Scandinavian Journal of Clinical & Laboratory Investigation, 2010; 70: 225–228

informa
healthcare

ORIGINAL ARTICLE

Oxidative stress and antioxidant defense in oral lichen planus and oral lichenoid reaction

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Abstract

Background. Oral Lichen Planus (OLP) is an inflammatory disease of unknown etiology while Oral Lichenoid Reaction (OLR) is a condition mimicking OLP. As these conditions are exposed to oxidative stress, they could release reactive oxygen species (ROS) which are implicated in the pathogenesis of a plethora of inflammatory conditions to lethal diseases. We evaluated and compared the levels of a series of oxidative stress markers in patients with OLP and OLR with that of normal controls and tried to identify the role of these oxidative stress markers in these conditions. ***Methods.*** Protein thiol oxidation, malondialdehyde (MDA) and total antioxidant activity were estimated in both the groups (OLP and OLR) and compared with that of normal subjects. ***Results.*** There were significantly lower levels of serum protein thiols in OLP ($p < 0.005$) while in patients with OLR the difference was not statistically significant ($p < 0.489$) when compared with controls. Serum MDA levels were significantly higher in OLP ($p < 0.001$) and OLR ($p < 0.001$) than in controls. However, there was no significant difference in serum MDA levels between OLP and OLR patients ($p>0.05$), but with a significant difference in serum thiol levels between the two ($p < 0.047$). Total antioxidant levels were lower in OLP ($p < 0.016$) and OLR ($p < 0.017$) when compared to normal subjects, while between the study group total antioxidant levels were not significantly different ($p < 0.632$). ***Conclusions.*** The findings from the present study demonstrate involvement of ROS in the pathogenesis of OLP and OLR, though both these disease conditions have a different clinical course.

Key Words: *Inflammatory conditions, malondialdehyde, protein thiol, reactive oxygen species, total antioxidant activity*

Introduction

Lichen planus is a chronic inflammatory mucocutaneous condition which most commonly affects the skin, genitalia and oral mucous membrane and has a varied presentation [1]. Oral Lichen Planus (OLP) affects 0.1% to about 4% of the population and is a disease of middle age being more common in women. Though the etiology is unknown, it is likely that both endogenous (genetic) and exogenous (environmental) components interact to elicit the disease [2]. However, a delayed hypersensitivity immune reaction to an altered keratinocyte antigen expression and function, where in release of various cytokines by activated T cells leading to the attraction of keratinocyte by cell-mediated cytokines has been implicated in its pathogenesis [3]. Oral Lichenoid Reaction (OLR) may represent reactions triggered by drugs or dental materials. The majority of them are seen in direct topographic relation to dental restorative materials while those associated with drug intake (oral hypoglycemic agents) could represent oral/cutaneous lesions [1].

It has been proved that reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an important role in inflammation-mediated carcinogenesis. ROS can generate 8-oxo-7, 8-dihydro-2'deoxyguanosine (8-oxodG), an indicator of oxidative damage whereas RNS can mediate the formation of 8-nitroguanine, a marker of nitrative DNA damage [4]. A raised level of ROS and RNS has been implicated in pathogenesis of various skin diseases such as atopic dermatitis [5], psoriasis [6], vitiligo [7], diabetes mellitus [8] and rheumatoid arthritis [9]. OLP seems to be a major concern, as it is considered to be a premalignant lesion with transformation into a squamous cell carcinoma, though there is much controversy regarding this issue [10].

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(Received 15 September 2009; accepted 30 December 2009)

ISSN 0036-5513 print/ISSN 1502-7686 online
DOI: 10.3109/00365511003602455

The present study was carried out to assess and evaluate the role of oxidative stress and antioxidant defense systems in patients with OLP and OLR by measuring serum malanodialdehyde, protein thiol and total antioxidant activity levels and comparing them with normal subjects (controls). To the best of our knowledge this seems to be the first study on OLR.

Subjects and methods

The study protocol and procedure were approved by the ethical committee of a tertiary referral University hospital and informed consent was obtained from patients after explaining the aim and objectives of the study.

Subjects (patients and controls)

The study was carried out on 47 subjects (32 patients and 15 healthy volunteers). Of the 32 patients (20 males and 12 females), 22 were diagnosed as having OLP and 10 OLR. In the OLP group, 17 (77.2%) of the 22 were diagnosed to have only OLP and the remaining 5 (22.7%) had combined lesions (oral and skin). The age of presentation of OLP ranged from 19–68 years with a median age of 47 years. In the OLR group, the age ranged from 25–48 years with a median age of 40 years (Table I). The inclusion criteria required a diagnosis by a biopsy of active and untreated OLP and OLR with onset of the symptoms of 6 weeks duration. The exclusion criteria included the ingestion of any oral medication such as immunosuppressive agents, non steroidal anti inflammatory drugs, history of trauma, surgery, alcohol ingestion and the presence of a systemic disease. The control group comprised of 15 healthy volunteers, 8 males and 7 females with ages ranging from 23–58 years with a median age of 43 years (Table I).

Methods

Venous blood samples (5 ml) were obtained from patients with OLP, OLR and healthy controls after 12 hours of fasting and were allowed to clot for 30 min and then centrifuged at 2000 *g* for 15 min for clear separation of the serum. Samples of these groups were stored and kept frozen at −40°C until analysis.

Table I. Mean age distribution between the groups.

Cases	Number	Age (median, years)	Range (years)
Controls	15	43.00	23–58
OLP	22	47.00	19–68
OLR	10	40.00	25–48

Biochemical determinants

All chemicals were purchased from Sigma Chemical Co (St. Louis, MO, USA).

Measurement of serum protein thiol

Serum protein thiol levels were measured by a spectrophotometric method using 5′5′ dithiobis (2–nitrobenzoic acid, DTNB) [11]; 900 µL of 0.2 M Na_2HPO_4 containing 2mM Na_2 EDTA, 100 µL serum and 20 µL of 10mM DTNB in 0.2 M Na_2HPO_4 were taken in an Eppendorf tube and warmed to 37°C. The solution was mixed in a vortex mixer and transferred to a cuvette, and the absorbance was measured at the end of 5 min at 412 nm in a Genesys 10 UV spectrophotometer. Both sample and reagent blanks were prepared and absorbances were noted at 412 nm. The absorbance values of sample and reagent blanks were subtracted from serum absorbance values to obtain the correct values. The calibration curve was produced using gluthione dissolved in phosphate buffered saline (PBS). The protein thiol concentration in serum was determined from the standard curve using the corrected absorbance values for serum. The protein thiol groups in plasma were calculated from total thiol levels.

Measurement of serum malondialdehyde levels

The serum MDA levels were determined by a method based on reaction with thiobarbituric acid (TBA) at 90–100°C [12], in which MDA and TBA react together under acidic conditions to produce a pink pigment having an absorption maximum at 532 nm. The results were given as MDA (µmol/L) and tetramethoxypropane was used as an external standard.

Estimation of antioxidative activity (AOA)

The method advocated by Koracevic et al. [13] was used in the estimation of total AOA. A standardized solution of Fe-EDTA complex reacts with H_2O_2 by a Fenton type reaction, leading to the formation of hydroxyl radicals (OH). These ROS degrade benzoate resulting in the release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction was measured spectrophotometrically and the inhibition of color development was defined as the AOA.

Statistical analysis

One way analysis of variance (ANOVA) test, followed by Student's *t*-test was applied to determine the statistical significance of serum protein thiol levels, MDA and total antioxidant levels in both patient and

control groups. A statistical p-value of less than 0.05 was considered to be significant.

Results

In the present study we found that serum MDA levels were high in patients with OLP ($p < 0.001$) and patients with OLR ($p < 0.001$, Table II) when compared with the normal control group. There was no significant difference between the patient groups ($p > 0.05$, Table II). Similar to the examination of serum protein oxidation, protein thiol levels were measured and were significantly lower in OLP patients than in controls ($p < 0.005$) and when compared between OLR patients and controls, they were non-significant ($p < 0.489$). Also, between the patient groups there was a significant difference in serum thiol levels ($p < 0.047$, Table II). The serum total antioxidant levels were significantly lower in OLP patients ($p < 0.016$) than in those of OLR patients ($p < 0.017$) and when compared to controls, there was no significant difference between the patient groups ($p < 0.632$, Table II).

Discussion

It has been evident from recent studies that oxidative stress plays an important role in the pathogenesis of several inflammatory and autoimmune diseases [5–9]. ROS (superoxides, hydroxyl radicals) can cause damage to the cellular components via protein peroxidation of nucleic acids, free amino acids and lipoproteins. These radicals can also induce gene mutation and post transitional modification of cancer-related proteins, which in turn are said to disrupt cellular processes such as DNA repair and apoptosis [14,15]. It has been found that ROS produced by keratinocytes, fibroblasts and various inflammatory cells could result in disequilibrium between the pro-oxidants and antioxidants [16]. One of the biomarkers which indicate the levels of oxidative stress is serum MDA levels, which are produced as a result of oxidation of membrane-associated polyunsaturated fatty acids of phospholipids. This study showed a higher level of serum MDA in patients with OLP and OLR when compared to controls. These results were in accordance to studies by Sander et al. [17,18] who indicated that increase in the production of ROS will result in increase in lipid peroxidation, which in turn can cause profound alteration in the function of cell membrane and structural organization of DNA leading to mutation. Since OLP and OLR are chronic inflammatory diseases, during respiratory bursts, inflammatory cells are known to release a large quantity of ROS that are injurious to the surrounding tissue [19]. Proteins are expected to be major targets for oxidative damage since they are major components of most tissues, cells and plasma and exhibit rapid rates of reaction with many oxidants [20]. Oxidized proteins are known to cause major physiologic perturbation including loss of structure or function [21]. The long-lived nature and slow rates of removal of many oxidized proteins [22,23] may make these materials valuable quantitative markers of oxidative stress.

Our observation on total thiol levels showed lower levels in OLP and OLR when compared to controls. These findings were in line with that of Prakash et al. [24]. It has been found that plasma thiols are the major oxidative target and that albumin via its thiol group provides the major antioxidant protection against hypochlorous acid in human plasma [15]. Further it has been found that proteins with a substantial conformational loss are a potential source of 'neo antigen' and these factors exacerbate immune reaction leading to autoimmunity. However our study has been contradictory to that of Baskol et al. [25] and Kiziltunc et al. [26] who found significantly higher levels of total serum thiol levels. This could be in response to the continued production of the ROS, which needs thiols for detoxification and this may be true in the case of OLR which could reflect that the unionized total serum thiol levels in these patients may have the ability to protect against oxidative stress. Cells also use various other mechanisms to modulate their intra as well as extracellular levels of ROS, so as to compensate the oxidative injury of which enzymatic as well as non-enzymatic defense mechanism play an important role.

Alteration in these antioxidant enzymes have been observed in many conditions such as aging, photo aging and carcinogenesis [26,27]. In our study we observed the total antioxidant activity in OLP and OLR to be low and these observations were in line with those of Chapple et al. [28]. Similar findings were observed in cervical cancer [29] and skin diseases such as atopic dermatitis [5] suggesting that the free radicals generated may cause structural and functional damage to antioxidant enzymes, and impair the efficiency of these enzymes, thus alteration in the antioxidant system is believed to play a

Table II. Comparison of MDA, protein thiol and serum total antioxidant levels in patients with OLP, OLR and Controls.

	MDA (μmol/L)	Thiol levels (μmol/L)	Total antioxidant activity levels (mmol/L)
OLP	0.7595 ± 0.536^1	378.26 ± 1.50^1	1.054 ± 0.3013^1
OLR	0.4890 ± 0.216^1	380.56 ± 70.62^2	1.019 ± 0.2435^1
Controls	0.2187 ± 0.054	472.13 ± 54.27	2.037 ± 0.1382

Student t-test: $^1p < 0.05$ vs control, $^2p > 0.05$ vs control.

key role in carcinogenesis. Though our study is with the view that oxidative stress could play an important role in the disease process of OLP and OLR, the fact remains that both these conditions have different clinical courses. Larger sample size and other diagnostic modalities in combination may offer a better understanding of the disease process.

Source of funding: Routine patient diagnostics, no extra funds.

Declaration of interest: None.

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