Efficacy of oral brush cytology cell block immunocytochemistry in the diagnosis of oral leukoplakia and oral squamous cell carcinoma.

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Abstract

Objectives: This study assessed the efficacy of using oral liquid-based brush cytology and cell block immunocytochemistry in the diagnosis of oral leukoplakia as minimally invasive diagnostic adjuncts.

Methods: Seventy-two patients diagnosed clinically with either oral leukoplakia (OLK) or oral squamous cell carcinoma were included. Oral brush samples using Orcellex® brushes were obtained from all participants directly before undergoing surgical biopsy. Cell blocks were prepared for all samples and assessed for cytomorphology and immunocytochemistry of DNA mismatch repair proteins (MSH-6, MSH-2, MLH-1, and PMS-2). A combined index score of immunocytochemistry expression and cytology grading was compared against the gold standard (histopathological diagnosis).

Results: A significant association was observed between the cytological assessments of oral liquid-based brush cytology samples and the histopathological diagnosis ($p < 0.005$). In addition, there was a significant inverse correlation between the grade of oral epithelial dysplasia and the cumulative score of the studied DNA mismatch repair proteins ($p < 0.005$). Grading criteria for both oral liquid-based brush cytology and immunocytochemistry cumulative index scores are proposed based on the analysis of receiver operating characteristic curve coordinates. The diagnostic accuracy of this approach was outstanding in terms of discrimination between the presence
presence or absence of oral epithelial dysplasia (0.961) and squamous cell carcinoma (0.977) separately.

**Conclusion:** Oral liquid-based brush cytology cell block immunocytochemistry provides a reliable strategy to investigate oral mucosal epithelial disorders. This approach presents a minimally invasive, highly accurate, and non-technically demanding method for the surveillance of oral potentially malignant disorders and squamous cell carcinoma.

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1. Introduction

Oral squamous cell carcinoma (OSCC) is the most predominant histological type of oral malignancy and is commonly preceded by oral potentially malignant disorders (OPMDs) that may harbour histopathological changes termed oral epithelial dysplasia (OED). Histopathological assessment alone however does not provide an accurate assessment of malignant transformation (MT) risk, and is both invasive and subjective in nature, in addition to being poorly accepted by patients.

The lack of highly specific and sensitive, validated clinical, histopathological or molecular biomarkers to predict the MT of OPMDs has contributed to the high mortality and morbidity rates of OSCC. OED alone does not inevitably indicate progression to malignancy, simultaneously; the absence of dysplastic features does not necessarily preclude the likelihood of the MT. In addition to being invasive and associated with high morbidity, the accurate diagnosis by incisional biopsy requires correct site selection, which often relies on an experienced clinician or specialist. This issue is amplified in the case of large-sized and/or multiple lesions, where multiple biopsies are needed, especially taking into consideration that the presence and grade of OED and molecular heterogeneity vary within a lesion. Thus, this sampling bias can lead to misdiagnosis.

There is an urgent need for developing non-invasive and non-technically sensitive approaches
assessing lesions coupled with objective predictive biomarkers to identify those at higher risk of malignancy.\(^7\) One method that has gained increased interest recently for the detection of OPMDs is oral liquid-based brush cytology (OLBC).\(^8,9\) OLBC provides a minimally invasive alternative to surgical biopsies in harvesting representative oral epithelial cells.\(^9,10\) However, despite the advantages of OLBC, it shares some general drawbacks with conventional cytology; the most striking is the limited amount of cellular material used or preserved for further downstream testing. Preparing cell blocks from cytology samples provides solutions to treat them in a similar way to tissue samples and with comparable cost.\(^11\) In this regard, the cell block is a type of cytological preparation where cytological material is collected in a pellet and paraffinized to form formalin-fixed paraffin-embedded (FFPE) cell blocks.\(^11\)

It is well known that DNA damage accumulates in cells over time as a result of various exogenous agents and endogenous reactive metabolites. Failure to repair this damage leads to cellular

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mutations which can potentially cause disease and dysfunction. Among many cellular systems to repair such damage, a pivotal one is DNA damage mismatch repair (MMR). MMR proteins (MutSa and MutLa) are a family of proteins that repair DNA base substitution mismatch and insertion/deletion mismatch that circumvent the editing functionality of DNA polymerases. MMR proteins work in a complementary manner by correcting DNA mismatch during DNA replication and therefore, they prevent permanent mutations in dividing cells, and play a critical role in cell cycle arrest and programmed cell death. Any alteration of these functions decreases apoptosis, increases cell survival, and leads to chemotherapy resistance. Studies have shown that a reduction in these proteins is associated with an increased predisposition for cancer. Moreover, we have previously shown that a converse correlation exists between the expression of these proteins and the severity of oral disease demonstrating the diagnostic role for MMR proteins in OED and OSCC.

In this current study, we aimed to assess the feasibility of using cell blocks prepared from OLBC samples in the diagnosis of oral leukoplakia (OLK) and OSCC, in which the diagnostic accuracy of the immunocytochemical expression of MMR proteins combined with the cytological assessment using cell blocks was determined.

2. Materials and Methods
2.1 Study design

This semi-quantitative comparative study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. The study received ethical approval from the University of Western Australia Human Ethics Committee (RA/4/20/4530 and RA/4/1/8562), and was conducted in accordance with the principles of the Declaration of Helsinki.

Samples were selected from those accumulated by us for other projects evaluating the utility of OLBC in the diagnosis of oral mucosal lesions. Cases were included if the subject was aged more than 18 years and provided informed consent. Subjects were diagnosed clinically with either OLK or OSCC. Patients with a history of radiotherapy or chemotherapy were excluded.

2.2 Sample preparation

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All patients underwent oral brush biopsy using Orcellex® brush (Rovers Medical Devices, The Netherlands) under local anaesthesia before surgical biopsies as described by Kujan et al. All OLBC samples were preserved in methanol-based preservative vials (ThinPrep®PreservCyt, Hologic Inc., MA, USA) and transported to the laboratory to prepare liquid-based cytology slides using the ThinPrep® 2000 processor (Hologic Inc., MA, USA) according to the manufacturer’s instructions. Surgical biopsies were performed on all lesions and histopathological diagnosis was considered as the standard point of reference for definitive diagnosis. All samples were histopathologically re-examined and cases with OED were classified as a either low-risk or high-risk.

2.3 Cell block preparation

Following preparation of ThinPrep® slides (Hologic Inc., MA, USA), all remaining cells were processed to form individual FFPE cell blocks according to the thromboplastin-plasma method. Cell block cellularity was evaluated using hematoxylin and eosin (H&E) stain and light microscopy. Samples were considered adequate if they showed at least $1 \times 10^6$ cells.

2.4 Cytological assessment

Cytological assessment of all Papanicolaou ThinPrep® slides and H&E stained cell block slides was carried out using the modified 2014 Bethesda Cervical Cytology grading system as described by us previously. Accordingly, cases were classified as atypical squamous cells of undetermined significance.
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index scores of the four proteins. For antibody optimization, three cell lines prepared as cell blocks were used: PE/CA-PJ15 (OSCC), DOK (dysplastic oral keratinocytes), and HOK (normal oral keratinocytes).20

2.6 Statistical analysis

Statistical tests were performed using IBM SPSS Statistics version 23.0 software (IBM Corporation, Armonk, NY). Chi-square test of independence was used to analyze the relationship between histopathological diagnosis, lesion site and cytological assessment. Post hoc analysis of pairwise comparisons using multiple z-tests of two proportions was used to assess the association between each pair of variables in the previously mentioned relationship. One-way ANOVA was used to study the association between continuous and categorical variables. Histopathological diagnosis was graded as: no dysplasia = 1, low-risk dysplasia = 2, high-risk dysplasia = 3, and OSCC = 4.

For chi-square tests of independence and one-way ANOVA, statistical significance was defined as $P < 0.05$. For post hoc analysis, the $p$-value of 0.05 was adjusted by dividing by 16 (the total number of pairwise comparisons) to be $p \leq 0.003$.

Associations between the index scores were assessed using Spearman’s product-moment correlation coefficient interpreted as: 0.9 - 1 = very strong, 0.7 - 0.89 = strong, 0.4 - 0.69 = moderate, 0.1 - 0.39 = weak.21 A receiver operating characteristic (ROC) curve was used to study disease progression.
diagnostic accuracy. The level of discrimination according to the area under the curve (AUC) was defined as: 0.5 \( \geq \) AUC none, 0.5 \( < \) AUC \( < \) 0.7 poor, 0.7 \( \leq \) AUC \( < \) 0.8 acceptable, 0.8 \( \leq \) AUC \( < \) 0.9 excellent, and AUC \( \geq \) 0.9 outstanding.\textsuperscript{22}

3. Results

3.1 General characteristics of study subjects

A total of 110 cell blocks were stained with H&E, of which 38 (34.5\%) were excluded as a result of inadequate cellularity. The remaining 72 (63.5\%) cases included samples from 33 males.
(45.8%) and 39 females (54.2%) (Table 1). Age ranged from 36 to 90 years, with a mean of 64.75 ± 11.7 years. The mean age of males was significantly higher than that of females, 68.3 ± 10.6 and 61.7 ± 11.8 respectively (p = 0.017). Smoking and alcohol consumption were not associated with patient gender, p = 0.179, and p = 0.79, respectively. The histopathological diagnosis of about a third of cases was either ortho-keratosis or para-keratosis without dysplasia. The most common location of lesions was the lateral tongue.

A chi-square test of independence showed a statistically significant association between the histopathological diagnosis and lesion site, X² (9) = 21.30, p = 0.011. Post hoc analysis revealed that the number of lesions diagnosed with hyperkeratosis with dysplasia was significantly greater on the lateral tongue, p = 0.001. In addition, hyperkeratotic lesions without dysplasia were significantly greater on labial/buccal mucosa, p = 0.002. No other pairwise comparisons were statistically different.

Neither smoking nor alcohol consumption was associated with histopathological diagnosis, p = 0.244, and p = 0.903, respectively. Similarly, histopathological diagnosis was not affected by age or gender, p = 0.211 and p = 0.341, respectively.

3.2 Accuracy of OLBC

Of the 72 OLBC slides, one case from a female patient was excluded due to a defect in Papanicolaou staining. A chi-square test of independence between cytological assessment and histopathological diagnosis showed a statistically significant association, X² (9) = 21.30, p = 0.011.
ological diagnosis was significant, $X^2 (9) = 143.33, p < 0.005$ (Table 1). Post hoc analysis of pairwise comparisons showed statistically significant associations (Table 1). The accuracy of using this approach in predicting histopathology diagnosis was 95.07\% (95\% CI = 91.87\% to 97.28\%), while the associated sensitivity and specificity were 90.14\% and 96.17\%, respectively (Table 2).

### 3.3 Correlation between MMR protein expression and histopathological/cytological diagnoses

A total of 72 cell blocks were prepared from the OLBC vials for MMR staining. Figure 1 shows the ICC expression of MMR proteins in representative samples. Cumulative protein scores (MSH-6, MSH-2, MLH-1, PMS-2) were independently significantly associated with histopathological and cytological diagnoses, where the highest score was noted in cases without dysplasia and the lowest in OSCC, $p < 0.005$ (Figure 2).
There were significant negative associations between the expression of each protein and the grade of disorder, (Spearman’s correlation coefficient ranged between -0.757 and -0.809, \( p < 0.005 \)).

### 3.4 Proposed index scores for the determination and grading of oral epithelial dysplasia

To determine the efficacy of using the cumulative MMR index score and cytology in the diagnosis and grading of OED, two indices were proposed. (1) MMR cumulative index score referred to as ‘cumulative index’, and (2) a combination of cumulative MMR index and OLBC grading scores termed the ‘combined index’ (**Table 3**). The cut-off value for each histopathological category (no dysplasia, low-risk OED, high-risk OED, and OSCC) was obtained based on Youden’s index after calculating the coordinates on ROC. The highest score represents the most appropriate cut-off value. The formula for calculating Youden’s index \(^{25}\) is:

\[
[(\text{true-positive rate}) + (1- \text{false-positive rate}) - 1]
\]

The accuracy of the combined index was higher than that of the cumulative index, 92.25% and 75.81%, respectively. However, the accuracy of OLBC grading alone was the highest at 95.07% (**Table 3**).

### 3.5 The ability to differentiate between the presence and absence of OED or OSCC

In order to determine the efficacy of the proposed approaches in the differentiation between oral lesions based on a binomial means (presence or absence of a specific condition), the AUC was analyzed for all approaches. The true positive rate (TPR) and false positive rate (FPR) were calculated accordingly.
for all approaches. The true positive rate (TPR) and false positive rate (FPR) were calculated as follows:

\[
\text{True positive} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}}
\]

\[
\text{False positive} = \frac{\text{False positive}}{\text{True negative} + \text{False positive}}
\]

The ability of the studied indices (OLBC, cumulative index, combined index) in discriminating between the presence or absence of OED or OSCC was outstanding according to general rules of thumb defined by Hosmer et al.\textsuperscript{22} \textbf{Table 4} demonstrates the ability of each approach in differentiating between conditions.

4. Discussion

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Cytology has advanced significantly over recent years providing a technically non-sensitive, rapid, and cost-effective diagnostic approach. Early studies in oral cytology suffered from major drawbacks of poor sensitivity and specificity for detecting OED and OSCC due to the inability to obtain representative samples, and presence of artefacts. The introduction of liquid-based cytology preparation and oral brush biopsy has improved sample quality, reproducibility, sensitivity, and specificity. The correlation of cell block and cytology material has provided several advantages to overcome the limitations of conventional cytology by offering archival material for ancillary tests. Moreover, it is widely accepted that this approach enhances diagnostic accuracy and shows superior sensitivity over other cytological preparations. A study comparing immunostaining among three cytological preparations (air-dried cytospins, liquid-based thin-layer ThinPrep, and cell blocks) found that cell blocks were associated with the closest approximation to surgical biopsies, the least background staining, and the lowest cost in comparison to other preparations. Similarly, other studies have revealed a significant improvement in diagnostic accuracy using cell blocks over other cytological preparations in the diagnosis of various pathologies. To our knowledge, this is the first study to assess immunoreactive protein expression utilizing paraffinized cell blocks from OLBC samples in a cohort of OLK samples.

The major aim of this study was not only to provide a minimally invasive adjunct for the diagnosis of OPMD and OSCC, but also to assess the ability to archive these samples to be used for future investigation.
tions. Initially thin-preparation slides were prepared from the original samples for cytological assessment using the modified Bethesda system. 8 34.5% of the prepared cell blocks were inadequate due to low cellularity given the original OLBC preservative had been used for the preparation of multiple OLBC slides that were used in previous studies. 8,16 This suggests that future studies should prepare cell blocks directly after collection of brush biopsies.

The accuracy of cytological diagnosis in the current study was 95.07%, which is higher than that reported previously by us (75%). 8 This can be seen in the post hoc analysis where each histopathological diagnostic category significantly coincided with its analogous cytological diagnostic category. This improvement can be attributed to the strict inclusion criteria in the present study as only samples diagnosed clinically with OLK or OSCC were included. Therefore, we believe there is a need to develop diagnostic criteria inclusive of all categories of OPMDs for wider adoption of this approach in clinical pathology practice.

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MMR proteins were selected for ICC analysis because the negative trend of these proteins with the severity of OED had previously been affirmed. A significant inverse correlation between MMR proteins and grades of OED was found once again in the current study. This provides additional evidence on the utility of these biomarkers in stratifying OLK cases, and supports the role of genetic instability in the malignant transformation of OLK.

Moreover, we developed two grading systems for the diagnosis of OED, one based on the cumulative index scores of MMR proteins, and the other a combination of the cumulative and OLBC scores. The accuracy of the grading systems was 75.81% and 92.25% for cumulative and combined indices, respectively. Although this level of accuracy was lower than the accuracy of OLBC grading alone, this may be attributed to the relatively limited sample size in the current study.

The diagnostic accuracy of OLBC alone and the MMR cumulative score alone in the discrimination between the presence and absence of OLK or OSCC was outstanding in this study. These results are comparable with a recent review among 1,110 patients where the diagnostic accuracy of using MMR proteins in detecting endometrial cancer was 0.988. Although this does not preclude the necessity of surgical biopsies, the presented approach may inevitably aid in the surveillance of OLK.

There was strong co-expression correlation between the MMR proteins according to Spearman’s coefficient.
These correlations were higher than that demonstrated by Jessri et al. in relation to oral epithelial diseases \(^1^3\) and comparable to that of Chen et al. in relation to colorectal cancer.\(^2^8\) The progression of oral epithelial disorders from hyperkeratosis without dysplasia to OSCC was significantly related with the reduction in the immunoexpression of all MMR proteins in a similar fashion. Although these findings have previously been reported,\(^1^2,^2^8\) the current study is the first to assess the utility of MMR protein expression in cell block samples.

Defective MMR proteins produce microsatellite instability (MSI), a phenomenon known to be associated with several solid human cancers,\(^1^4\) and disease severity.\(^2^9\) The ability of cells to respond to DNA damage determines their ability to restore genomic alterations, hence and can be used as predictors of malignant transformation as highlighted by Farah et al. recently.\(^3^0\) This may explain the observed gradual inverse relationship between the grade of oral epithelial disorders and the expression of these proteins.

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There are potential limitations associated with the current study. The relatively small sample size and the retrospective nature of the study design could hinder the applicability of the technique in a clinical setting. Also, the study may have suffered from selection bias by only including clinically diagnosed cases of OLK. Further prospective studies that include a wide variety of OPMDs would be required to support the implementation of this approach in daily diagnostic pathology practice. Furthermore, the investigation of additional molecular markers using cell blocks is recommended.

In conclusion, the results of this study confirm the feasibility of utilising cell blocks from OLBC samples as a reliable adjunct for the accurate determination of the underlying histopathology of oral leukoplakia. This approach provides a minimally invasive adjunct to surgical biopsies for the surveillance of OPMD and the early detection of OSCC.
Conflict of interest

Authors declare no conflict of interests.

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Authors’ contribution

OK and CSF conceived and devised the study; CSF collected clinical and histopathological samples; MI, NA, BS, EW collated data; MI and OK analysed data; all authors drafted the manuscript; OK and CSF edited and revised the manuscript; all authors approved the final manuscript.

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Table 1: Patient clinical demographics and cytological diagnoses correlated with histopathological diagnoses

<table>
<thead>
<tr>
<th>Variables</th>
<th>Histopathology diagnosis</th>
<th></th>
<th></th>
<th>Total</th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ortho-/para-keratosis</td>
<td>Low-risk</td>
<td>High-risk</td>
<td>OSCC</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>10</td>
<td>6</td>
<td>5</td>
<td>33</td>
<td>0.341</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>6</td>
<td>13</td>
<td>6</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Lesion site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral tongue</td>
<td>3</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Floor of the mouth</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>16</td>
<td><strong>0.017</strong></td>
</tr>
<tr>
<td>Labial/buccal mucosa</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Alveolar ridge and palate</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Conventional cytology*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetermined significance</td>
<td>24</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>N</td>
<td>M</td>
<td>V</td>
<td>F</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Low-grade</td>
<td>2</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>0.000</td>
</tr>
<tr>
<td>High-grade</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>1</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>OSCC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>26</td>
<td>16</td>
<td>19</td>
<td>11</td>
<td>72</td>
<td></td>
</tr>
</tbody>
</table>

* One case was excluded

N: number of subjects

OSCC: oral squamous cell carcinoma

SIL: squamous intraepithelial lesion
Table 2: The accuracy of proposed indices in predicting the histo-/cyto-pathological diagnosis of oral leukoplakia and oral carcinoma

<table>
<thead>
<tr>
<th>Index</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV (%)</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
</table>

The accuracy of proposed indices in predicting the histo-/cyto-pathological diagnosis of oral leukoplakia and oral carcinoma.
<table>
<thead>
<tr>
<th>Index</th>
<th>(%)</th>
<th>(%)</th>
<th>PPV (%)</th>
<th>(%)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLBC grading</td>
<td>90.14</td>
<td>96.71</td>
<td>90.14</td>
<td>96.71</td>
<td>95.07</td>
</tr>
<tr>
<td>Cumulative criteria</td>
<td>58.33</td>
<td>82.95</td>
<td>62.5</td>
<td>87.5</td>
<td>75.81</td>
</tr>
<tr>
<td>Combined criteria</td>
<td>84.51</td>
<td>94.84</td>
<td>84.51</td>
<td>94.84</td>
<td>92.25</td>
</tr>
</tbody>
</table>

PPV: positive predictive value
NPV: negative predictive value

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Table 3: Proposed indices and scores used to diagnose and grade oral epithelial dysplasia

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<table>
<thead>
<tr>
<th>Indices</th>
<th>Score</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OLBC grading scores</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>No dysplasia</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Low-grade SIL</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>High-grade SIL</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>OSCC</td>
</tr>
<tr>
<td><strong>Cumulative index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.76 – 12</td>
<td>No dysplasia</td>
</tr>
<tr>
<td></td>
<td>5.51 – 7.75</td>
<td>Low-risk lesion</td>
</tr>
<tr>
<td></td>
<td>2.76 – 5.5</td>
<td>High-risk lesion</td>
</tr>
<tr>
<td></td>
<td>0 – 2.75</td>
<td>OSCC</td>
</tr>
<tr>
<td><strong>Combined index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.26 – 12</td>
<td>No dysplasia</td>
</tr>
<tr>
<td></td>
<td>5.76 – 8.25</td>
<td>Low-grade lesion</td>
</tr>
<tr>
<td></td>
<td>3.76 – 5.75</td>
<td>High-grade lesion</td>
</tr>
<tr>
<td></td>
<td>0 – 3.75</td>
<td>OSCC</td>
</tr>
</tbody>
</table>
Table 4: Diagnostic accuracy of proposed indices in discriminating between the presence and absence of OED or OSCC

<table>
<thead>
<tr>
<th>Cumulative index</th>
<th>Presence/absence of OED</th>
<th>Presence/absence of OSCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>OIIBC grading</td>
<td>AUC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>AUC&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>SE&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;i&gt;P&lt;/i&gt;-value</td>
<td>&lt;i&gt;P&lt;/i&gt;-value</td>
</tr>
<tr>
<td>0.942</td>
<td>0.921</td>
<td>0.986</td>
</tr>
<tr>
<td>0.028</td>
<td>0.032</td>
<td>0.016</td>
</tr>
<tr>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Combined index</td>
<td>0.961</td>
<td>0.025</td>
</tr>
<tr>
<td>----------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>a Area under curve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Standard error</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Figure legends

Figure 1:

Representative photomicrographs of oral leukoplakia and OSCC lesions (× 400). Selective photomicrographs (highlighted in bold) are presented as full images in Supplementary Figure 1. A) Papanicolaou stained OLBC slide of undetermined significance squamous intraepithelial lesion (SIL), B) haematoxylin & eosin (H&E) stained cell block of undetermined significance SIL, C) positive expression of MSH-2 stained cell block of undetermined significance SIL, D) weak expression of MSH-6 stained cell block of undetermined significance SIL, E) positive expression of MLH-1 stained cell block of undetermined significance SIL, F) positive expression of PMS-2 stained cell block of undetermined significance SIL, G) Papanicolaou stained OLBC slide of low-grade SIL, H) haematoxylin & eosin (H&E) stained cell block of low-grade SIL, I) positive expression of MSH-2 stained cell block of low-grade SIL, J) negative expression of MSH-6 stained cell block of low-grade SIL, K) negative expression of MLH-1 stained cell block of low-grade SIL, L) positive expression of PMS-2 stained cell block of low-grade SIL, M) Papanicolaou stained OLBC slide of high-grade SIL, N) haematoxylin & eosin (H&E) stained cell block of high-grade SIL, O) negative expression of MSH-2 stained cell block of high-grade SIL, P) negative expression of MSH-6 stained cell block of high-grade SIL, Q) weak expression of MLH-1 stained cell block of high-grade SIL, R) weak expression of PMS-2 stained cell block of high-grade SIL, S) Papanicolaou stained OLBC slide of OSCC, T)
Figure 2: Association between cumulative MMR protein expression and (A) histopathology diagnosis ($p < 0.005$), and (B) cytology diagnosis ($p < 0.005$).
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References


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Histopathology diagnosis

OSCC
High-risk keratosis with no dysplasia
Low-risk

MMR proteins cumulative score 10.00 8.00 6.00 4.00 2.00 .00
Cytological assessment

MMR proteins cumulative score

Cytological assessment:
- Undetermined significance
- Low-grade dysplasia
- High-grade dysplasia
- SCC

MMR proteins cumulative score:

- 10.00
- 8.00
- 6.00
- 4.00
- 2.00
- 0.00
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