Assessment of serum and salivary ceruloplasmin level in patients with oral lichen planus

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ABSTARCT

Background: Oxidative stress is a deleterious process that can be an important mediator of damage to cell structures and consequently various disease states. Exposure to free radicals from a variety of sources has led organisms to produce a series of defense mechanisms. The antioxidant ceruloplasmin is a copper-containing ferroxidase that can oxidize ferrous iron (Fe^{2+}) to its nontoxic ferric (Fe^{3+}) form. Ferrous iron (Fe^{2+}) is extremely damaging because of its ability to generate toxic free radicals. Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease of unknown etiology. Previous studies reported that reactive oxygen species may be involved in the pathogenesis of lichen planus. The aim of this study was to estimate the role of oxidative stress in pathogenesis of OLP through the study of serum and saliva ceruloplasmin as a marker of antioxidant status.

Methods: Forty eight patients with histologically confirmed OLP by oral pathologist were included in this study. The sample group was split up in to two groups according to the clinical presentation of the lesions, 21 patients with reticular formation and 27 patients with erosive form together with 32 healthy looking volunteers that were age-matched with the patients. Serum and saliva ceruloplasmin activity was determined by oxidation of P-Phenylenediamine to give a blue - violet color that measured spectrophotometricaly at 525 NM.

Results: Statistically, there was a substantial increase in serum and saliva ceruloplasmin levels of OLP patients group as compared to controls ($p<0.01$) and there was no statistically significant differences in serum and saliva ceruloplasmin when compared between reticular and erosive forms ($p>0.05$). The study showed that there was no statistically significant correlation between serum and saliva ceruloplasmin levels in OLP patients group ($r=-0.029$, $p>0.05$).

Conclusion: Oxidative status play a role in the pathogenesis of oral lichen planus represented by increased serum and saliva ceruloplasmin levels.

Keywords: Oral lichen planus, Ceruloplasmin, Serum, Saliva. (J Bagh Coll Dentistry 2014; 26(3):53-57).

INTRODUCTION

Reactive oxygen species (ROS) are highly reactive molecules and can damage cell structures. The shift in the balance between oxidants and antioxidants is termed oxidative stress. Aerobic organisms have integrated antioxidant systems, which include enzymatic non enzymatic antioxidants that are usually effective in blocking harmful effects of ROS. However, the antioxidant systems can be overwhelmed [1] which may play a key role in the onset and development of several inflammatory oral pathologies [2].

The potent antioxidant activity of normal human plasma has been shown to be chiefly dependent upon the copper-containing protein Ceruloplasmin (CP) and the iron-binding protein transferrin [3,4].

Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease of unknown etiology [5]. Basically there are two categories of oral lesions; reticular and erosive [6]. The atrophic, ulcerative, and bulbous forms of the disease are referred to as erosive lichen planus [7].

Reticular pattern is the most frequent clinical presentation and appears in the form of a network of connections and overlapping white lines [8] combined with a few symptoms and reflecting a milder stage of the disease [9,10]. Erosive/ulcerative OLP constitute the most destructive pattern and causes a great oral discomfort [8,10].

The purpose of this study is to assess the antioxidant status and its role in the pathogenesis of OLP through the study of serum and saliva CP levels.

MATERIALS AND METHODS

Forty eight patients with histologically confirmed OLP were included in this study. A diagnosis of oral lichen planus was made based on clinicopathologic correlation according to the modified WHO diagnostic criteria for OLP [11]. The sample group was split up in two groups according to the clinical presentation of the lesions, 21 patients with reticular formation and 27 patients with erosive form together with 32 healthy looking volunteers that were age-matched with the patients.

Serum and unstimulated whole saliva were collected from each subject then the supernatant serum and saliva was obtained by centrifugation at 3000 RPM for 10 minutes then aspirated and

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transferred immediately into Eppendorf tubes and frozen at -20 °C for subsequent analysis.

Ceruloplasmin activity was determined by oxidation of P-Phenylenediamine to give a blue-violet color that measured spectrophotometricaly at 525 nm [12].

RESULTS

The mean age of the patient group was 50.96 ±10.55 with female predilection 52.1%. The present study revealed that the mean of serum CP in patients with OLP (0.408±0.101 g/l) was significantly higher ($p<0.001$) by using t-test than that of control group (0.311±0.105 g/l). (Table 1) (Figure 1)

The mean of saliva CP in patients with OLP (0.014±0.009 g/l) was significantly higher ($p<0.01$) by using t-test than that of control group (0.009±0.009 g/l). (Table 2) (Figure 1)

Table 1: Mean of serum ceruloplasmin in OLP patients and controls

Serum Ceruloplasmin (g/l)	Patients	Controls
No	48	32
Mean±SD	0.408±0.101	0.311±0.105
Standard Error of Mean	0.015	0.018
Mode	0.299	0.224
Range	0.274-0.710	0.188-0.619
Percentile 05th	0.299	0.202
25th	0.338	0.240
50th (Median)	0.393	0.292
75th	0.448	0.346
95th	0.613	0.618
99th	0.710	.619
P value	0.0001*	

*** Highly significant using Students-t-test for difference between two independent means at 0.01 level**

Table 2: Mean of saliva ceruloplasmin in OLP patients and controls

Saliva Ceruloplasmin (g/l)	Patients	Controls
Mean±SD	0.014±0.009	0.009±0.009
Standard Error of Mean	0.001	0.002
Mode	0.013	0.003
Range	0.003-0.056	0.002-0.035
Percentile 05th	0.004	0.003
25th	0.008	0.003
50th (Median)	0.013	0.006
75th	0.018	0.010
95th	0.034	0.034
99th	0.056	0.035
P value	0.01*	

*** Highly significant using Students-t-test for difference between two independent means at 0.01 level**

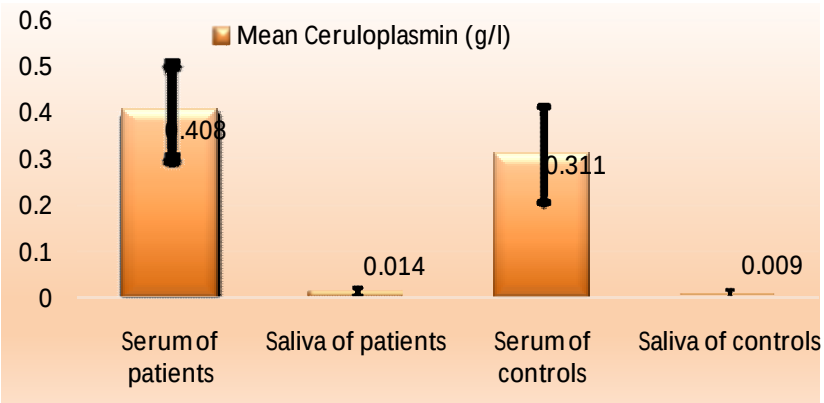

Figure 1: Mean of ceruloplasmin conc. in serum and saliva of OLP patients and control groups

The present study showed that there was no statistically significant difference in serum and saliva CP between patients with reticular and patients with erosive form of OLP. (Table 3).

This study showed that there was no statistically significant correlation (r =-0.029, p>0.05) between serum and saliva measurements of CP in patients with OLP. (Figure 2)

Table 3: Comparison of serum and saliva Ceruloplasmin levels between reticular and erosive forms of OLP patients group

	Type		
	Reticular	**Erosive**	**P value**
Ceruloplasmin (g/l) Serum	0.418±0.130	0.400±0.072	0.549
Saliva	0.013±0.007	0.015±0.011	0.394

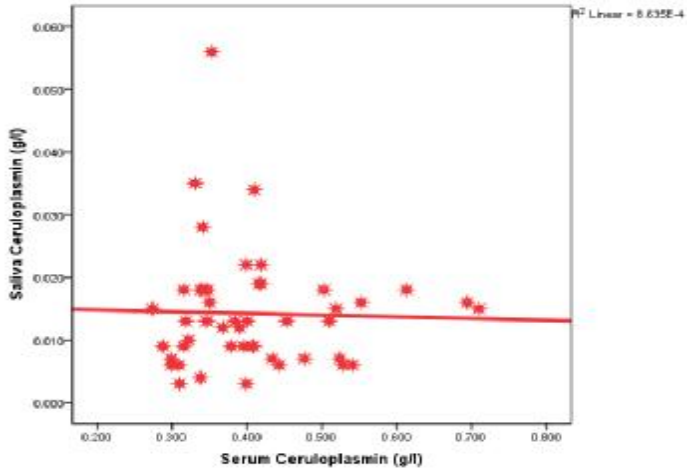

Figure 2: Correlation between serum and saliva ceruloplasmin in OLP patients group

DISCUSSION

The exact etiology of OLP is unknown. Cell mediated immune deregulation has been associated with the pathogenesis of this condition [13]. Anshumalee et al in 2007 reported that oxidative stress may play a role in OLP [14].

Reactive oxygen species (ROS), superoxide anions, hydroxyl radical, hydrogen peroxide and nitric oxide, are highly reactive, diffusible molecules [15]. Cells generate ROS intracellularly and may release them extracellularly [16] which may damage surrounding tissues and promote inflammatory processes [17].

Metals such as iron and copper are capable of redox cycling in which a single electron may be accepted or donated by these metals. This action catalyzes reactions that produce reactive radicals [18].

Iron is essential for a variety of cellular functions, but its levels and bioavailability must be tightly regulated because of its toxic redox activity. The multi-copper ferroxidase CP converts toxic ferrous iron (Fe^{2+}) to its nontoxic ferric form (Fe^{3+}) and is required for iron efflux from cells [19].

Multiple mechanisms have been proposed to explain ceruloplasmin antioxidant activity, including scavenging of superoxide and other reactive oxygen species [20], and inhibiting the Fenton reaction by conversion of Fe^{2+} to Fe^{3+} (CP is also called "ferroxidase") [21,22]. The latter mechanism is backed by a considerable body of evidence, but the ability of CP to block Cu^{2+}-mediated lipid oxidation suggests that alternate antioxidant mechanisms must also pertain [23]. There is evidence that CP as an antioxidant blocks protein [24] and DNA damage [25], and that it gives protection against free radical-initiated cell injury and loss [26].

The source of circulating CP has been almost exclusively assigned to CP secreted by hepatocytes [27]. Human monocytic cells have also been shown to produce and secrete their own CP on activation [28].

Human peripheral blood lymphocytes express the transcripts for both CP molecular isoforms. During infection and inflammation characterized by active proliferation of circulating lymphocytes, CP concentration in serum increases, suggesting that the expression of the CP gene represents an

essential part of host response to immunological stress [29].

The significant increase of serum CP level in this study may represent a compensatory antioxidant defense system to counteract oxidative stress.

Antioxidants are present in all body fluids including saliva. Saliva may constitute a first line of defense against oxidative stress and has protective effects against microorganisms, toxins and oxidants [2,30].

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 [31].

In spite of this, levels of certain markers in saliva are not always a reliable reflection of the levels of these markers in serum. The transfer of serum components which are not part of the normal salivary constituents into saliva is related to the physicochemical characteristics of these molecules. Salivary composition can be influenced by the method of collection and the degree of stimulation of salivary flow. Furthermore, salivary proteolytic enzymes can affect the stability of certain diagnostic markers [32]. Blood still remains the best body fluid for evaluation of many biomarkers reflecting systemic processes and substitution should be used with caution [31].

The facts mentioned may reflect what the present study showed that there was no significant correlation between serum and saliva CP levels in OLP patients group.

Therefore, saliva is not always a reliable indicator of the internal environment of the body. On the extent of our knowledge, there were no previous studies dealing with CP in both serum and saliva of OLP patients.

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