Salivary Peroxidase Levels in Patients with Oral Lichen Planus

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Abstract

Oral lichen planus (OLP) is a chronic mucocutaneous disease, well described clinically and histologically, but the etiology remains unknown. Various systemic and/or local predisposing factors have been implicated in the etiopathogenesis of OLP. Saliva and its constituents play an important role in the homeostasis of the oral cavity, and alterations either in its quantity or quality might contribute to various oral diseases and disturbances. In 30 patients with OLP (age range 30-62, mean 52), diagnosis was made on the basis of histopathological and clinical findings. The control group consisted of 28 participants (age range 23-27, mean 24). Each participant collected saliva using the simple method of spitting unstimulated whole saliva into calibrated tubes (0.1 ml) during a five minute period between 8-11 A.M. Salivary peroxidase levels were determined according to Putter and Becker. Statistical analysis was performed using descriptive statistics, and Student t-test p-values below 0.05 were considered statistically significant. No significant differences between patients with OLP and controls in salivary flow rate as well as in salivary peroxidase levels were found. Our results might indicate that neither salivary flow rate nor salivary peroxidase level contribute to the pathogenesis of OLP.

Keywords:
salivary peroxidase, oral lichen planus

Introduction

Oral lichen planus (OLP) is one of the most common dermatoses affecting the oral mucosa and may appear in different forms. The cause of OLP is still not clear (1). The role of salivary constituents especially salivary peroxidase in the pathogenesis and the clinical course of oral mucosal diseases have yet not been studied comprehensively. Human whole saliva contains two peroxidase enzymes that are important mucosal defense factors. Major salivary glands secrete salivary peroxidase, which is structurally somewhat different but catalytically quite similar to bovine milk lactoperoxidase (2). Oral polymorphonuclear leucocytes release myeloperoxidase into gingival crevicular fluid and whole saliva in amounts proportional to the degree of gingival inflammation (3, 4). The salivary peroxidase (SP) system is composed of the salivary peroxidase enzyme, thiocyanate (SCN), and hydrogen peroxide. SP and SCN are normal components of human saliva. The enzyme is synthesized and secreted by acinar cells, whereas SCN is concentrated in the salivary glands from serum and subsequently secreted. Hydrogen peroxide (H₂O₂) is derived from bacterial and leucocytic metabolism. In saliva SP catalyzes the oxidation of SCN to produce hypothiocyanous acid (HOSCN) and the hypothiocyanate anion (OSCN). This reaction has an important consequence for the host because the HOSCN and OSCN inhibit the growth and metabolism of many species of pathogens. In addition to its antimicrobial properties, the SP system also protects the host cells from hydrogen peroxide toxicity (5, 6). Salivary peroxidase can reversibly inhibit bacterial enzyme and transport systems by oxidation of protein sulphydryl groups; with extended incubation this effect can be made irreversible (7, 8).

The aim of our study was to evaluate salivary
peroxidase values in patients with oral lichen planus.

**Materials and Methods**

Prior to the investigation informed consent according to Helsinki II was obtained from each participant. This study included 30 patients with pathohistologically confirmed oral lichen planus, age range 30–62 (mean 52 yrs.) (25 women, 5 men), and 28 controls (age range 23–27, mean 24 yrs.) (18 women, 10 men) with clinically healthy appearance of the oral mucosa.

The unstimulated whole saliva was obtained by the simple method of the participant spitting the collected saliva into calibrated tubes (0.1 ml) for five minutes. The results were expressed per one minute. Saliva samples were centrifuged (800 turns) during ten minutes and then frozen at −20°C (9). The salivary peroxidase values were determined according to Putter and Becker (10). The reagents used for salivary analysis were 20 mM 2.2 azino-di-(3-etilbenzotiazolin-(6)-sulphonic acid) diammonium acid (ABTS) in 67 mM phosphate buffer pH value 6.0 and 10 mM hydrogen peroxide and peroxidase in quantity of 250 J/kg. The assay was based on 0.5 ml of saliva sample, which was diluted with 1.5 ml of phosphate buffer pH value 6.0. Reactive mixture consisted of 2 ml of diluted saliva sample, 0.2 ml of ABTS solution, and 0.2 ml of hydrogen peroxide, which were mixed in the reactive civette and put into spectrophotometer 405 nm wavelength and temperature of 25°C. The absorbance was read after the first and fifth minute with reagent as a blind trial. The absorbance difference (delta T = 5 min) is used for reading the activity of peroxidase in saliva from the calibrated curve. Calibrated curve is done from the basic solution of peroxidase (2500 J/ml) in 6 dilutions, which contain 5, 10, 15, 20, 30, 50 J/ml. According to the process described above the absorbance difference is measured for every sample in duplicate, and a calibrated curve was made. Statistical analysis consisted of Student t-test, and values below 0.05 were considered statistically significant.

**Results and Discussion**

Rudney (11) reported that salivary levels of lactoferrin, lysozyme, and salivary peroxidase may increase in some persons with bacterial or viral infections. Cockle and Harkness (12) observed longitudinal increase in peroxidase as a sequel to vaccination or respiratory infection. However, Goecce et al. (13) found that patients with OLP had significantly lower levels of total peroxidases and salivary perox-

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SP OLP: salivary peroxidase levels in patients with oral lichen planus.

SP CONTROLS: salivary peroxidase levels in control group.

SFR OLP: salivary flow rate in patients with oral lichen planus.

SFR CONTROLS: salivary flow rate in control group.
idases in resting whole saliva as well as other salivary components. These authors suggested that patients with OLP had a lower anti-oxidant activity and that the excess oxidative substances attacking keratinocytes might be responsible for modifying surface proteins, which, in turn, could trigger the autoimmune response. Lundstrom et al. (14) also found significantly lower salivary flow rates in patients with OLP. Gandara et al. (15) could not find OLP-related changes in flow rates or in concentrations of salivary components, such as immunoglobulins A and G, albumin, amylase, lysozyme, lactoferrin, and total proteins. Results of our study indicate that salivary peroxidase levels were not significantly increased in patients with oral lichen planus when compared to the healthy controls. Also, no significant differences in salivary flow rates between patients with OLP and controls could be found. Although patients with OLP and controls were not of the same age distribution, we might conclude that neither age nor gender differences in this study influenced salivary peroxidase levels. Our data show that salivary peroxidase levels do not have a role in the pathogenesis of OLP. Further studies are needed in order to evaluate salivary peroxidase levels in patients with different types of OLP (erosive, reticular form) and to examine in particular to the acute phase and remission period of OLP.

References