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Nitric Oxide as an Inflammatory Biomarker in Oral and Systemic Diseases-A Systematic Review

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Abstract: A biomarker or biologic marker is a substance that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Nitric oxide, a free radical gas, is a noxious chemical in the atmosphere but in small controlled concentrations in the body, acts as a physiological and pathophysiological mediator and plays an important role in biological systems. Nitric oxide (NO) is an ubiquitous intercellular messenger molecule with important cardiovascular, neurological and immune functions. This paper highlights the systematic review on a role of nitric oxide in systemic diseases.

Key words: Nitric Oxide-NO · Biomarker · Diabetes Mellitus-DM

INTRODUCTION

Nitric oxide is synthesized from L-arginine by a family of enzymes called nitric oxide synthases. There are three forms [1]

- Type 1 nitric oxide synthase-neural enzyme (n NOS)
- Type 2 nitric oxide synthase-inducible enzyme (iNOS), found in macrophages
- Type 3 nitric oxide synthase-endothelial cell enzyme (e NOS)

Nitric oxide (NO) is a short living product of nitrogen metabolism, produced by may cells in the organism with much important physiological function [2]. Endothelial and neural cells constitutively produce NO. Macrophages and other inflammatory cells can induce its synthesis and release. The most important inductors of NO synthesis are bacterial products[3].

The known biological functions of nitric oxide can be divided into two categories. First, it acts as an endothelial-derived relaxer of vascular smooth muscle, an inhibitor of platelet aggregation and adhesion and a neuronal messenger. Secondly, the nitric oxide synthesized in large amounts by activated macrophage is a cytotoxic molecule influencing the ability of cells to kill bacteria, viruses, protozoa as well as tumour cells. In addition, it is well established that nitric oxide secreted by macrophages has damaging effects against cellular proteins, DNA and lipids leading to periodontitis [4].

Methods of Detection of Nitric Oxide: There are various methods to detect salivary nitric oxide in samples [5]. They are

- Griess Reaction
- Electrochemical Detection
- Chemiluminescent Detection of NO

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- Arginine-Citrulline Conversion
- Hemoglobin Oxidation

Nitric Oxide in Human Autoimmune and Chronic Inflammatory Diseases

Nitric Oxide in the Cardiovascular and Pulmonary Circulation: In the study by Chen H *et al*.[6]described the role of NO in hypertension, pulmonary disorders, sepsis and to some extent, the endothelial factors on the arterial baroreceptors and cerebral blood flow. This study indicate the vasodilatory effects of endogenous NO on the small resistance vessels. The large conduit vessels are less affected. In contrast to the earlier work suggesting that NO or endothelial function is impaired in hypertension.The study provided evidence to indicate that the NO release or function is enhanced in rats with hypertension. Recent clinical investigations have revealed that the inducible NO synthase (iNOS) expression was increased in patients with enterovirus and other infections, suggesting a detrimental role of iNOS and NO in the acute lung injury.

Rheumatoid Arthritis: To assess NO production in Rheumatoid Arthritis (RA) patients, the authors compared levels of serum, urine and salivary nitrite and nitrate (NO2) in patients with RA and normal subjects and examined the relationships of these measures to disease activity. Serum, urine and NO2 levels as well as renal creatinine, NO2 clearance and fractional excretion rates were compared in 25 RA patients and 20 age and gender-matched healthy controls. Subjects were hospitalized for 3 days and placed on a NO2 restricted diet. NO2 was assayed using nitrate reductase and the Griess reagent. RA activity was assessed using standard clinical and laboratory measures. They found that salivary NO2 levels did not differ between normal and RA subjects [7].

Crohns Disease: It has been postulated that oxidative stress, nitric oxide (NO) and transforming growth factor beta(1) (TGF- beta(1)) have major roles in the pathophysiology of Crohn's disease (CD). The aim of this study was to determine the salivary levels of total antioxidant capacity (TAC), specific antioxidants (*i.e.*, uric acid, albumin, transferrin and thiol molecules), lipid peroxidation (LPO), NO and TGF- beta (1) in CD patients. Control subjects were also subjected. Twenty-eight patients with confirmed diagnosis of CD were enrolled and whole saliva samples were obtained. Smokers, diabetics, those who suffered from periodontitis and those consuming antioxidant supplements were excluded from the study. The Crohn's Disease Activity Index (CDAI) was used to determine the severity of the disease. Twenty healthy subjects were also recruited. In CD patients significant reductions in salivary levels of TAC (0.248 +/- 0.145 vs. 0.342 +/- 0.110 mmol/L), albumin (1.79 +/- 0.42 vs. 2.3 +/- 0.2 microg/mL) and uric acid (3.1 +/- 1.4 vs. 4.1 +/- 2.0 mg/dL) were found. TGF-beta (1) was significantly increased in CD patients compared to healthy subjects (3.02 +/- 1.54 vs. 2.36 +/- 0.52 ng/mL). A fourfold increase in NO levels (198.8 +/- 39.9 vs. 50.2 +/- 21.3 micromol/L) along with a fivefold increase in LPO concentration (0.146 +/- 0.064 vs. 0.027 +/- 0.019 micromol/L) was documented in CD patients in comparison to the control group. CDAI significantly correlated with the TAC, LPO and the interaction between TAC and LPO ($r(2) = 0.625$, $r(2) = 0.8$, F-test's $P < 0.00005$). Saliva of CD patients exhibits an abnormal feature with respect to oxidative stress, NO and TGF-beta(1). TAC and LPO modify the effect of each other in determination of CD severity, which underlines the importance of oxidative stress in the pathogenesis of CD [8].

Sjogrens Syndrome: A Study by Konttinen Y.T. *et al*. [9], was done to measure levels of salivary nitrite (NO2-) and to localize nitric oxide synthases (NOS) in the labial salivary glands (LSGs) of patients with Sjögren's syndrome (SS). NO2 was measured by the Griess reaction. LSGs were analyzed using NADPH-diaphorase histochemical and immunohistochemical studies to determine the constitutive NOS (neuronal [ncNOS] and endothelial [ecNOS]) and inducible NOS (iNOS) isoforms. Results showed that the NO2- concentration (mean +/- SEM 307 +/- 51 microM versus 97 +/- 16 microM; $P < 0.05$) and output (166 +/- 46 nmoles/minute versus 37 +/- 7 nmoles/minute) were increased in SS patients compared with healthy control subjects. NADPH-diaphorase was found in some nerve fibers and endothelial cells and, in SS, was found in myoepithelial, acinar and ductal epithelial cells, but in only a few inflammatory cells. In SS, ncNOS-immunoreactive nerve fibers were sparse and eNOS was found in a minority of the CD31-positive vascular endothelial cells and acinar cells, whereas iNOS was localized in myoepithelial, acinar and ductal epithelial cells, often together with tumor necrosis factor alpha. The authors concluded Nitrite was found in normal human saliva. NO produced by ncNOS probably acts as a

nonadrenergic, noncholinergic neurotransmitter, whereas that produced by ecNOS exerts a vasodilatory effect. SS patients had increased NO2- concentrations, with most of the superfluous salivary NO being produced not by the immigrant inflammatory cells, but rather, by the resident salivary gland cells. NO may contribute to inflammatory damage and acinar cell atrophy in SS [9].

To investigate a role for the inflammatory mediator, nitric oxide (NO) in SS, an autoimmune condition characterized by salivary and lacrimal gland hypofunction resulting from failure of acinar cells to secrete. FURA-2 microfluorimetry was used to measure agonist-evoked changes of [Ca(2+)] in isolated mouse and human salivary acinar cells following exposure to NO donors. Results showed that NO had a biphasic effect on salivary acinar function. Acute exposure to NO (2 min) caused a cyclic guanosine monophosphate (GMP)-dependent, 1-H-[1,2,4]oxadiazolo[4,3-a]quinoxalin sensitive increase in the Ca(2+) signal elicited in response to acetylcholine (ACh) stimulation, consistent with stimulation of ryanodine receptors by cyclic adenosine diphosphate ribose. Prolonged exposure to NO (>40 min) significantly reduced the ACh-evoked Ca(2+) signal by a mechanism independent of cyclic GMP. The authors found no differences between the responses of human and mouse acinar cells. This data showed that chronic exposure to NO, which is known to be elevated in SS, could have a role in salivary gland hypofunction. The authors note a similarity in the response to stimulation of salivary acinar exposed to NO and that has previously reported in salivary acinar cells isolated from patients with SS. This may speculate that NO-mediated nitrosylation of one or more elements of the signal transduction pathway could underlie down-regulation of salivary function in SS [10].

Diabetes Mellitus: The study by Astaneie F *et al*. [11] discussed how type 1 diabetic patients have an altered levels of lipid peroxidation, antioxidant defense, NO and EGF in their plasma and saliva. The authors tested the differences in lipid peroxidation level, antioxidant power and concentrations of epidermal growth factor (EGF) and nitric oxide (NO) in saliva and blood of type 1 diabetic subjects in comparison to healthy control subjects. Nineteen subjects with type 1 diabetes mellitus and 19 healthy age-and sex-matched control subjects were included in the study. Blood and saliva samples were obtained and analyzed for thiobarbituric reactive substances (TBARS) as a marker of lipid peroxidation, ferric reducing ability (total antioxidant power), EGF and NO levels. Results showed NO level increased in both saliva and plasma of diabetic patients in comparison to those of healthy subjects. The authors concluded that increased salivary EGF and NO levels in association with elevated TAOP is interesting and should be further studied.

The aim of a study by Skaleric U. *et al*. [12] was to evaluate expression of NO in gingivae of type I diabetic patients presenting with periodontal disease and to correlate the level of NO with *P.* intermedia infection. Gingival tissues were obtained during modified Widman flap surgery from diabetic patients (three males and two females; mean age [standard deviation], 48.2-6.9 years) diagnosed with moderate (probing depth of-5 mm) or advanced (probing depth of-5 mm) periodontitis. Noninflamed gingival tissue was obtained during the crown-lengthening procedure of diabetic patients (two females, aged 44 and 51 years). Fixed and embedded tissue sections were stained with haematoxylin-eosin (H&E) or antibodies against iNOS. Results showed an intense inflammatory infiltrate composed predominantly of mononuclear cells, including lymphocytes and macrophages, was observed in H&E-stained gingival tissues from periodontally involved type 1 diabetic patients. The authors concluded that in diabetic patients, increased inducible NO synthase in inflamed gingiva correlated with NO in gingival crevicular fluid. Although increased NO reflected more-severe inflammation, it was associated with reductions in CFU of Prevotella intermedia, a major periodontopathogen, highlighting dual roles for NO.

Role of Nitric Oxide in Periodontitis: The study by Reher VG *et al*. [13] compared nitric oxide (NO) levels in stimulated whole saliva from individuals with and without generalized chronic periodontitis (GCP) and in role to evaluate correlations between these levels with a clinical diagnostic parameter. According to specific criteria, 30 individuals were divided into three groups: one comprising individuals without periodontitis (GC), second comprising individuals with moderate GCP (GM) and a third comprising individuals with advanced GCP (GA). Samples were collected and NO levels measured. NO in the GCP group (GM: 7.78 microM; GA: 15.79 microM) was higher than in the GC group (5.86 microM). NO levels in the GA group were significantly higher ($P < 0.0001$) than in the GC group and could also differentiate ($P < 0.0001$) the moderate and advanced forms of the disease.

In addition, positive correlations between NO level and the number of teeth with a probing depth of > or = 4 mm (r = 0.54) and > or = 7 mm (r = 0.68) were observed. In conclusion, NO levels are elevated in individuals with GCP and are correlated with a periodontal clinical parameter. These results revealed that this form of periodontal disease and its severity are related to salivary nitrite concentration, indicating that NO may serve as a potential biological marker for detection and/or monitoring of GCP.

The study by Menaka KB *et al*. [14] was to assess the level of NO in serum in chronic periodontitis and correlate these levels with the severity of periodontal disease. Sixty subjects participated in the study and were divided into two groups. Control group or Group 1, comprised of clinically healthy gingival tissues (*n* = 30) and Case group or Group 2, subjects of chronic periodontitis with pocket depth >5 mm and clinical attachment loss of > 4 mm (*n* = 30). Subjects' consent was taken prior to the collection of samples. About 2 mL of venous blood was drawn from the patient's arm to estimate the serum nitric oxide levels. The blood samples were centrifuged at 3000 rpm for about 10 min to collect the serum, followed by biochemical estimation of nitric oxide. NO levels were assayed by measuring the accumulation of stable oxidative metabolite, nitrite with Griess reaction. Results showed subjects with periodontitis had significantly high nitrite in serum than healthy subjects. The authors concluded that NO production is increased in periodontal disease, this will enable us to understand its role in disease progression and selective inhibition of NO may be of therapeutic utility in limiting the progression of periodontitis.

Endothelial nitric oxide synthase (NOS3) is involved in key steps of immune response. Genetic factors predispose individuals to periodontal disease. This study's aim was to explore the association between NOS3 gene polymorphisms and clinical parameters in patients with periodontal disease. Genomic DNA was obtained from the peripheral blood of 23 subjects with aggressive periodontitis (AgP), 26 with chronic periodontitis (CP), 31 with gingivitis (G) and 50 healthy controls. Probing depth (PD), clinical attachment loss (CAL), plaque index (PI) and gingival index (GI) were recorded as clinical parameters. We genotyped NOS3 polymorphisms using the PCR and/or PCR-RFLP method. Genotype frequencies differed significantly among periodontal diseases and controls for these polymorphisms. A significant association was detected between NOS3 +894 polymorphism and PD and CAL in the CP and AgP patient groups; whereas NOS VNTR analysis detected no associations with clinical parameters in the CP and AgP groups. However, a significant association was detected between the AA genotype and both PI and GI in patients with gingivitis; and a significant association was shown between the BB genotype and PI [15].

Oral Cancer: Nitric oxide metabolism was studied in cancerous and non-cancerous oral gingival mucosal tissues. The study included 19 tissues from human subjects, 11 malignant and 8 benign lesions. Results showed that NO level and NOS activity were increased in the malignant lesions compared with the benign lesions. The authors concluded that decreased NO synthesis may be an attempt to suppress angiogenesis, which provides nutrients to the cancerous lesion [16].

Oral Mucosal Diseases and Nitric Oxide Levels: A study by Ohashi M. *et al*. [17] was conducted to measure salivary NO levels in 39 patients with oral mucosal disorders. 21 patients had oral lichen planus (OLP) and 18 had recurrent aphthous ulceration (RAU). NO was assayed using the Griess reagent, which measures nitrite (NO2), the byproduct of NO. NO2 was detected in all tested samples and the results reveal that levels in the NO in saliva of patients were significantly increased relative to those of healthy subjects.

The study conducted on 30 patients of oral lichen planus determined by immunohistochemistry whether or not the expression of the inducible form of nitric oxide synthase was increased compared to 10 normal patients on buccal mucosa biopsies, using primary antibodies to i NOS and CD68 . Results showed CD68 expression was significantly increased in the cellular infilterate of all 30 cases of oral lichen planus compared to normal tissue biopsies [18].

The aim of a study by Yildirim M *et al*. [19] was to investigate the serum NO levels in patients with active and inactive BD (behcet's disease) and RAS (recurrent aphthous stomatitis). Forty-six patients with BD, 30 patients with RAS and 30 healthy controls were enrolled in the study. The patients with BD were separated into two groups: clinically active (n = 24) and inactive (n = 22). A blood sample was collected from all subjects in order to determine their serum NO levels. In patients with active BD, higher serum levels of NO metabolite were found in comparison with patients with inactive BD, in patients

with RAS, or healthy controls ($p < 0.05$). We also found higher serum NO metabolite levels in patients with RAS than in healthy controls ($p < 0.05$). In patients with inactive BD, statistically significant higher levels of serum NO levels were found in comparison with the control group ($p < 0.05$). However, the authors found no statistically significant difference between the patients with inactive BD and RAS, which indicated that inactive BD cannot be distinguished from RAS by serum NO levels. They conclude that serum NO levels may be an important marker for estimating the severity of BD. However, further studies are needed to confirm our findings.

The study by Gurel A. *et al*. [20] was to compare serum xanthine oxidase (XO) and adenosine deaminase (AD) activities and malondialdehyde (MDA), nitric oxide (NO) and uric acid (UA) levels in a group of patients affected by RAU (recurrent aphthous ulceration) and in a group of healthy controls. A total of 26 patients with minor RAU were included in the study. Twenty-six healthy volunteers were selected to form the control group. AD and XO activities and UA, NO and MDA levels were studied in the serum samples of all patients and controls. Serum XO and AD activities and MDA, NO and UA levels were significantly higher in RAU patients than in controls. Increased XO and AD activities, NO and UA levels and lipid peroxidation were thought to take part in the pathogenesis of RAU.

CONCLUSION

Nitric oxide (NO) plays multiple roles in both intracellular and extracellular signalling mechanisms with implications for health and disease. In addition it has been postulated that pharmacological inhibition of NO or its actions may be therapeutically valuable in disease management. Levels of nitric oxide may provide clues about severity and state of underlying disease process. It could be an inflammatory biomarker that may enable clinicians to direct environmentally based prevention or treatment programmes.

REFERENCES

1. Sundar, N. Mani, V. Krishnan, S. Krishnaraj, V.T. Hemalatha and M.D. Nazish Alam, 2013. "Comparison of the Salivary and the Serum Nitric Oxide Levels in Chronic and Aggressive Periodontitis: A Biochemical Study." J. of Clinical and Diagnostic Research: JCDR 7(6): 1223-27. doi:10.7860/JCDR/2013/5386.3068.
2. Biomarkers Definitions Working Group. Biomarkers and surrogateendpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther, 2001, 69(3): 89-95.
3. Michael, T. and O. Feron, 1997. Nitric oxide synthesis: which, where, how and why? J. Clin. Invest, 100(9): 2146-52.
4. Sunitha, M. and S. Shanmugam, 2006. Evaluation of salivary nitric oxide levels inoral mucosal diseases: a controlled clinical trial, Ind. J. Dent Res., 17(3): 117-120.
5. Margaret, M., T. Fridovich and I. Methods, 2001. Of Detection of Vascular Reactive Species: Nitric Oxide, Superoxide, Hydrogen Peroxide and Peroxynitrite. Circ. Res., 89: 224-236.
6. Hsing, I. Chen, Huai-Ren Chang and Chia-Yen Wu, 2007. Nitric Oxide in the Cardiovascular and Pulmonary Circulation-A Brief Review of Literatures and Historical Landmarks. Chinese Journal of Physiology, 50(2): 43-50.
7. Weinberg, J.B., T. Lang, W.E. Wilkinson, D.S. Pisetsky and E.W. St Clair, 2006. Serum, urinary and salivary nitric oxide in rheumatoid arthritis: complexities of interpreting nitric oxide measures. Arthritis Res. Ther., 8(5): R140.
8. Rezaie, A., F. Ghorbani, A. Eshghtork, M.J. Zamani, G. Dehghan, B. Taghavi, S. Nikfar, A. Mohammadirad, N.E. Daryani and M. Abdollahi, 2006. Alterations in salivary antioxidants, nitric oxide and transforming growth factor-beta 1 in relation to disease activity in Crohn's disease patients. Ann N Y Acad Sci. Dec., 1091: 110-22
9. Konttinen, Y.T., L.A. Platts, S. Tuominen, K.K. Eklund, N. Santavirta, J. Törnwall, T. Sorsa, M. Hukkanen and J.M. Polak, 1997. Role of nitric oxide in Sjögren's syndrome. Arthritis Rheum. May; 40(5): 875-83.
10. Caulfield, V.L., C. Balmer, L.J. Dawson and P.M. Smith, 2009. A role for nitric oxide-mediated glandular hypofunction in a non-apoptotic model for Sjogren's syndrome. Rheumatology (Oxford), 48(7): 727-33.
11. Astaneie, F., M. Afshari, A. Mojtahedi, S. Mostafalou, M.J. Zamani, B. Larijani and M. Abdollahi, 2005. Total antioxidant capacity and levels of epidermal growth factor and nitric oxide in blood and saliva of insulin-dependent diabetic patients. Arch. Med. Res. Jul-Aug., 36(4): 376-81.

12. Skaleric, U., B. Gaspirc, N. McCartney-Francis, A. Masera and SM.Wahl, 2006. Proinflammatory and antimicrobial nitric oxide in gingival fluid of diabetic patients with periodontal disease. Infect Immun., 74(12): 7010-3.
13. Reher, V.G., E.G. Zenóbio, F.O. Costa, P. Reher and R.V.Soares, 2007.Nitric oxide levels in saliva increase with severity of chronic periodontitis. J. Oral Sci., 49(4): 271-6.
14. Menaka, K.B., A. Ramesh, B. Thomas and N.S. Kumari, 2009. Estimation of nitric oxide as an inflammatory marker in periodontitis. J. Indian Soc. Periodontol., 13: 75-8.
15. Kamile Erciyas, Sacide Pehlivan, Tugce Sever and Mehri Igci, 2010. Endothelial nitric oxide synthase gene polymorphisms associated with periodontal diseases in Turkish adults. African Journal of Biotechnology, 9(21): 3042-3047.
16. Avci, A., A.M. Tüzüner-Öncül, M.K. Gökcan, M. Namuslu, A. Öztürk and I. Durak, 2009. Nitric oxide metabolism in cancerous and non-cancerous oral gingivomucosal tissues: possible implications of nitric oxide in cancer process. J. Oral Pathol. Med., 38: 304-306.
17. Ohashi, M., M. Iwase and M. Nagumo, 1999. Elevated production of salivary nitric oxide in oral mucosal diseases. J. Oral Pathol Med., 28(8): 355-9.
18. Brennan, P.A., T. Umar, M. Palacios-Callender, A.V. Spedding, T.K. Mellor, J. Buckley and J.D. Langdon, 2000. A study to assess inducible nitric oxide synthase expression in oral lichen planus. J. Oral Pathol Med., 29(6): 249-54.
19. Yildirim, M., V. Baysal, H.S. Inaloz and D. Doguc, 2004. The significance of serum nitric oxide levels in Behçet's disease and recurrent aphthous stomatitis. J. Dermatol. Dec., 31(12): 983-8.
20. Gurel, A., H.C. Altinyazar, M .Unalacak, F. Armutcu and R. Koca, 2007. Purine catabolic enzymes and nitric oxide in patients with recurrent aphthous ulceration. Oral Dis., 13(6): 570-4.