samples through deep-learning neural networks [5]. Given that the epithelium may appear as normal mucosa despite harboring mutations at the DNA level and other molecular changes that have not yet translated into a dysplastic phenotype [43, 44], in high-risk patients with epithelial alterations strongly suggesting progression to malignancy, further analysis of DNA content [45, 46], and biomarkers defined for the oral carcinogenesis process can be conducted [47, 48].

1.3 | Detailed Procedures for Lesion Evaluation During Follow-Up Visits

The importance of follow-up in surveillance of OED lesions has been emphasized previously [12, 13, 30, 31], however, the specific procedures to be administered for lesion evaluation during each control have not been clarified step-by-step. Based on a review of the literature on OED follow-up studies in PubMed between the years of 1995–2024 via utilizing the search terms "oral

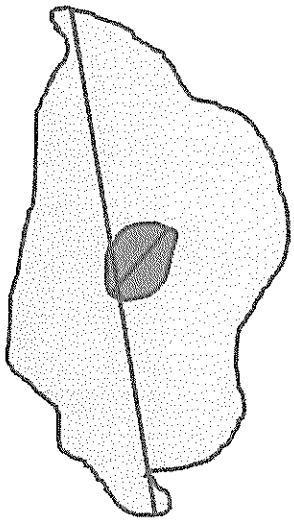

FIGURE 1 | The illustration of an oral epithelial cell presenting the morphometric parameters that are utilized for evaluation of the cytological samples of oral suspicious lesions. As the ratio of the nuclear diameter and area to those of cellular diameter and area gets closer to 1, the likelihood of having a malignant cell escalates. [Color figure can be viewed at wileyonlinelibrary.com]

potentially malignant disorders" OR "oral epithelial dysplasia" AND "follow up," the authors of this paper propose a protocol for the clinical assessment of patients with OED that needs to be tested in future research settings (Figure 2). It is acknowledged that some steps presented here may not be evidence-based findings:

1. In order to evaluate and compare the morphological appearance of a lesion during follow up, high-quality intraoral clinical images of the lesion should be acquired using an intraoral camera, adhering to standardized guidelines [49] for intraoral clinical imaging [11]. Given that the presence of redness in a suspected lesion as seen in erythroleukoplakia or erythroplakia may suggest a more aggressive behavior [43, 50], it is recommended to place a white paper with a 5 mm diameter as a calibration material adjacent to the lesion. This not only provides the quantitative evaluation of the lesion's color and size through digital image analysis, but also enables assessment of progression between follow-up sessions (Figure 3).
2. As there is growing data linking chronic inflammation and tumor progression, the potential role of periodontal pathogens including *Fusobacterium nucleatum* and periodontal inflammation in cancer warrants addressing gingivitis and periodontal disease. Patients' oral hygiene and periodontal status shall be evaluated at each

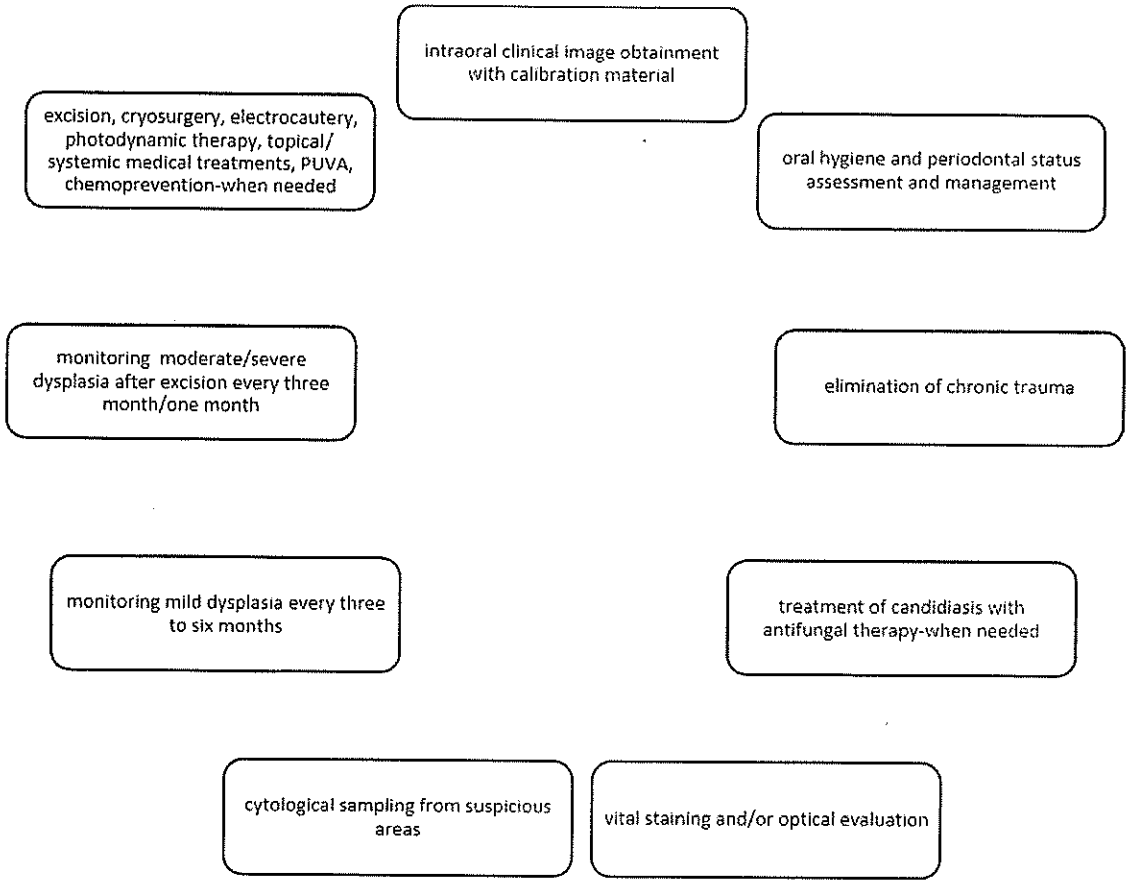

FIGURE 2 | Diagram of follow-up procedures for clinical monitoring of OED.

FIGURE 3 | The original and TB stained clinical images of a chronic oral mucosal lesion at the lateral border of the tongue, which was clinically diagnosed as leukoplakia and histologically established as mild OED. The white round paper (5×5 mm) was used for color and light calibration of digital clinical images, as well as for size determination of the lesion. [Color figure can be viewed at wileyonlinelibrary.com]

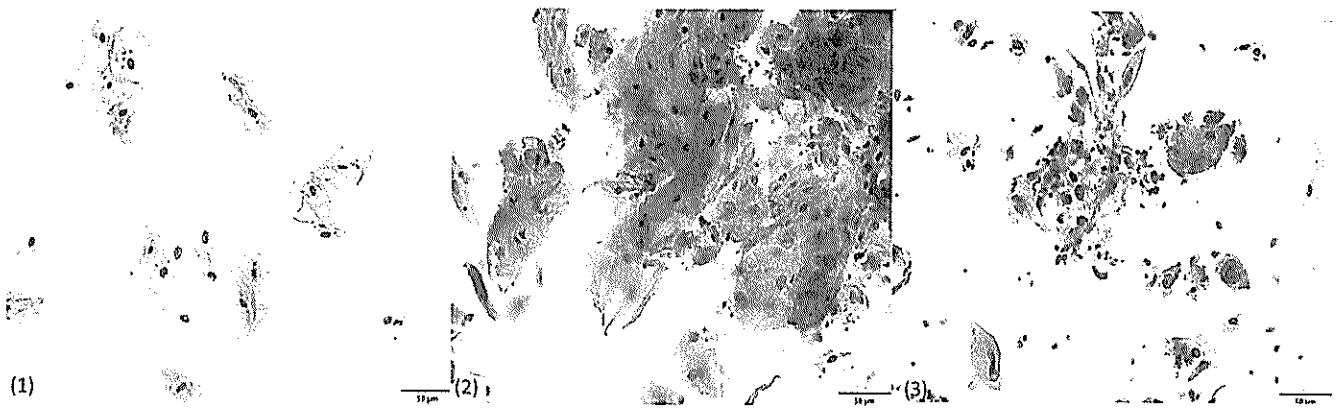

FIGURE 4 | (1) Negative for tumor cells: The sample shows predominantly normal superficial and intermediate squamous cells. The nuclei are round or oval with smooth contours and uniformly distributed chromatin. (2) Suspicious samples: Atypical squamous cells are present, with nuclei showing disorganized orientation and non-parallel alignment. Orangeophilic cells display nuclear size variation and hyperchromasia. (3) Positive for tumor cells: Loosely cohesive sheets of highly atypical squamous cells with a high nuclear-to-cytoplasmic ratio. Nuclei are hyperchromatic, with irregular contours and coarse chromatin. Atypical keratinization is evident, with some cells showing nearly black, smudged nuclei [58]. [Color figure can be viewed at wileyonlinelibrary.com]

appointment, and necessary measures shall be taken to ensure optimal oral and periodontal health in all OED patients to reduce the inflammatory burden on the dysplastic mucosa [15–17, 51, 52].

3. Although not evidence-based, the potential contribution of chronic mechanical irritation to the development of carcinoma in a risk site via altering the microenvironment of the chronic inflammation may be considered [53, 54], and elimination of the causes of chronic trauma such as damaged/fractured teeth or replacement of ill-fitting prostheses could be undertaken by the referring dentist.

4. Microbial chronic inflammation has been attributed as a contributor for tumor development [55]. Thus, considering the additive role of Candidal infection in the process of malignancy development, the patients found positive for candidiasis should receive appropriate antifungal therapy [55].

5. Taking the concept of "field cancerization" into account, vital staining with solutions like TB as an oral rinse and/or optical evaluation methods can be employed to examine the complete oral mucosa to enhance the visual detection of recurrences and/or secondary malignancies [11].

6. In instances where areas appear stained or optically suspicious, brush biopsy may be employed to collect cell samples from these suspected areas for nuclear morphometric evaluation [56]. For all oral cytology specimens, a standard and objective classification shall be used as [56, 57]: (1) negative (no atypical cells observed), (2) suspicious (presence of atypical cells), (3) positive (presence of potentially malignant cells), and (4) insufficient (inadequate cell sample for diagnosis) (Figure 4).

7. In cases demonstrating atypical or suspicious cytology and/or belonging to the high-risk group, molecular analyses like DNA ploidy analysis using DNA image cytometry on formalin-fixed paraffin-embedded specimens of archived tissue samples, or flow cytometry for the analysis of fresh cell nuclei in suspension [59] may be conducted [46]. DNA ploidy analysis involves assessing the cellular DNA content to detect abnormal ploidy which could serve as a biomarker for assessing the risk of cancer development of lesions with OED [60], and to provide insights

into the genetic stability of the cells and their potential for malignant transformation [59, 60].

8. After assessing each patient individually in terms of general and medical conditions alongside the severity of OED, a rigorous follow-up regimen should be instituted as follows: patients at low-risk with no or mild dysplasia should undergo clinical monitoring using the aforementioned procedures every 3–6 months.

9. Those with moderate or severe dysplasia may be considered for surgical or laser excision based on a thorough assessment of all factors. Following surgery, those with moderate dysplasia should be recalled every 3 months. Patients with severe dysplasia require more frequent monitoring and should be followed up monthly. Notwithstanding any of the above interventions, as studies show that surgical management does not predict reduced progression to cancer [25], rigorous ongoing follow-up remains essential, along with the implementation of strategies to mitigate known risk factors for disease progression. Ongoing follow-up routine should be maintained for a period of 2 years, but given the unpredictable nature of the OED prognosis, lifelong monitoring is considered the optimal approach [30].

10. The treatment of choice for OED depends on several factors, including the severity of dysplasia, the patient's overall medical and dental status, and the findings of follow-up examinations [12, 19], recurrence following excision, broad mucosal involvement and critical sites of involvement not amenable to surgical management. Some of the OPMDs with moderate or severe OED may require further chemoprevention with antioxidants (Vitamin A, B complex, lycopene, Vitamin E), minerals (iron, zinc, magnesium), polyphenols, various enzymes and immune modulators such as mTOR, Cox-2, and EGFR inhibitors and potentially prevention of DNA hypermethylation [61]. Management options to be tested include surgical or laser excision, photodynamic therapy, topical or systemic medical treatments, and long-wave ultraviolet light (PUVA), tailored to individual patient needs and circumstances.

Some reports in the literature indicate no link between the progression rate to cancer and the grading or site of OED [2, 6], while others have noted the development of OSCC in younger patients, contrary to previous findings [62]. In this context, a more comprehensive approach shall be used during follow-up of OEDs, regardless of the severity of the dysplasia. This paper offers a step-by-step guide to develop protocols for each control, considering also oral dysbiosis—an imbalance in oral microbiota linked to tumor development in recent decades. Unfortunately, the oral hygiene status of patients has not been sufficiently considered in the follow-up monitoring of OED in the literature [51, 52]. Therefore, we propose new research that in addition to the severity of OEDs, other confounding factors including the general immune status, dietary habits, tobacco and alcohol use, genetic predisposition, chronic inflammation causing factors encompassing especially oral microbial load of the patient shall be considered to prepare a monitoring/management plan. Consequently, by developing a more comprehensive algorithm that incorporates additional patient-related parameters for OED evaluation during follow-up examinations, we hope that the patient-centered care for OEDs would be further enhanced in the future with contributions of the findings of the forthcoming researches.

Author Contributions

Conceptualization: Pelin Güneri, Gaye Bolukbasi, Betul Ilhan, Joel B. Epstein, and Saman Warnakulasuriya. Investigation: Pelin Güneri, Gaye Bolukbasi, and Betul Ilhan. Supervision, visualization, and roles/writing – original draft: Pelin Güneri, Joel B. Epstein, and Saman Warnakulasuriya. Writing – review and editing: Gaye Bolukbasi, Betul Ilhan, Joel B. Epstein, and Saman Warnakulasuriya.

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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