Histopathological aspects and local implications of oxidative stress in patients with oral lichen planus

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Abstract
Aerobic life is connected with continuous production of free radicals, particularly reactive oxygen species (ROS). Cells possess an enzymatic and non-enzymatic antioxidant system to maintain redox homeostasis. Oxidant–antioxidant imbalance resulting in excessive accumulation of ROS is defined as oxidative stress. Several researchers suggest that oxidative stress is implicated in the pathogenesis of this disorder. The aim of this study was to evaluate the histopathological alterations and the status of local oxidative stress and antioxidant defense system in patients with OLP. We evaluated and compared the local levels of oxidative stress biomarkers malondialdehyde (MDA) and glutathione (GSH) in patients with OLP with that of normal controls. Increased levels of MDA and decreased levels of GSH suggest the idea of oxidative stress implication in the pathogenesis of oral lichen planus.

Keywords: oxidant–antioxidant imbalance, local oxidative stress, oral lichen planus.

Introduction
Aerobic life is connected with continuous production of free radicals, particularly reactive oxygen species (ROS) [1]. The five principal targets for ROS are: small organic biomolecules, proteins, nucleic acids, gene activation and unsaturated fatty acids [2]. Cells possess an enzymatic and non-enzymatic antioxidant system to maintain redox homeostasis [2].

Oxidant–antioxidant imbalance resulting in excessive accumulation of ROS is defined as oxidative stress [3, 4]. The consequence of installing the oxidative stress is interfering with apoptosis, proliferation, growth arrest and senescence [5].

Oxidative stress have been implicated in a variety of diseases including inflammation, cancer, kwashiorkor (predominantly protein deficiencies), seizure, Parkinson’s disease, sickle cell anemia, liver disease, cystic fibrosis, heart attack, stroke, diabetes, infection, HIV, AIDS in which ROS were involved in the initiation stage or are produced during its course [4, 6–8].

Oral lichen planus (OLP) is a chronic inflammation of unknown etiology. It is recognized as an autoimmune disease and it is hypothesized that antigen-specific and non-specific mechanisms are involved in his pathogenesis. Antigen-specific mechanisms in OLP include antigen presentation by basal keratinocytes and antigen-specific keratinocyte killing by CD8+ cytotoxic T-cells. Non-specific mechanisms include mast cell degranulation and matrix metalloproteinase activation in OLP lesions [9, 10].

Several studies evaluated the blood and saliva oxidative stress biomarkers in oral lichen planus and suggest that oxidative stress is implicated in the pathogenesis of this disorder [11–13]. The aim of this study was to evaluate the histopathological alterations and the status of local oxidative stress and antioxidant defense system in patients with OLP.

Materials and Methods
Tissues specimens
The study included nine patients with OLP and four healthy volunteers. All subjects were retrieved from Department of Oral, Cranio-Maxillary and Cervico-Facial Surgery of “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca. This work has been approved by the ethical committee of “Iuliu Hatieganu” University of Medicine and Pharmacy and all of the participants gave their informed consent prior any investigation was conducted.
The diagnosis was established after all patients were subjected to a thorough history interview, clinical and histopathological examination. Biotic specimens from the lesions of patients and normal mucosa of controls were utilized one fragment for histopathological exam and the other was stored at -80°C until it was processed for the evaluation of oxidative stress.

Microscopic examination

Microscopic examination of oral biopsies was performed from formalin fixed paraffin embedded tissue. Samples were sectioned using a Leica RM 2125 RT microtome and stained using the standard Hematoxylin–Eosin method (HE). Tissue analysis was performed using an Olympus system for image acquisition and analysis, respectively an Olympus BX51 microscope equipped with Olympus Cell B software.

Assays

We assessed from the preserved oral tissue malondialdehyde (MDA) as a marker of oxidative stress, using the fluorometric method according to Conti M et al. [14] and as a marker of antioxidant defense glutathione (GSH), using the fluorometric method according to Ellman GL [15]. The results were expressed in nmol/mg protein.

Statistical analysis

Data normality was assessed using Kolmogorov–Smirnov test. Data were analyzed using a nonparametric Kruskall–Wallis test for overall groups’ comparison, Mann–Whitney U-Test/Student for the comparison of two groups. The correlations between the qualitative variables were analyzed by chi-square test. Adjustments were used using stratification of groups to exclude the effect of possible error factors. SPSS 17.0 (Chicago, IL, USA) statistical package was used to analyze all data.

Results

General characteristics and correlations

The average age of the participants was 47.27±17.83 years, significantly higher in patient’s group (p=0.007) (Table 1). One toxic substance (alcohol/nicotine/caffeine) was reported to be consumed by the subjects in the control group while subjects in the tested group consume one to three types of drugs. The correlation between the use of drugs and the group origin was not statistically significant (p=0.246) (Table 1).

Table 1 – Characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Tested group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median±SD) [years]</td>
<td>28.25±2.98</td>
<td>54.18±15.67</td>
</tr>
<tr>
<td>Toxic substances, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>0 (0%)</td>
<td>2 (16.7%)</td>
</tr>
<tr>
<td>One substance</td>
<td>4 (100%)</td>
<td>5 (41.7%)</td>
</tr>
<tr>
<td>Two substances</td>
<td>0 (0%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td>Three substances</td>
<td>0 (0%)</td>
<td>4 (33.3%)</td>
</tr>
</tbody>
</table>

White symmetrical reticular pattern of OLP was found in all subjects in the tested group.

Histopathological exam

The most important histological features in oral lichen planus include: liquefaction of the basal cell layer accompanied by apoptosis of the keratinocytes (destruction of the epithelial basal cell layer, Figures 1–4), followed by a marked layered predominantly lymphocytic infiltrate immediately underlying the epithelium (Figures 1–4); the presence of numerous eosinophilic colloid bodies, which represent degenerating keratinocytes, along the epithelial-connective tissue interface (Civatte bodies, Figures 2 and 4); absent (Figure 1), hyperplasic or, more frequently, sawtooth-shaped interpapillary ridges; variable thickness of the spinous layer; and variable degrees of orthokeratosis (Figure 1) or parakeratosis.

Figure 1 – Lichen planus: tongue. Dense band-like infiltrate predominantly of lymphocytes in the lamina propria extending to the epithelium, absence of interpapillary ridges, basement layer lichenification, presence of necrotic and enlarged keratinocytes, mild epithelial hyperparakeratosis (HE stain, 100×).

Figure 2 – Lichen planus: jugal. Presence of both orthokeratosis and parakeratosis, presence of a granular layer, artificial cleft between the epithelium and lamina propria (Max–Joseph space), pink-staining necrotic keratinocytes (Civatte bodies – arrowhead), vacuolar alteration of the basal layer, lymphocytic inflammatory infiltrate in the lamina propria (HE stain, 100×).
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Figure 3 – Lichen planus: jugal. The epithelium shows basement layer liquefaction, necrotic keratinocytes, and a layered dense band-like marked lymphocyte infiltrate immediately underlying the epithelium (HE stain, 200×).

Figure 4 – Lichen planus: jugal. The epithelium shows large keratinocytes with prominent nuclei and nucleoli, vacuolar alteration of the basal layer (arrow), a layered marked lymphocyte infiltrate immediately underlying the epithelium. Civatte body – arrowhead (HE stain, 400×).

Tissue

Median MDA levels were significantly increased in the tested group: 2.67 (0.26–3.40) vs. control group 0.44 (0.19–0.70). The simple comparison between groups has returned a value of 0.021. After age adjustment the difference was highly significant (p<0.0001) (Figure 5).

Figure 5 – Tissue MDA levels in tested group and controls.

GSH medium level was significantly decreased in patients tissue compared to controls: 2.3 (1.25–5.70) vs. 9.56 (6.5–12.5). Non-adjusted significance was p=0.005. After the age adjustment, p<0.0001 (highly significant) (Figure 6).

Figure 6 – Tissue GSH levels in tested group and controls.

Discussion

The classic histopathological aspects of oral lichen planus liquefaction of the basal cell layer accompanied by apoptosis of the keratinocytes, a predominantly lymphocytic infiltrate immediately underlying the epithelium, eosinophilic colloid bodies, sawtooth-shaped interpapillary ridges, variable thickness of the spinous layer, orthokeratosis, parakeratosis [16–20] were found in the mucosal biopsies prevailed from our subjects. Other conditions may, however, present similar histopathological findings to those of oral lichen planus. These include lichenoid reactions to dental amalgam and drugs, lupus erythematosus, leukoplakia, erythroleukoplakia, and proliferative verrucous leukoplakia [21]. In establishing the diagnosis of OLP, the histopathological exam was correlated with the clinical criteria, which is of equal importance, the presence of white bilateral reticular lesions [22].

So far, some factors have been implicated in induction of apoptosis and the presence of an interface lymphocytic infiltrate in lichen planus including p53, the BCL-2 family proteins, TNF-α, the Fas/FasL pathway, granzyme B–perforin system, proteases of the matrix metalloproteinase-9 type (MMP-9), and caspase-3 [23].

Intensive research has reported that oxidative stress is a major physiological inducer of p53 on one hand and ROS appear to be generated downstream of p53 activation where they play a role in mediating apoptosis on the other hand [24–26]. In the mean time, p53 is known to down-regulate repressors of apoptosis such as BCL-2 [27].

ROS also seem to play an important role in mediating Fas-dependent apoptosis [28] sustained by the observation that Fas-induced apoptosis was completely abolished by antioxidants such as glutathione [29].
Other studies have shown that oxidative stress is involved in both pro- and anti-apoptotic signaling in response to TNF-α [30]. It was found that oxidative stress and increased inflammatory mediators produce the release of effector molecules including granzyme that can promote local tissue damage [31].

ROS production was also observed in Jurkat system in response to granzyme B and perforin [32]. A recent study demonstrated that ROS stimulate MMP-9 secretion in human fetal membranes [33]. These factors implicated in the induction of histological alteration present in oral lichen planus are influenced by oxidative stress.

ROS’s major damage to cells is produced via lipid peroxidation (LP) with direct effects on the membrane structure, fluidity, cross-linking, and function [34]. The final product of lipid peroxidation, MDA, is considered a biomarker of oxidative stress [35]. In our study, the level of MDA was higher in patients’ oral tissue homogenate vs. healthy subjects. This point out to the involvement of oxidative stress in the pathogenesis of oral lichen planus.

Glutathione is a tripeptide present in nearly all animal cells. He is considered the most powerful non-enzymatic antioxidant with an important role in antioxidant defense, nutrient metabolism, and the regulation of pathways essential for the whole body homeostasis [8, 36, 37]. We found lower local GSH levels in tested group compared to controls. This may be due to the rate of GSHA turnover augmented as a defense mechanism against oxidative stress.

There is little evidence in evaluating the level of lipid peroxidation and antioxidant capacity in LP tissue specimens. Our results are in concordance with another study where raised levels of lipid peroxidation product MDA, increased oxidative DNA damage, increased protein oxidation, and alteration of enzymatic antioxidant defenses were found in erosive lichen planus of the vulvae tissue specimens [38].

Conclusions

OLP patients have higher concentration of MDA within the oral mucosa. This suggests a local increase of lipid peroxidation in oral lichen planus. In OLP patients’ tissue homogenate, decreased levels of GSH suggest a local antioxidant system alteration. The data obtained support the idea of oxidative stress implication in oral lichen planus. The results of our study may serve in further studies aimed at investigating the possible protective ways of oxidative stress counteraction with positive effects on the evolution of this disorder.

References


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