

Salivary Endothelin-1 Level in Patients with Oral Lichen Planus

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Abstract:

Background: Oral Lichen Planus (OLP) is “a chronic disorder commonly occurs in elderly people usually women with or without skin involvement”. Endothelin-1 (ET-1) is a potent vasoconstrictor implicated in vascular biology and tumor carcinogenesis. The current study investigates the level of salivary ET-1 in OLP patients and a control group.

Patients and Methods: saliva samples were collected from 32 OLP patients and 30 apparently healthy subjects. ET-1 levels were measured by ELISA, and the results were evaluated with a Mann-Whitney U Test for statistical analysis.

Results: the mean level of ET-1 in patients with OLP estimated 1.15 ng/ml versus 0.47 ng/ml in control group ($p < 0.001$); moreover, the highest mean ET-1 level was found in the erosive type (1.67 ng/ml) of OLP.

Conclusion: salivary ET-1 levels may be a useful diagnostic tool in OLP to indicate cases with a poor prognosis.

Keywords: Endothelin-1; ELISA; Oral Lichen Planus; Saliva.

I. Introduction

Oral lichen planus (OLP) is —a disease of stratified squamous epithelia with unknown etiology [1]. OLP is a relatively common, chronic, immuno-inflammatory, and potentially premalignant condition which is a rather common disease in the middle-aged and elderly populations [2]. Clinically, several subtypes have been characterized, but the following six clinical types of OLP lesions are the most prevalent either individually or mixed: atrophic, bullous, erosive, papular, plaque-like, and reticular [3]. The prevalence rate of OLP —varies from 0.5% to 4% of the general population, and the malignant transformation rate is (0–2%)” [4]. It tends to be more chronic in nature than the cutaneous disease, affecting women more commonly than men, with age of onset on average around 60 years; meanwhile, it is rare in childhood [5]. —The relative risk in people with mixed oral habits and in non-smokers is low (0.3%), but higher (13.7%) in those smoke or chew tobacco [6]. World Health Organization (WHO) in their Global Oral Health Program designated —OLP as a premalignant condition with undefined malignant transformation risk and offer that OLP patients should be under continuous checking” [7]. OLP has been suggested as an ideal pattern of inflammation that gives rise to tumors [8]. Endothelin-1 (ET-1) is a powerful vasoconstrictor that belong to the endothelin family and is involved in diverse pathological cases such as inflammation, wound healing, and carcinogenesis [9, 10, 11]. The contribution of ET -1 to tumor growth and progression has been evaluated in —prostatic, ovarian, renal, pulmonary, colorectal, cervical, breast, lung, bladder, endometrial cancer and oral squamous cell carcinoma (OSCC) [12, 13, 14, 15]. Keratinocytes have the ability to —translate and excrete —ET-1 protein into keratinocyte certain media, and that oral epithelial cells can produce ET-1 [16]. Increased level of ET-1 mRNA and ET-1 protein have been previously reported in tissue affected by OSCC [17]. Salivary ET-1 is —a useful biomarker for OLP patients towards bad prognosis and those who have a previous history with OSCC [18, 19]. Therefore, this study investigated the level of salivary ET-1 in OLP patients and its correlation to different clinical types of OLP.

II. Patients And Methods

2.1. Study groups:

This is a case-control study. The protocol was certified by the Commission Board of Ethical Standards in the College of Medicine, Al-Nahrain University. Informed consent was taken from each patient before samples collections, which agrees with the terms of Ethical Considerations of the Iraqi Ministry of Health. All patients and apparently healthy donors were enrolled from June 2014 to February 2015. Thirty-two patients (32) were diagnosed by a specialist physician at the Dermatological Outpatient Clinic, Medical City Hospital, Baghdad-Iraq. The exclusion criteria were systemic diseases such as —hepatitis C, lupus erythematosus or Sjögren’s syndrome and bone marrow transplants as well as those who used —any corticosteroids or immunosuppressant a week before sample collection at a minimum. Thirty (30) apparently healthy volunteers who were age and gender match with the study group enrolled as control group.

2.2. Sample Collection

Un-stimulated saliva was collected from the patients and donors as mentioned by a previously published protocol [20, 21]. The patients were instructed to drool saliva into a 50 ml Falcon tube kept on ice. Nearly 5 ml were collected within 15 minutes.

2.3. Sample processing:

The Samples were processed immediately after collection according to a previous mentioned protocol [22]. Then 0.2 μL/ml of proteinase inhibitor cocktail (Promega/ USA) was added to the supernatant to block —protein degradation; then kept at -80 °C until next step.

2.4. Endothelin-1 level estimation

An Enzyme-Linked ImmunoSorbent Assay kit (ELISA) (MyBiosource, USA, Cat No: MBS726557) was used to estimate ET-1 levels according to the manufacturer’s guides. A humanreaders® HS —microplate reader (Human Diagnostic, Wiesbaden, Germany, Cat No. 16670) was used to quantify the —optical density at 450 nml. The concentration of ET-1 in each sample was estimated based on the standard curve.

2.5. Statistical analysis:

“The statistical package for social sciences (SPSS) for Windows, version 21.0 and Microsoft Excel 2013 were used to analyze the results. Categorical data were expressed as count and percentage. A Chi-square test used to characterizes the association of these data. The “frequency, mean and standard deviation were used to characterize the study variables in each group. A Mann-Whitney U test was used to define if whether —a significant difference in the levels of ET-1 between the study and the control group was estimated. Statistical significance was defined as values equal to or below 0.05

III.Results

The patients mean age was 48.16±13.56 years, while there were females predominance over males it comes with no significant differences statistically in gender between groups (p>0.05). Twenty OLP patients were females (62.5%); only 12 patients were males (37.5%). Thirteen OLP patients were smokers (41%), there were four smokers in the control group (13%) with a significant difference between the groups (p =0.016). Reticular OLP was the most common type (44%), followed by the erosive type with (34%), and finally plaque-like type (22%), (P > 0.05) as in (Table 1).

Table 1: Demographic data and clinical types of oral lichen planus patients and control group .

Characters	OLP	Healthy subjects	P value
Age groups (years)	(20-30)	4 (12.5%)	0.102 ^{NS}
	(31-40)	4 (12.5%)	
	(41-50)	10 (31%)	
	> 50	14 (44%)	
Age Range (years)	(22-73)	(27-67)	-
Mean □ SD	48.16 □ 13.56	43.67 □ 10.94	0.075 ^{NS}
Gender	Male	12 (37.5%)	0.523 ^{NS}
	Female	20 (62.5%)	
Smoking	Smoker	13 (41%)	0.016*
	Non-smoker	19 (59%)	
Clinical type	Reticular	14 (44%)	> 0.05 ^{NS}
	Erosive	11 (34%)	
	Plaque-like	7 (22%)	

NS = Non-Significant; * = Significant

The mean ET-1 salivary level was 1.15 ng/ml in patients and 0.47 ng/ml in controls (P<0.001) as in (Table 2).

Table 2: The ET-1 level in oral lichen planus patients and apparently healthy controls.

Variables	OLP	Healthy subjects	P value
Mean of Endothelin-1 (ng/ml)	1.15	0.47	<0.001**
Median (CI 25-75)	1.03 (0.71-1.29)	0.48 (0.34-0.57)	

** = Highly significant differences

Interestingly, this study showed increment in the mean levels of ET-1 among the disease clinical types. The highest ET-1 levels were in erosive type (1.67) versus plaque-like type (1.14) and reticular type (0.75) (p<0.001) as in (Table 3).

Table 3: Endothelin-1 level in different clinical types of oral lichen planus.

OLP patients		Mean of Endothelin-1 (ng/ml)	Median	Percentile 25	Percentile 75	P value
Clinical types	Reticular	0.75	0.71	0.63	0.91	< 0.001**
	Erosive	1.67	1.75	1.16	2.08	
	Plaque-like	1 .14	1.22	0.90	1.31	

** = Highly significant differences

IV. Discussion

The results showed that OLP is predominant in patients older than 50 years, which comes inconsistent with (Munde et al, 2013) [23]. The results revealed that there was a predominance of OLP in women relative to men which are comparable to results by (Yu et al., 2003), which in his study specified that women are more influenced with oral lichen planus [24]. The women:men ratio was 2:1 similar to (Patil et al, 2012) [25] in an Indian cohort. Hormonal differences (e.g. estrogen) contributes to higher incidence in women, which affects immune responses. Those hormones cause women to mount more —robust immune responses, and these responses are higher than TH2 responses. Hence, this enhances the development of autoimmune phenomena [25]. This study investigates the effect of smoking on the salivary level of ET-1, the results is inconsistent with (Milnerowicz et al, 2009) who established that ET-1 concentration in plasma is higher in smokers than non-smokers [27]. The *in vivo* studies showed that intense exposition to tobacco smoke increased the —expression of ET-1 mRNA, nevertheless; continuous exposition to tobacco smoke have been found insignificant [28]. Furthermore, activated platelets are higher in smoker's blood flow, and these platelets provoke the "expression of pre pro-ET mRNA and ET-1 biosynthesis from cultures of the endothelial cell". In another word, the cigarette smoking effect on ET-1 stimulation is complicated in the cellular level [29]. Different clinical types of OLP were present, however; in the current study reticular type was the most common, which similar to earlier reports [30, 31]. In this study, the mean level of salivary ET-1 in patients revealed significant increment comparing to controls which, agrees with (Cheng et al, 2011) who stated in his study that salivary ET-1 is —a useful biomarker for OSCC development in OLP patients regardless of its clinical degree [18]. Of note, ET-1 provokes the production of IL-6 in —endothelial cells and TNF- α in macrophages. These have a potent inflammatory influence that initiates the local disease and intensifies the inflammation [32]. A correlation between chronic inflammation and the increase risk of malignancy is well known [33]. The present study revealed that the highest mean level of ET-1 was among the erosive type of OLP, this agrees with (Cheng et al, 2011) [18]. The —Genetic variability occurring in atrophic and erosive forms of OLP might be associated with a higher malignant transformation risk as reported in clinical forms of the disease [34].

V. Conclusion

Salivary ET-1 has a prospect use as a biomarker for OLP patients towards poor prognosis of the disease. Large-scale community screening based-studies are required to inspect its feasibility.

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References

- [1]. Gangeshetty N and Kumar BP. Oral lichen planus: Etiology, pathogenesis, diagnosis, and management. *World J Stomatol.* 2015; 4(1): 12–21.
- [2]. Lavanya N, Jayanthi P, Rao UK, and Ranganathan K. Oral lichen planus: An update on pathogenesis and treatment. *J Oral Maxillofac Pathol.* 2011; 15: 127–32.
- [3]. Mahboobi N, Agha-Hosseini F and Lankarani KB. Hepatitis C Virus and Lichen Planus: The Real Association. *Hepat Mon.* 2010; 10(3) :161–64.
- [4]. McCartan BE and Healy CM. The reported prevalence of oral lichen planus: a review and critique. *J Oral Pathol Med.* 2008; 37(8): 447–53. doi: 10.1111/j.1600-0714.2008.00662.x. Epub 2008 Jul 9.
- [5]. Boorghani M, Gholizadeh N, Zenouz AT, et al. Oral lichen planus: Clinical features, etiology, treatment and management; A review of literature. *J Dent Res Dent Clin Dent Prospect.* 2010; 4: 3–9.
- [6]. Shafer WG, Hine MK, Levy BM. *Shafer's textbook of oral pathology.* 6th ed. Elsevier publications: Noida, India 2009. pp. 800.
- [7]. Petersen PE and Yamamoto T. Improving the oral health of older people: the approach of the WHO Global Oral Health Programme. *Comm Dent Oral Epidemiol.* 2005; 33(2):81–92.

- [10]. Georgakopoulo EA, Achantari MD, Achantaris A, et al. Oral Lichen Planus as a Preneoplastic Inflammatory Model. *J Biomed Biotech.* 2012; Volume 2012, Article ID 759626, 8 pages.
- [11]. Shah R. Endothelins in health and disease. *Eur J Intern Med.* 2007; 18(4):272–82. [PubMed:17574100].
- [12]. Khimji AK, Rockey DC. Endothelin--biology and disease. *Cell Signal.* 2010; 22(11):1615–25. [PubMed: 20466059].
- [13]. Elisa T, Antonio P, Giuseppe P, et al. Endothelin Receptors Expressed by Immune Cells Are Involved in Modulation of Inflammation and in Fibrosis: Relevance to the Pathogenesis of Systemic Sclerosis. *J Immunol Resea.* 2015; (2015) Article ID 147616, 11 pages <http://dx.doi.org/10.1155/2015/147616>.
- [14]. Bagnato A, Spinella F and Rosanò L. The endothelin axis in cancer: the promise and the challenges of molecularly target therapy. *Can J Physiol Pharmacol.* 2008; 86:473–84.
- [15]. Bhalla A, Haque S, Taylor I, et al. Endothelin receptor antagonism and cancer. *Eur J Clin Invest.* 2009; 39(S2):74–7.
- [16]. Abdel-Gawad IA, Hassanein HM, Bahgat NA, et al. Study of endothelin-1 and vascular endothelial growth factor in patients with cancer colon. *J Egypt Natl Canc Inst.* 2008; 20: 216–23.
- [17]. Smollich M, Götte M, Yip GW, et al. On the role of endothelin-converting enzyme-1 (ECE-1) and neprilysin in human breast cancer. *Breast Cancer Res Treat.* 2007; 106:361–9.
- [18]. Yamamoto E, Awano S, Koseki T, et al. Expression of endothelin-1 in gingival epithelial cells. *J Periodontal Res.* 2003; 38: 417–21.
- [19]. Pickering V, Jordan RC, and Schmidt BL. Elevated salivary endothelin levels in oral cancer patients—a pilot study. *Oral Oncol.* 2007; 43(1):37–41. [PubMed: 16757207].
- [20]. Cheng YL, Reesb T, Jordana L, et al. Salivary Endothelin-1 Potential for Detecting Oral Cancer in Patients with Oral Lichen Planus or Oral Cancer in Remission. *Oral Oncol.* 2011; 47(12): 1122–26.
- [21]. Choontharu MM, Binda A, Bhat S, et al. Role of tumor markers in oral squamous cell carcinoma: Review of literature and future consideration. *SRM Journal of Rese in Dent Scie.* 2012; 3(4): 251–56.
- [22]. Hu S, Li Y, Wang J, et al. Human saliva proteome and transcriptome. *J Dent Res.* 2006; 85(12):1129–33. [PubMed: 17122167].
- [23]. Navazesh M. Methods for collecting saliva. *Ann NY Acad Sci.* 1993; 694:72–77. [PubMed: 8215087].
- [24]. St John MA, Li Y, Zhou X, et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg.* 2004; 130(8):929–35. [PubMed: 15313862].
- [25]. Munde A, Karle R, Wankhede P, et al. Demographic and clinical profile of oral lichen planus: A retrospective study. *Contemp Clinic Denti.* 2013; 4(2):181–5.
- [26]. Yu TC, Kelly SC, Weinberg JM, et al. Isolated lichen planus of the lower lip. *Cutis; Cutaneous medicine for the practitioner.* 2003; 71(3):210–12.
- [27]. Patil S, Khandelwal S, Rahman F, et al. Epidemiological Relationship of Oral Lichen Planus to Hepatitis C Virus in an Indian Population. *Oral Health Dent Manag.* 2012; 11(4):199–205.
- [28]. Kindt TJ, Goldspy RA and Osborne BA. Tolerance an autoimmunity. In: —Kuby Immunology, 6th edition (2007). W.H. Freeman and Company, New York. pp. 408–11.
- [29]. Milnerowicz S, Śliwińska-Mossoń M, Milnerowicz H, et al. The effect of smoking on plasma level of insulin and endothelin in pancreatitis or/and diabetes mellitus. In: FOCIS 2009: the ninth Annual Meeting of the Federation of Clinical Immunology Societies. San Francisco (2009). Abstract supplement addendum, 4.
- [30]. Barua RS, Ambrose JA, Eales-Reynolds LJ, et al. Heavy and light cigarette smokers have similar dysfunction of endothelial vasoregulatory activity: an in vivo and in vitro correlation. *J Am Coll Cardiol.* 2002; 39(11):1758–63.
- [31]. Lee SD, Lee DS, Chun YG, et al. Cigarette smoke extract induces endothelin-1 via protein kinase C in pulmonary artery endothelial cells. *Am J Physiol Lung Cell Mol Physiol.* 2001; 81:403–11.
- [32]. Jayavelu P and Sambandan T. Prevalence of hepatitis C and hepatitis B virus infection(s) in patients with oral lichen planus. *J Pharm Bioallied Sci.* 2012; 4(2):S397–405.
- [33]. Gupta SB, Chaudhari ND, Gupta A, et al. Lichen planus – An update. *Int J Pharm Biomed Sci.* 2013; 4(2):59–65.
- [34]. Andrzejewska A and Długosz JW. The endothelin-1 receptor antagonists ameliorate histology and ultrastructural alterations in the pancreas and decrease trypsinogen activation in severe taurocholate pancreatitis in rats. *Int J Exp Path.* 2003; 84:221–9.
- [35]. Wu J, Yi C, Chung H, et al. Potential biomarkers in saliva for oral squamous cell carcinoma. *Oral Oncol.* 2010; 46: 226–31.
- [36]. Merry R, Belfield L, McArdle P, et al. Oral health and pathology: a macrophage account. *Br J Oral Maxillofac Surg.* 2011; 50(1):2–7.