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Invited Review: Association of nitric oxide with oral lichen planus

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ABSTRACT:

Background: The small signalling molecule nitric oxide (NO) has been postulated to have a mediator role in the pathogenesis of several diseases including oral lichen planus (OLP). This systematic review aimed to quantify the existing literature and assess the association of NO and OLP.

Methods: The focused question being addressed was *“Is there an association between nitric oxide and OLP?”* PubMed, EMBASE, Scopus, and Web of Science, and grey literature from January 1990 to August 2018 were searched. Two independent reviewers performed the study selection using specified eligibility criteria.

Results: Seven studies that met the eligibility criteria were included. All of these were case-control studies and 151 patients with OLP were evaluated (mostly females), with an age ranged from 20 to 75 years. The included studies showed a significant higher NO levels in OLP patients compared to the healthy controls, with two studies demonstrated a higher NO levels in erosive OLP compared to non-erosive OLP .

Conclusion: These findings support that an association exists between higher NO concentration and OLP. However, larger high-quality studies with refined methodological design are needed to confirm the role of NO in the aetiology and pathogenesis of OLP.

Key words: *Biomarkers; Nitric Oxide; Oral Lichen Planus; Systematic review.*

INTRODUCTION

Oral lichen planus (OLP) is a chronic inflammatory disorder that affects 0.1 to 2% of population worldwide, mainly middle-aged and elderly females, although it can occur in any age, and be associated with skin LP.¹⁻³ It usually presents as white striations and/or erosions mainly affecting the buccal mucosa, tongue and gingiva. It is usually asymptomatic, but may also cause soreness which can be severe.^{4,5} Although the aetiology of OLP is unknown, it is proposed that a T-cell mediated response causing apoptosis of oral epithelial cells triggers the onset of the disease. lichenoid reaction, genetic, and psychological factors may play a significant role in the initiation of this auto-immune mediated response.⁶⁻⁸

The small free radical and signalling molecule, nitric oxide (NO), has been implicated in the aetiology and pathogenesis of several diseases, including OLP.⁹ Nitric oxide (NO) is a reactive nitrogen free radical produced by the nitric oxide synthase (NOS) isoenzymes that participates in virtually every cellular and organ function.^{10,11} Over the last 25 years, there has been an increased understanding of the complex role of NO, and it is now known to have both protective and damaging actions. NO in low (physiological) concentrations influences blood flow, immune functions, and platelet aggregation, while higher concentrations (produced by the isoenzyme NOS2 or inducible NOS) are associated with pain, atherosclerosis, cancer, inflammatory and immunological disorders.⁹ NO may have a role in tumour progression due to involvement of mechanisms such as DNA damage, tumour angiogenesis and tumour invasion and metastasis is also well appreciated.

Overall, the reported literature on different oxidative and inflammatory biomarkers in OLP and their potential role in the development of OLP is scarce and poorly documented. Since NO has oxidative, inflammatory, and immunological properties, it is appropriate to investigate its potential role in OLP. The aim of this review was to present the current evidence on the potential role of NO as a biomarker of OLP.

MATERIAL AND METHODS:

Study Protocol

The current systematic review was registered as a protocol with international prospective register of systematic reviews (PROSPERO) platform (ID: 118591) and it was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Eligibility Criteria

Inclusion criteria

PECOS framework (Population, Exposure, Comparison, Outcomes, Studies) was used to formulate the focused question of the review, of which: P) Patients with diagnosis of OLP; E) NO detection; C) Patients/participants with no history of OLP; O) Assessing association between NO and OLP; S) Observational studies.

Observational studies including case-control, cross-sectional or population-based that recruited patients with clinically and/or histologically confirmed diagnosis of OLP and evaluated the association of NO and OLP were included. Articles published in English language only were included. The study had a focused question. *“Is there an association between nitric oxide and OLP?”*

Exclusion criteria

The following exclusion criteria were applied: (1) Studies that did not evaluate the association between NO and OLP; (2) case-reports, reviews, experimental studies, short communications and personal opinions, letters to the editor, and conference abstracts.

Search strategy

Detailed automated literature searches were performed in PubMed, EMBASE, Scopus, and Web of Science from January 1990 up to and including July 2018. An additional search of the grey literature was carried out on Google Scholar, ProQuest, and OpenGrey. Reference lists of all included articles were manually searched to identify any potential relevant articles. End-Note software (End-Note X7®, Thomson Reuters, Philadelphia, USA) was used to manage the references and remove any duplicate articles.

Various combinations of descriptors extracted from Medical Subject Headings (MeSH) and free terms were used; “Oral Lichen Planus” [MeSH] OR “Lichen Planus, Oral” [MeSH] AND “Nitric Oxide” [MeSH] AND “Saliva” [MeSH] AND “Serum” [MeSH].

Study selection and data extraction

The study selection process was completed in two stages. Firstly, titles and abstracts of all identified articles were screened by two independent reviewers (KHA and SP). This was followed by retrieval of full texts for studies that met the eligibility criteria. These were reviewed independently by the same two reviewers using a standardized and pilot tested form. Any disagreements on study selection were mutually discussed and a consensus was made before inclusion of the study.

The two reviewers (KHA and SP) independently collected the data on study characteristics (author, year of study and country), study design, sample population, NO detection and analysis methods, and outcome.

Quality assessment of included studies

The quality of included studies was assessed using the Newcastle Ottawa scale (NOS) (Table 1).¹¹ Two reviewers (KHA and SP) independently evaluated the quality of studies based on the following parameters: Selection, Comparability, and Outcome/Exposure. A maximum of 4 stars in the selection domain, 2 stars in the comparability domain and 3 stars in the outcome/exposure domain were given. The included studies were qualified as “Good”, “Fair” and “Poor” quality based on the total NOS score they achieved. Studies with a NOS score ≥ 7 and were considered good-quality studies.

Statistical analysis

Cohen’s kappa statistic was used to calculate the agreement between the two reviewers (KHA and SP). Statistically significant differences in NO levels between the two groups (case and control) were reported. NOS scores based on the assessment of quality of each study were also reported

RESULTS

Study selection

Of 32 full texts assessed, 24 articles were excluded, and only seven papers that met the eligibility criteria were included (Figure. 1). The inter-examiner agreement (Kappa) was 0.98 in the first stage (title and abstract screening stage) and 1.00 in the second stage (full-text reading stage).

Studies characteristics

All of the included studies were case-control in nature and 151 patients with OLP were evaluated (mostly females), with an age ranged from 20 to 75 years. Of the 7 included studies,¹²⁻¹⁸ four were from India,¹³⁻¹⁶ and one each from Spain,¹⁸ Iran,¹⁷ and Japan.¹² The majority of studies used whole unstimulated saliva^{13-16,18} and one study used unstimulated gland saliva from parotid and submandibular glands collection of NO.¹² Since NO is so reactive, it cannot be measured directly and detection is by its reaction products. Griess assay was the most common method used for the analysis of NO.^{12-16,18} Only one study used serum and ELISA kit for the extraction and analysis of NO.¹⁷

Table 2 provides the detailed characteristics of the included studies.

Main findings

All of the included studies showed a significant higher NO levels in OLP patients compared to healthy controls. Two studies showed evidence for a higher NO levels in erosive than in non-erosive OLP.^{13,14}

The NOS score for the quality of the included studies ranged from 6 to 7. Only two studies^{12,15} had a NOS score of '7' to be considered good-quality studies, while the remaining five studies^{13-14,16-18} recorded a NOS score of '6'. The majority of studies scored high in the selection domain and comparability domain was the least scored domain. The majority of studies also scored high in the outcome/exposure domain.

DISCUSSION:

In this study we systematically reviewed the literature on the association of NO and OLP based on the view that cellular immune-mediated mechanisms could act as predisposing factors for OLP. In addition, detection of these inflammatory biomarkers in OLP patients might aid the diagnosis and prognosis of the disease as well as identifying most effective therapeutic agents.

All of the included studies demonstrated a high NO saliva levels in OLP compared to healthy controls. The majority of studies collected saliva for the analysis of NO concentrations, while only one study¹⁷ reported serum concentrations of NO via blood samples. Saliva is a biofluid rich in antioxidants and can act as the first line of defense against oxidative stress.¹⁹⁻²³ The majority of studies used Griess assay method for the measurement of NO levels, while one study¹⁷ employed Human NO ELISA kit (Cusabio; USA) and ELISA apparatus (ELISA micro plate reader; BIOTEK, Winooski, VT) to measure the NO levels.

Although the exact role of NO in the aetiology and pathogenesis of OLP is not known, a possible role has been proposed.^{12,24,25} OLP patients often report increased levels of stress during the onset or exacerbation of their symptoms. NO and the stress pathophysiology are closely related and under certain conditions share similar molecular pathways or mechanisms. Psychological stress causes an increased activity of nitric oxide synthase (NOS) which results in higher concentration of NO.^{26,27} The increased NO in saliva could result in oxidative stress on reactive nitrogen species (RNS) by increasing the oxidant generation and decreasing the antioxidant protection, thus correlating indirectly to OLP.²⁸⁻³⁰ In addition, increased NO leads to cell damage, tissue injury, and organ failure. Studies have reported that increase in NO can lead to damage to fibroblasts, keratinocytes, and oral epithelial cells in vitro.^{11,12}

While assessing quality of the included studies using NOS score, only two studies were classified as good-quality.^{12,15} Both of these reported OLP cases that were confirmed either through clinical and/or histopathological analysis, had consecutive representative series of cases and controls with no history of disease. In addition, the same method of ascertainment was used for both patients and controls. In contrast, studies that scored low on NOS did not match the controls based on age/sex and location use hospital patients for their controls.

There are a few limitations to the current review. Firstly, the level of evidence was low in the included studies. None of the studies had recruited controls from the same community as the patients and failed to match them in terms of age, sex and location. In addition, the inclusion criteria were not very stringent in many of the studies presented. Lastly, lack of the assessment of heterogeneity and a fully comprehensive meta-analysis further undermine the quality of this review.

CONCLUSION: The findings support an association of increased salivary NO in OLP patients. Assessment of these biomarkers in OLP patients could be valuable in clinical practice to classify the risk of new events, and to identify the most effective therapeutic targets. Further longitudinal studies with larger samples are warranted to confirm the role of nitric oxide in oral lichen planus.

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Conflict of interest

None declared.

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Ethical approval

Not required.

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Author et al. (year)	Country	Study type and setting	Sample population	NO collection method	NO detection method	Outcome
Ohashi et al. (1999) ¹²	Japan	<ul style="list-style-type: none"> • Case-control • Second Department of Oral and Maxillofacial surgery, School of Dentistry, Showa University, Tokyo, Japan 	<ul style="list-style-type: none"> • Group I: 21 patients with OLP (erosive type); Mean age 44.5 (range 31-73) years; 6M, 15F • Group II: 8 patients with RAU; Mean age 37.7 (20-64) years; 7M, 11F • Controls: 9 healthy adults (without any inflammatory oral lesions); Mean age 41.6 (24-69) years; 6M, 13F 	<ul style="list-style-type: none"> • Unstimulated gland saliva from parotid and submandibular glands 	<ul style="list-style-type: none"> • Griess 	Higher salivary NO levels in OLP than in healthy controls
Sunitha and Shanmugam (2006) ¹³	India	<ul style="list-style-type: none"> • Case-control • Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College, Chennai, India 	<ul style="list-style-type: none"> • Group I: 20 patients with OLP (erosive type); Mean age 37.7 (22-51) years; 8M, 12F • Group II: 20 patients with RAU; Mean age 29 years (20-48) years; 9M, 11F • Controls: 20 healthy patients (without inflammatory oral lesions and systemic diseases); Mean age 33.4 (20-51) years; 8M, 12F 	<ul style="list-style-type: none"> • Whole unstimulated saliva 	<ul style="list-style-type: none"> • Griess 	Higher salivary NO levels in OLP than in healthy controls
Japtap and Baad (2012) ¹⁴	India	<ul style="list-style-type: none"> • Case-control • Outpatient Department of Govt. Dental College and Hospital, Aurangabad, India 	<ul style="list-style-type: none"> • Group I: 15 patients with OLP (clinically confirmed); Mean age 37.2 (24-55) years; 9M, 6F • Group II: 20 patients with RAU (clinically confirmed); Age 11-40 years; Mean age 30.8 (15-66) years; 	<ul style="list-style-type: none"> • Whole unstimulated saliva 	<ul style="list-style-type: none"> • Griess 	Higher salivary NO levels in OLP than in healthy controls

			<p>15M, 5F</p> <ul style="list-style-type: none"> • Controls: 30 healthy patients; Age 11-40 years; Mean age 28.1 (15-55) years; 15M, 15F 			
Kapoor et al. (2013) ¹⁵	India	<ul style="list-style-type: none"> • Case-control • Department of Oral and Maxillofacial Pathology and Microbiology, India; November 2009 to June 2011 	<ul style="list-style-type: none"> • Cases: 25 patients with OLP (clinically and histologically confirmed), Age 20-45 years • Controls: 25 health patients (undergoing prophylactic removal of tooth, free of any inflammation, and free of any systemic disorders, Age 20-45 years 	<ul style="list-style-type: none"> • Whole unstimulated saliva 	<ul style="list-style-type: none"> • Griess 	Higher salivary NO levels in OLP than in healthy controls
Panjwani et al. (2013) ¹⁶	India	<ul style="list-style-type: none"> • Case-control • Department of Oral Medicine and Radiology, KLE VKI Institute of Dental Sciences, Belgaum, India 	<ul style="list-style-type: none"> • Cases: 30 patients with OLP (clinically and histologically confirmed); Age 25-55 years • Controls: 30 healthy subjects free of any deleterious habits and periodontal disease 	<ul style="list-style-type: none"> • Whole unstimulated saliva 	<ul style="list-style-type: none"> • Griess 	Higher salivary NO levels in OLP than in healthy controls
Mehdipour et al. (2014) ¹⁷	Iran	<ul style="list-style-type: none"> • Case-control • Department of Oral Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences, Iran 	<ul style="list-style-type: none"> • Cases: 20 patients with OLP (clinically and histologically confirmed); Mean age 34.1 (18-60) years • Controls: 20 healthy patients; Mean age 35.6 years 	<ul style="list-style-type: none"> • Serum were isolated from 5 cc blood samples 	<ul style="list-style-type: none"> • Human NO ELISA kit and ELISA apparatus 	Higher serum NO levels in OLP than in healthy controls
Tvarijonavičiute et al. (2017) ¹⁸	Spain	<ul style="list-style-type: none"> • Case-control • Dental clinic at the Morales Meseguer University Hospital, University of Murcia, Spain 	<ul style="list-style-type: none"> • Group I: 20 patients with OLP (clinically and histologically confirmed); Mean age 57.5 (37-75) years; 20F • Group II: 19 with BMS (Scala and the International Classification of Headache Disorders); Mean age 70 (54-83) 	<ul style="list-style-type: none"> • Whole unstimulated saliva 	<ul style="list-style-type: none"> • Griess 	Higher salivary NO level and nitrite in OLP than healthy controls

			years; 1M, 19F • Controls: 31 healthy patients; Mean age 33 (18-67) years; 11M, 20F			
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OLP – Oral lichen planus; BMS – Burning mouth syndrome; NO – Nitric oxide; RAU – Recurrent Aphthous ulcers; M – Male; F – Female

Table 1. Characteristics of the included studies

Table 2. Assessment of the quality of studies included using Newcastle-Ottawa scale (NOS)¹¹

Study	Selection	Comparability	Outcome/exposure	NOS
Ohashi et al. ¹²	○ ● ● ●	○ ●	○ ● ●	7
Sunitha and Shanmugam ¹³	● ● ○ ●	○ ●	○ ● ●	6
Japtap and Baad ¹⁴	○ ● ● ●	○ ●	○ ● ●	6
Kapoor et al. ¹⁵	● ● ○ ●	● ●	○ ● ●	7
Panjwani et al. ¹⁶	○ ● ● ●	○ ●	○ ● ●	6
Mehdipour et al. ¹⁷	○ ● ● ●	○ ●	○ ● ●	6
Tvarijonavičiute et al. ¹⁸	○ ● ● ●	● ●	○ ● ●	6

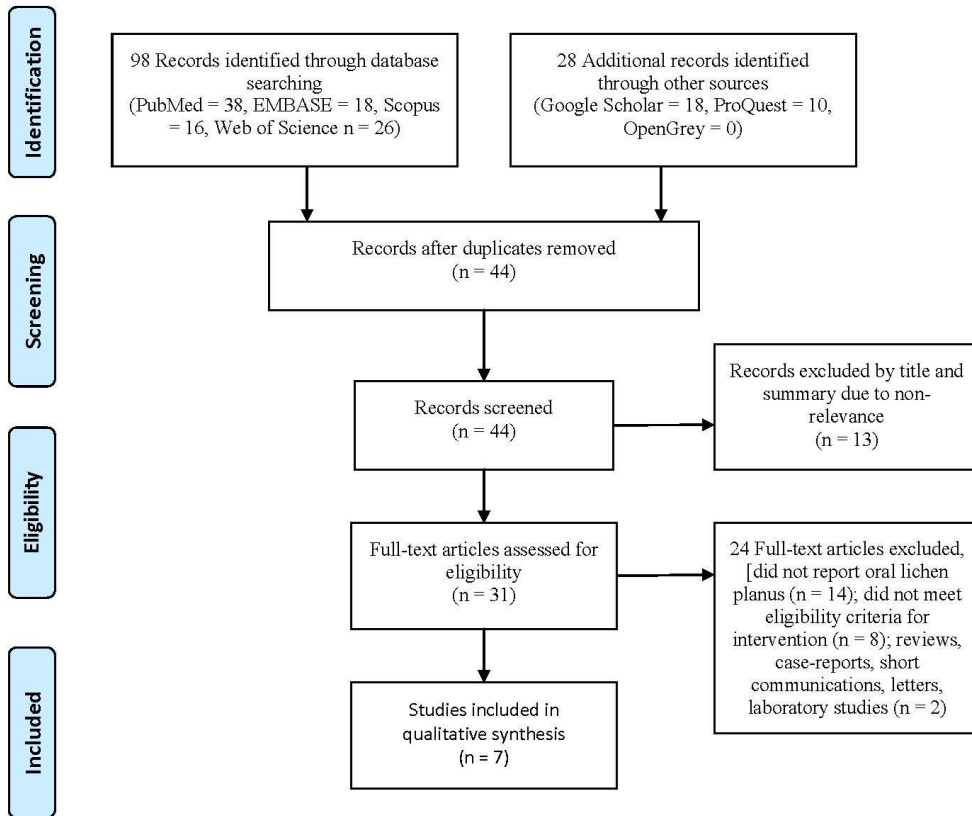


Fig. 1. Flow diagram of literature search and selection criteria.