

**HPV+ oral epithelial dysplasia: A new clinical entity?**

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High-risk human papillomavirus (HR-HPV) infection is associated with oropharyngeal squamous cell carcinoma (OPSCC) whereas oral cavity squamous cell carcinoma (OSCC) is generally associated with other risk factors such as alcohol consumption and smoking [1, 2]. Recently, HPV+ oral epithelial dysplasia (OED) has been recognised in the oral cavity. HPV infection of the oral epithelium initiates in the basal epithelial cells, and subsequently spreads to the suprabasal layer. This mechanism of infection means that even in the absence of clinically detectable mucosal changes, changes have already occurred at the DNA level within the basal cells [3]. There is currently an unmet clinical need to develop more reliable, accessible and cost-effective testing for HPV+ OED and related predictive/prognostic biomarkers. HPV+ OED can remain stable, spontaneously resolve or undergo malignant transformation [2]. Due to a lack of long-term studies and longitudinal follow-up, the role of HR-HPV and other potential contributing factors in the clinical outcome of OED is still unclear at present. Improved characterisation of the progression pathway is imperative. A number of differences in the transcriptomic profiles are expected, but to date there are no comparative transcriptomic studies.

The initial aims of my project were to investigate the clinico-pathological features and gene expression profile of HPV+ OED in comparison to conventional OED using Nanostring technology. Sub- analysis of the HPV+ OED cohort based on clinical outcome (e.g. malignant transformation vs no malignant transformation) would then be undertaken with the aim of potentially finding a gene signature associated with specific clinical outcomes. Selected candidate genes would have been validated using immunohistochemical staining.

Given the drastic change in circumstances due to the Covid-19 pandemic, the project could not maintain a laboratory component and data could not be obtained prior to lockdown due to personal health circumstances. The amended aims were to review the literature on the development, aetiology and molecular pathogenesis of oral cancer with a particular focus on HPV+ oral epithelial dysplasias and cancers. We anticipated that assembling the current understanding will allow for a better insight into this rare subset of oral cancers and inform further research into its aetiopathology and diagnosis. In summary, the project discussed the histopathological and molecular similarities and differences between the two types of OED as recognized by pre-existing studies and will form the basis for future work to develop prognostic and predictive biomarkers.

With the support of my supervisor, I have been able to complete some of the initial aims. Under the circumstances, we managed to extract the RNA and completed the Nanostring experiment. We are currently analysing the data and I’m intending to stay involved in the project and contribute to the publication of our findings. I have also received basic training in the histopathology of OED and OSCC, and this knowledge will benefit the development of my career.

Whilst I might have been disappointed not to be undertaking a lab-based project as initially intended, I soon recognised the clinical value of undertaking this literature review. The clinical features of patients with HPV+ OED compared to patients with conventional OED are still not well defined and the role of HR-HPV infection in development and clinical outcome of OED is also unclear due to an absence of long-term follow-up studies. As a dental professional, there can often be uncertainty around diagnosing OED, and advising patients of the long-term impact of these diagnoses on their lives. The identification of HR-HPV associated OED as a potentially premalignant subset of oral potentially malignant disorders (OPMDs) and a histopathologically distinct entity as compared to conventional OED has been a significant advancement in a critical area of research, and forms a valid basis for further research into potential gene signatures which may be associated with specific clinical outcomes. Development of prognostic and predictive biomarkers and earlier diagnosis of OSCC and OPSCC will be instrumental in improving treatment outcomes and patients may be provided with a clearer insight into the potential implications of said conditions. If HPV+ OED is determined to have significant societal impact, national screening programmes could be developed and implemented. Diagnostic improvements will allow for a reduction in cost to the NHS not only through the primary saving of less intensive treatments, but also a reduced burden on the mental health system by reducing the psychological impact of late stage cancers [4].

Previous studies considered as part of my literature review have identified and partially validated potential diagnostic biomarkers and combinatorial use of potential biomarkers, however sample sizes were too low to have any predictive power. Some of the main candidates for studies have been aberrant expression of p16 and Ki-67, alongside the recognised histopathologic profile [5, 6]. Histopathological analysis alone is unreliable due to the opportunities for inter- and intra-observer variance. ProExC has recently been identified as a potential surrogate marker of HPV+ OED, however as only one study has been undertaken, it is clear that this necessitates further investigation as to its merit [7].

Currently accepted diagnosis protocols are ISH for HPV DNA alongside IHC staining for p16 [1]. The primary limiting factors to studies are limited sample sizes and inconsistent selection criteria (limiting the value of inter-study comparisons). As diagnostic protocols and criteria become more established, multicentric studies will become a viable method to increase samples sizes whilst maintaining consistent selection criteria [1]. One area in which the benefit of multicentric studies would be substantial would be in determining whether there is a true preferential nature of HPV+ OED and the site affected, and whether certain sites have a higher propensity to undergo malignant transformation. This could then be used to improve diagnosis and treatment outcomes.

As outlined in the initial aims of this project, validation of selected candidate genes using immunohistochemical staining and identification of key morpho-molecular similarities and differences between the two types of OED will form the basis for future studies to develop prognostic and predictive biomarkers. Cohort analysis based on clinical outcomes (e.g. malignant transformation vs no malignant transformation) is a necessary area of further research, and may uncover a gene signature associated with specific clinical outcomes. Therefore, future studies must be unbiased and use modern molecular screening platforms. Single markers are unlikely to predict clinical outcomes. Gene signatures have higher specificity and sensitivity as they are better suited for capturing complex molecular changes that are common to all patients of a subgroup. Nanostring nCounter technology is one means by which the gene expression profile of HPV+ OED could be analysed and compared to conventional OED, as well as comparing clinical outcomes of previous datasets to identify potential biomarkers [8].

Having undertaken this project, I can return to clinical practice with a much better informed sense of the current research around conventional OED and HPV-associated OED. This will, in turn, lead to better informed discussions with patients at the point of diagnosis and may act to reassure them further. Whilst determining which lesions are likely to undergo malignant transformation has clear clinical benefit, this should be complemented by suitable treatments, ideally non-invasively and with minimal associated morbidity. This will result in clearer diagnosis, clearer understanding of future sequelae and more effective treatment protocols with superior outcomes. In conclusion, the project as undertaken in line with the amended aims demonstrates a clear clinical benefit to further research and forms a clear basis for future work to develop prognostic and predictive biomarkers.

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