DNA methylation patterns of genes related to immune response in the different clinical forms of oral lichen planus

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Background: The oral lichen planus is a chronic inflammatory disease. Although its aetiology is not well understood, the role of T lymphocytes in its inflammatory events is recognised. Identifying the epigenetic mechanisms involved in the pathogenesis of this immune-mediated condition is fundamental for understanding the inflammatory reaction that occurs in the disease. The purpose of this work was to evaluate the methylation pattern of 21 immune response-related genes in the different clinical forms of oral lichen planus.

Methods: A cross-sectional study was performed to analyse the DNA methylation patterns in three distinct groups of oral lichen planus: (i) reticular/plaque lesions; (ii) erosive lesions; (iii) normal oral mucosa (control group). After DNA extraction from biopsies, the samples were submitted to digestions by methylation-sensitive and methylation-dependent enzymes and double digestion. The relative percentage of methylated DNA for each gene was provided using real-time polymerase chain reaction arrays.

Results: Hypermethylation of the STAT5A gene was observed only in the control group (59.0%). A higher hypermethylation of the ELANE gene was found in reticular lesions (72.1%) compared to erosive lesions (50.0%).

Conclusion: Our results show variations in the methylation profile of immune response-related genes, according to the clinical type of oral lichen planus after comparing with the normal oral mucosa. Further studies are necessary to validate these findings using gene expression analysis.

Keywords: epigenetic, inflammation, lichen planus, methylation, oral lichen planus

1 INTRODUCTION

Lichen planus is a chronic immunoinflammatory mucocutaneous disease, which can affect the skin, mucous membranes, scalp and nails. It affects about 0.5% to 2% of the population and is classified by the World Health Organization (WHO) as a potentially malignant lesion. Oral lichen planus (OLP) lesions may precede the disease in other locations or can occur as a single manifestation of the disease. It can manifest in six different clinical forms: reticular, plaque, papular, erosive, atrophic and bullous (Figure 1A). Histopathologically, OLP shows degeneration of the basal layer in addition to subepithelial T lymphocytes infiltration (Figure 1B).

The events involved in the aetopathogenesis of OLP are complex, resulting from the dysregulation of the immune system caused by the interaction between genetic and environmental factors. Epigenetics comprises of the study of the interplay between environmental factors and the patterns of gene expression in cells. DNA methylation is an epigenetic mechanism that regulates gene
expression through effective inhibition of genomic transcription by adding a methyl group at the 5'-position of the cytosine ring.8

Previous studies found a hypermethylated profile of the promoter regions of the microRNA miR-1379,10 and the p16 gene10 in OLP compared to in normal mucosa. In oral carcinogenesis, both miR-137 and p16 gene are known to participate in cell cycle control.11,12 In another study, evaluation of the global methylation in lichenoid lesions, including OLP, showed no difference compared to the methylation levels in normal mucosa.13 Although the information regarding DNA methylation in OLP immunoinflammatory response is scarce,8-10 such phenomenon is being currently studied in several other inflammatory processes, including inflammatory periapical lesions,14 systemic lupus erythematosus15 and Addison’s disease.16

Here, we hypothesise that the DNA methylation profile of genes related to immune response varies according to the clinical forms of OLP, harbouring different DNA methylation patterns when compared to healthy, normal oral mucosa.

2 | MATERIALS AND METHODS

2.1 | Subjects and sample collection

This study was approved by the Research Ethics Committee of the Universidade Federal de Minas Gerais and Odilon Behrens Hospital (protocol number 57753916.3.0001.5129), and all subjects gave an informed consent in writing. Eligible case group subjects included those presenting clinical and histopathological diagnosis of OLP (Figure 1).17 The control group comprised of healthy patients submitted to impacted third molar extraction. Patients who reported a history of neoplastic, infectious or other immunoinflammatory disorders (except lichen planus for the case group) or had been treated with an anti-inflammatory drug in the last 3 months before the start of the study were excluded.

According to the clinical aspects of the disease, cases were divided into two groups: Reticular/plaque OLP group-patients with white plaque or reticular lesions; Erosive OLP group-patients with ulcerated or erosive lesions. Patients who exhibited more than one clinical type were classified according to the area of biopsy and sample collection.

Oral lichen planus biopsies were divided into two fragments. One fragment was fixed in 10% formaldehyde and sent for routine processing and confirmation of diagnosis. The other fragment was stored in RNA Later® solution (Thermo Fisher Scientific, Wilmington, DE, USA) at −80°C until the experiments were performed. In the case of the control group, a fragment of normal gingiva or alveolar mucosa, without clinical signs of inflammation such as pericoronitis, gingivitis or other local inflammatory process, was collected during tooth extraction.

All collected tissues were stored in RNA Later® (Thermo Fisher Scientific) for 24 h and frozen at −80°C in liquid nitrogen until processing time.

2.2 | DNA isolation

Genomic DNA (gDNA) was isolated from fresh frozen tissue samples using the DNeasy Blood and Tissue Kit (Qiagen Inc, Valencia, CA, USA) according to the manufacturer’s protocol. The quantification and evaluation of DNA purity were performed by spectrophotometry using NanoDropTM 2000 (Thermo Fisher Scientific, Waltham, MA).

2.3 | DNA methylation analysis

Pools of reticular/plaque OLP (n = 6), erosive OLP (n = 5) and normal mucosa (n = 5) were used for the DNA methylation analysis. For each assay, 1 µg of gDNA was used, as suggested by the manufacturer.

DNA methylation analysis was performed using the EpiTect Methyl DNA Restriction Kit (Qiagen Inc). The pools of gDNA were digested to prepare for methylation analysis using a methylation-sensitive and/or a methylation-dependent restriction enzyme that digests unmethylated and methylated DNA, respectively, according to the manufacturer’s protocol. DNA methylation profile of 21 cytokine genes was analysed using the EpiTect Methyl II Signature Human Cytokine Production PCR Array (SABiosciences, Qiagen, catalogue: EAHS 541Z-1). The genes were related to the following functions: T cell function regulators, B cell function regulators, transcriptional regulators, translational regulators, environment and intracellular stimuli response and cytokine production signalling molecules. Due to the location of the FOXP3 gene in X chromosome,18 its methylation levels were not evaluated. To perform this analysis, it would be necessary to match the patients by sex, but due to the small number of subjects, both genders were included in the same pool.

After the enzymatic cleavage, a quantitative real-time polymerase chain reaction (qPCR) was performed for detection of remaining
gDNA using a StepOnePlus instrument (Applied Biosystems, Foster City, CA, USA). Through the ΔCT values, a relative percentage of methylated DNA was provided for each gene in each group.

3 | RESULTS

Table 1 shows the primary clinical data of the subjects included in the study. The methylation levels of the promoter region of 21 immune response genes in OLP groups and in control group are shown in Figure 2.

STAT5A promoter region showed 59% methylation levels in normal oral mucosa, while reticular/plaque and erosive OLP showed a markedly reduced percentage of DNA methylation (5.4% and 2.8%, respectively) in the same gene (Table 2). When comparing the reticular/plaque OLP forms with erosive OLP, ELANE promoter gene showed a higher methylation percentage in reticular/plaque OLP (72.2%) compared to erosive OLP (50.0%).

4 | DISCUSSION

The probability of developing immune-mediated diseases depends on the interaction between genetic and environmental factors. Epigenetic mechanisms of gene regulation translate the modifications caused by the environment into stable modifications in gene expression. Although DNA methylation is one of the most studied and mechanistically understood epigenetic mechanisms, there is a paucity of data regarding the role of methylation in the regulation of the immune response in OLP. Therefore, this cross-sectional study aimed to analyse the DNA methylation patterns of immune response-related genes in the different clinical forms of OLP.

**TABLE 1** Clinical data of subjects with oral lichen planus (OLP), and control subjects included in the Methylation assay

<table>
<thead>
<tr>
<th>Case</th>
<th>Sample</th>
<th>Clinic clinical form</th>
<th>Sex</th>
<th>Age</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>Male</td>
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<td>Buccal mucosa</td>
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<tr>
<td>3</td>
<td>OLP</td>
<td>Reticular</td>
<td>Female</td>
<td>64</td>
<td>Buccal mucosa</td>
</tr>
<tr>
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<td>OLP</td>
<td>Reticular</td>
<td>Female</td>
<td>20</td>
<td>Buccal mucosa</td>
</tr>
<tr>
<td>5</td>
<td>OLP</td>
<td>Reticular</td>
<td>Female</td>
<td>55</td>
<td>Buccal mucosa</td>
</tr>
<tr>
<td>6</td>
<td>OLP</td>
<td>Plaque</td>
<td>Female</td>
<td>53</td>
<td>Buccal mucosa</td>
</tr>
<tr>
<td>7</td>
<td>OLP</td>
<td>Reticular</td>
<td>Female</td>
<td>55</td>
<td>Buccal vestibule</td>
</tr>
<tr>
<td>8</td>
<td>OLP</td>
<td>Erosive</td>
<td>Female</td>
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<td>Buccal mucosa</td>
</tr>
<tr>
<td>9</td>
<td>OLP</td>
<td>Erosive</td>
<td>Male</td>
<td>62</td>
<td>Upper lip mucosa</td>
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<tr>
<td>10</td>
<td>OLP</td>
<td>Erosive</td>
<td>Female</td>
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<td>Buccal mucosa</td>
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<tr>
<td>11</td>
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<td>Erosive</td>
<td>Female</td>
<td>60</td>
<td>Buccal mucosa</td>
</tr>
<tr>
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<td>Buccal mucosa</td>
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<tr>
<td>13</td>
<td>Control</td>
<td>Normal mucosa</td>
<td>Female</td>
<td>24</td>
<td>Gingiva</td>
</tr>
<tr>
<td>14</td>
<td>Control</td>
<td>Normal mucosa</td>
<td>Female</td>
<td>46</td>
<td>Alveolar mucosa</td>
</tr>
<tr>
<td>15</td>
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<td>Male</td>
<td>14</td>
<td>Gingiva</td>
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<td>Control</td>
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<td>37</td>
<td>Alveolar mucosa</td>
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<td>Normal mucosa</td>
<td>Male</td>
<td>45</td>
<td>Alveolar mucosa</td>
</tr>
</tbody>
</table>

In our study, we first compared the methylation status of distinct clinical forms of OLP with that of normal oral mucosa. There is evidence that the clinical form of OLP is related to the severity of the inflammatory lymphocyte reaction in the basal layer of the mucosal epithelium. When the reaction is mild, there is stimulation of epithelial activity, resulting in striations or white plaques. More intense lymphocyte activity may result in ulceration or atrophy of the epithelium. As this study proposed to evaluate genes related to inflammation, the reticular and plaque clinical forms of the disease were grouped in the same pool. The atrophic, bullous and papular OLP were not included in these analyses because access to patients with these variants was not possible.

Among 21 gene promoter regions relevant to immune response evaluated, STAT5A showed the most discrepant methylation profile in OLP compared to healthy oral mucosa. OLP samples, including the reticular/plaque and erosive clinical forms, showed a predominant hypomethylated status compared to the normal control group samples. STAT5A is a transcription factor member of the STAT family.

Our pilot study raises the hypothesis that the epigenetic modulation of STAT5A could be relevant to the OLP development. Data available in the literature shows increased gene expression of STAT5A in OLP compared to that in healthy mucosa. Although this information converges with results presented in this study, it is important to consider that other factors besides methylation may affect gene expression.

The STAT5A is activated by a variety of cytokines and hormones, and plays an essential role in the development of regulatory T cells (Treg). Previous studies have suggested the participation of STAT5A in other inflammatory diseases, such as asthma.

When the two groups of OLP clinical types were compared, ELANE promoter region showed higher levels of methylation in the reticular/plaque clinical forms compared to in the erosive form. There is no data about the methylation profile of ELANE gene in inflammatory processes but it codes the neutrophil elastase protein, which spreads neutrophilic inflammation by accelerating the production of pro-inflammatory cytokines. Previous studies suggested that OLP is probably related to functional changes in salivary neutrophils, reflecting the multifactorial profile of the pathophysiological mechanisms of the disease associated with functional changes of salivary neutrophils, reflecting the different pathophysiological mechanisms of the disease. Also, there is no data available about ELANE expression in OLP.

The first evidence that suggested the involvement of methylation in autoimmunity was published in 1986. These authors investigated the effect of DNA methyltransferase 5-azacytidine inhibitor in the induction of symptoms associated with autoimmunity. A hypomethylated genome was observed in patients with autoimmune diseases such as systemic lupus erythematosus and autoimmune Addison’s disease. Considering that autoimmune diseases are complex- and present-specific challenges to researchers seeking to define their aetiology and explaining the progression, several mechanisms involved in the pathogenesis of these diseases can serve as targets in therapeutics, such as DNA methyltransferase inhibitors, which have been applied in animal models and in clinical trials. Therefore, as gene methylation is an important mechanism of gene expression regulation, it is an attractive area for OLP patients’ management in the future.
The present study shows some limitations in the form of information bias in the collection of data on systemic condition and habits of subjects. Another limitation was the impossibility of matching the patients included in this study by characteristics inherent to them, such as age and sex, which may influence DNA methylation. Further studies are also necessary to validate some of the findings using qRT-PCR or immunohistochemistry analyses.

In conclusion, our data suggests that the methylation status of immune response genes in OLP differs from that of the normal oral mucosa, and it varies according to the clinical type of the disease.

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FIGURE 2 Percentage of methylation levels in immune response genes in reticular/plaque oral lichen planus (OLP), erosive OLP and normal mucosa pools. Note that STAT5A showed lower methylation levels in OLP compared to the control (normal mucosa) group.
REFERENCES


TABLE 2 Methylation levels of the promoter region of the genes ELANE and STAT5A

<table>
<thead>
<tr>
<th>GENE</th>
<th>Reticular/plaque OLP</th>
<th>Erosive OLP</th>
<th>Normal mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UM (%)</td>
<td>M (%)</td>
<td>UM (%)</td>
</tr>
<tr>
<td>STAT5A</td>
<td>94.57</td>
<td>5.43</td>
<td>97.24</td>
</tr>
<tr>
<td>ELANE</td>
<td>27.84</td>
<td>72.16</td>
<td>50.00</td>
</tr>
</tbody>
</table>

M, Methylated; UM, Unmethylated; OLP, oral lichen planus.

CONFLICT OF INTEREST

The authors of this study declare that they have no conflict of interest.

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