



## THE ANTIOXIDANT POTENTIAL OF SALIVA: CLINICAL SIGNIFICANCE IN ORAL DISEASES

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**Abstract.** The use of saliva as a diagnostic fluid is a relatively recent trend. Saliva is a mixed oral fluid derived from major and minor salivary glands. Oral fluid, often called the mirror of the body's health, is a perfect medium to be explored for health and disease surveillance. Saliva is considered to be the first line of defense against oxidative stress (OS), the main cause for many systemic and oral diseases. Important sources of oral free radicals and reactive oxygen species (ROS) are tobacco smoke, periodontitis and other oral diseases. Saliva is rich in antioxidants: uric acid, albumin, ascorbate and enzymes which constitute the antioxidant potential of saliva. **Aim of the study:** To test possible correlations between several salivary biomarkers and oral diseases associated with OS. **Material and methods:** We have determined the salivary antioxidant potential in patients with periodontitis (20), oral lichen planus (OLP) (20) smokers (20) versus controls (20). The following salivary biomarkers were evaluated: uric acid, albumin and total antioxidant capacity (TAC). Salivary biomarkers were performed using analysis kits on automatic analyzer. Analysis kits were provided by Biosystems Diagnostics (Spain) and by Randox (UK). **Results:** Analysis revealed statistically significant changes of the mentioned parameters in patients' saliva versus controls. **Conclusions:** Our results illustrate that OS caused a depletion of antioxidant status in the oral cavity. Uric acid, albumin and TAC are very important and promising salivary biomarkers for monitoring the oral OS. **Keywords:** saliva, oxidative stress, uric acid, albumin, total antioxidant capacity, salivary biomarkers

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### Introduction

Systemic diseases such as cancer, cardiovascular, metabolic and neurological diseases may be diagnosed using other biological fluids than blood [1]. Blood is the most commonly used fluid for laboratory diagnostic procedures which involve the analyses of the cellular and chemical constituents [2]. Saliva has been used in the past few decades as a new diagnostic fluid [1-3].

It is produced by the three major salivary glands (parotid, submandibular and sublingual) as well as by numerous minor salivary glands [3]. The salivary fluid contains water, normal proteins ( $\alpha$ -amylase, lysozyme, peroxidase, immunoglobulins IgA), post-translationally modified proteins (glycopro-

teins), peptides, lipids, minerals, and other small molecules [4,5].

Saliva contains locally produced substances and other molecules derived from the systemic circulation, such as serum products, gingival crevicular fluid (GCF), electrolytes, microorganisms and other foreign substances [6]. Even markers for hormonal, infectious, immunological and toxicological diseases can be determined in saliva. The oral cavity can be an alternative tool for monitoring the oral and systemic health [7]. Therefore, saliva is often called the mirror of the body [1,7].

The use of saliva as a diagnostic tool presents many advantages: it is easy to collect, by a non-invasive technique which can be performed at home; no special equipment is needed for collection. From children to seniors, saliva can be used as a diagnostic fluid because collection of this fluid is associated with fewer compliance problems compared with blood collection [2].

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Saliva being the first biological fluid met by external substances ingested as food, drinks, inhaled volatile cigarette smoke (CS), microorganisms, it represents the first line of defense against OS [8]. OS represents the imbalance between the production of highly reactive molecular species (ROS, reactive nitrogen species [RNS]) and antioxidant defense systems [9]. OS is implicated in various pathological conditions such as cancer, cardiovascular disease, neurological disorders (Alzheimer and Parkinson's diseases), rheumatoid arthritis, diabetes, ischemia/reperfusion, ageing [10].

Antioxidants represent one of the defense mechanisms against OS which are present in all body fluids and tissues [9]. Enzymatic antioxidant defenses include superoxide dismutase, glutathione peroxidase and catalase. Non-enzymatic antioxidants are represented by ascorbic acid,  $\alpha$ -tocopherol, flavonoids and  $\beta$ -carotene [9,10].

Uric acid, non-protein thiols, glutathione also act as antioxidants [9], as well as albumin which can be found in plasma and saliva [11,12]. Ascorbic acid is the major aqueous antioxidant, while  $\alpha$ -tocopherol protects against lipid peroxidation [13].

Saliva is also rich in antioxidants (AO). Uric acid, albumin, ascorbic acid, glutathione and antioxidant enzymes are present in saliva. Uric acid appears to be the dominant antioxidant present in saliva. The concentrations of albumin and ascorbic acid are lower than those of serum. This may indicate an active secretion system for salivary antioxidants rather than passive diffusion from the circulation [11].

TAC includes all salivary antioxidants and presents clinical significance in the evaluation of the antioxidant status of saliva under normal and pathological situations.

Stimulated saliva contains a lower concentration of antioxidants but when flow rates are taken into account, the antioxidant capacity is higher than in unstimulated saliva [11].

OS is implicated now in the pathology of several oral diseases such as periodontitis, OLP and oral cancer [14-21].

Periodontal disease, including both gingivitis and periodontitis, is among the most widespread chronic conditions affecting populations worldwide [22]. Periodontal disease is a chronic, tissue destructive inflammatory state, induced by gram-negative bacteria that colonize the gingival crevice [23,24]. Several studies emphasize implication of polymorphonuclear leukocytes (PMN) as the primary mediators of host response against the pathogens [25]. PMN produce a range of antimicrobial factors, such as ROS during phagocytosis.

ROS have been reviewed to be implicated in the pathogenesis of periodontitis [26]. It has been

suggested that PMN produce and release a big quantity of ROS, culminating in increased oxidative damage to gingival tissue, periodontal ligament and alveolar bone [27].

CS represents an important source of free radicals [20]. It is a complex mixture which includes compounds such as: nicotine, phenols, acetaldehyde, nitric oxide, and cadmium. Many of these can be oxidants or pro-oxidants [19,28].

Two different populations of free radicals have been identified in CS, one in the tar and the other in the gas phase [29].

OLP is a relatively common muco-cutaneous disease of unknown etiology [30]. It can affect about 0.5-2% of the world population, more often females at the mean age of onset in the fourth decade, although younger adults and children may be affected, too. In the complex etiopathogenesis of this disease, the cell-mediated immune dysfunction is believed to be involved [31].

Oral lesions of lichen planus appear in three forms. The reticular form consists of white, papular and linear lesions arranged in a network. It appears most commonly on the posterior buccal mucosa and lateral borders of the tongue. The erosive form produces large, irregular, ulcerated areas, most commonly on the tongue and buccal mucosa. Reticular lesions are often present at the periphery of the ulcerated areas. While reticular and plaque lesions are usually asymptomatic, erosive lesions are painful and require treatment [32].

OLP has been clinically associated with development to oral cancer [33]. There are a number of studies of OLP with regards to malignant transformation in the last few decades [34]. The World Health Organization has described OLP as a precancerous condition, i.e. "a generalized state associated with a significant increased risk of cancer" [35].

There are no universally accepted specific diagnostic criteria for OLP, this representing a problem in studying the malignant potential of OLP [36].

The aim of our study was to test possible correlations between several salivary biomarkers and oral diseases associated with OS.

## Material and methods

### Patient selection

We included in our study 20 patients with periodontitis (never-smokers), 20 patients with OLP (never-smokes), 20 smokers versus 20 controls (age 20-78 years).

Upon clinical examination, the control and the smoker groups did not present periodontal disease, gingival inflammation or OLP.

The patient smokers had smoked only for five years.

- number of cigarettes per day ranged from 1 to 10 cigarettes
- time elapsed until the first cigarette was in most patients after 60 minutes

All subjects included in this study had no history of systemic diseases, had not received periodontal therapy, had not used antibiotics, anti-inflammatories for at least 6 months.

Periodontal status was assessed by measuring three indicators of periodontal status: plaque index, gingival index (presence or absence of gingival bleeding), periodontal pockets.

The diagnosis of OLP was based upon clinical manifestation: plaque and/or reticular lesions alone or in association with erosive/ulcerative lesions, confirmed by incision biopsy. The study was approved by the local Ethical Committee and all volunteers gave their written informed consent to take part in this investigation.

### Saliva collection

We collected unstimulated whole expectorated saliva from each subject, into sterile tubes, between 9 and 10 a.m. after a single mouth rinse with 15.0 ml of distilled water to wash out exfoliated cells.

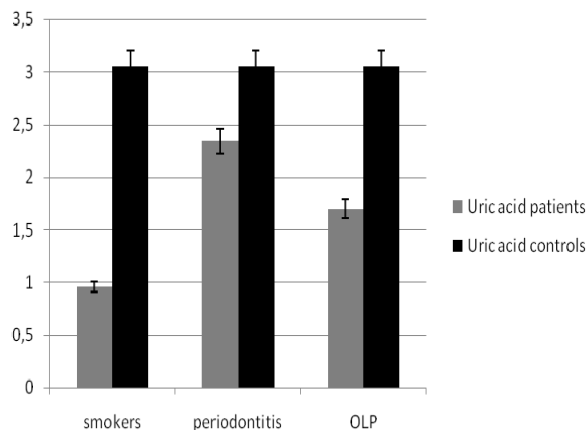
We placed collected samples on ice immediately, we then centrifuged them at 3.000 rpm and the supernatant was used for analysis of: uric acid, albumin and total antioxidant capacity (TAC). Analyses were performed using analysis kits on automatic biochemistry analyzer. Analysis kits were provided by Biosystems Diagnostics (Spain) and by Randox (UK). The principle of the method for salivary uric acid and albumin is the colorimetric method, using analysis kits on automatic analyzer EOS BRAVO–Hospitex Diagnostics.

TAC in saliva was measured by the 2,2'-azinobis (3- ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS<sup>•+</sup>) assay adapted to a flow injection (FI) system [37]. The method is based on the ability of antioxidant molecules to quench the long-lived ABTS<sup>•+</sup>, a blue – green chromophore with characteristic absorption at 734 nm, in comparison to that of Trolox, a water-soluble vitamin E analogue. The addition of antioxidants to the preformed radical cation reduces it to ABTS<sup>•+</sup> determining a colour change.

Two-tailed t-test was used for statistical comparisons.

### Results

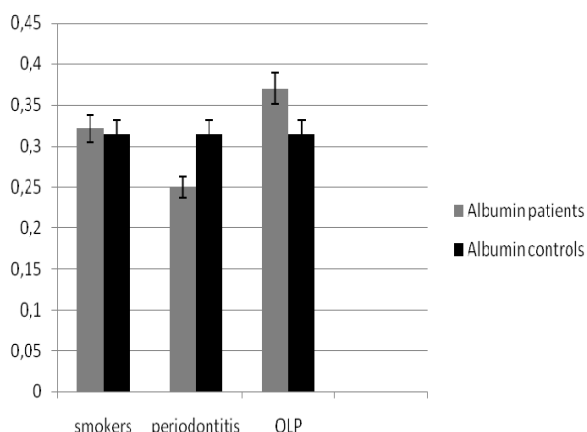
Uric acid, the major salivary antioxidant responsible for 85% of the total antioxidant capacity, was significantly decreased in smokers, OLP and periodontitis patients group, as compared with controls (Figure 1).



**Figure 1.** Salivary uric acid levels in smokers, periodontitis, OLP patients, versus controls, mg/dL (p<0.05)

In our study, we did not observe significant differences between salivary albumin values of smokers patients when compared with the control group (Figure 2).

In the case of periodontitis patients, salivary albumin was lower compared with controls. Salivary albumin from OLP patients showed a little increase versus controls (Figure 2).



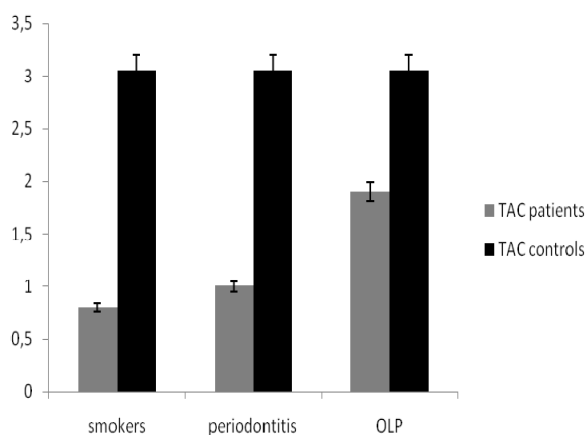
**Figure 2.** Salivary albumin levels in smokers, periodontitis, OLP patients, versus controls, g/dL (p<0.005)

TAC was significantly lower in periodontitis, smokers and OLP patients groups, versus controls (Figure 3).

### Discussion

Increased generation of ROS may cause toxic effects by oxidative damage of proteins, lipids and DNA. Oxidative damage of these biomolecules contributes to disease development [18].

The decrease of salivary uric acid levels in all three groups of patients versus controls illustrated the presence of oral OS, caused in the case of



**Figure 3.** Salivary TAC levels in smokers, periodontitis, OLP patients, versus controls, Trolox equivalent mM, ( $p < 0,005$ ).

smokers by CS free radicals from the tar and gas phase. Lower salivary uric acid levels in OLP and periodontitis patients versus controls can reflect increased oxygen radical activity during periodontal and OLP diseases progression.

Uric acid is an important salivary biomarker with clinical importance in monitoring the OS [38].

Albumin is a protein with antioxidant properties [39] and in our study this molecule was not sensitive for smokers, but was a good marker for periodontitis and OLP patients. The little increase of albumin in the case of OLP patients may represent a compensatory antioxidant defense system to counteract OS, because the level of the principal antioxidant, uric acid, is very low [39]. Lower salivary levels of albumin in the case of periodontitis patients reflect the presence of ROS in the progression of this disease. Uric acid and albumin appear to contribute to most of the antioxidant protection in whole saliva and albumin is considered a sacrifice antioxidant [12].

Salivary TAC activities were significantly lower in periodontitis, OLP and smokers patients compared with controls, this representing a significant oxidative process that occurs in the oral cavity.

The decrease in the levels of these important salivary antioxidants can be considered an important mechanism by which toxic effects of free radicals can initiate precancerous transformations, oral diseases and destroy the oral cavity homeostasis.

Investigating the role of OS in periodontitis, OLP, will not only be useful in clarifying the pathogenic mechanisms of this relationship, but will also focus on antioxidant therapeutic approaches and in the treatment of these oral diseases [15, 40, 41].

## Conclusions

The results of this study suggest that a significant OS occurs in the case of patients with OLP,

periodontitis and smokers. Uric acid, albumin and TAC are very important and promising salivary biomarkers for monitoring the oral OS. OS can be considered not only the cause of many oral diseases but also the biochemical link between systemic and oral diseases.

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