The expression of the nitric oxide synthase enzyme in periodontal disease and orofacial pain: systematic review

A expressão da enzima óxido nítrico sintase na doença periodontal e na dor orofacial: revisão sistemática

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ABSTRACT

BACKGROUND AND OBJECTIVES: The literature suggests that the enzyme nitric oxide synthase (NOS) plays an important role in both periodontal disease (PD) and orofacial pain (OP), although few studies associate these two conditions simultaneously. The objective of this study was to evaluate the expression of the NOS enzyme in PD and OP, in comparison to those patients who did not present PD and/or OP.

CONTENTS: Seven electronic databases (Pubmed, Web of science, Scopus, Cochrane Library, BBO, LILACS, Clinical trial) were used to identify the potentially relevant studies published until April 2021. The research resulted in 1,960 studies, of which 11 were selected to be critically evaluated. Two independent reviewers selected titles and abstracts using the criteria defined in the PECO question (Population, Exposure, Comparison, Outcome). The methodological evaluation of the selected studies was carried out according to the Effective Public Health Practice Project (EPHPP).

CONCLUSION: Scientific evidence demonstrated that NOS may play a key role in the pathogenesis of PD and OP, since its expression is increased in patients with these conditions. However, genetic studies on endothelial nitric oxide synthase (eNOS) gene mutations in patients with migraine and cluster headache did not indicate the expected susceptibility to pain. In addition, studies on the association of NOS expression in PD and OP, occurring simultaneously, are scarce. This review may support future research on NOS in two very common correlated diseases such as PD and OP.

Keywords: Orofacial pain, Nitric oxide, Nitric oxide synthase, Periodontal diseases.

RESUMO

JUSTIFICATIVA E OBJETIVOS: A literatura sugere que a enzima óxido nítrico sintase (NOS) desempenha um papel importante tanto na doença periodontal (DP) quanto na dor orofacial (DOF), embora poucos estudos associem estas duas condições simultaneamente. O objetivo deste estudo foi analisar a expressão da NOS em pacientes com DP e DOF em comparação a controles sem estas condições.

CONTEÚDO: Foram consultadas as bases de dados eletrônicas (Pubmed, Web of Science, Scopus, Cochrane Library, BBO, Lilacs e Clinical Trial) para identificar os estudos potencialmente relevantes publicados até abril de 2021. As pesquisas resultaram em 1.960 estudos, dos quais 11 foram selecionados para serem avaliados criticamente. Os critérios definidos na questão PECO (População, Exposição, Comparação e Desfecho) foram usados para selecionar títulos e resumos por dois revisores independentes. A avaliação metodológica dos estudos selecionados foi realizada de acordo com a ferramenta *Effective Public Health Practice Project* (EPHPP).

CONCLUSÃO: As evidências científicas demonstraram que a NOS desempenha um importante papel na patogênese da DP e da DOF, já que sua expressão está aumentada em pacientes com estas condições. Porém, estudos genéticos sobre mutações dos genes da óxido nítrico sintase endotelial (eNOS) em pacientes com migrânea e cefaleia em salvas não evidenciaram a esperada suscetibilidade a dor. Além disso, estudos sobre a associação da expressão da NOS na DP e na DOF, ocorrendo simultaneamente, são escassos. Este estudo pode subsidiar futuras pesquisas sobre NOS na correlação de duas doenças muito frequentes como a DP e a DOF.

Descritores: Doença periodontal, Dor orofacial, Óxido nítrico, Óxido nítrico sintase.

INTRODUCTION

Periodontal disease (PD) is an infectious immunoinflammatory condition with a multifactorial etiology in the adult population[1]. The association of PD with some systemic conditions, such as

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cardiovascular, metabolic, and neurovascular diseases, has been previously demonstrated[2-5]. Microbial factors and factors derived from host response, including various enzymes such as nitric oxide synthase (NOS), are associated with the destruction of the periodontium[6,7].

Another common condition in the general population is orofacial pain (OP)[8]. Its control and treatment depend on an accurate diagnosis[9]. Generally, the term "orofacial pain" refers to painful conditions related to structures of the oral cavity and the face itself. It includes painful conditions associated with the soft and hard tissues of both head and neck, as well as all structures that form the oral cavity[10].

There are several inflammatory mediators related to both pain and PD, such as interleukins, prostaglandins, and some non-adrenergic and non-cholinergic neurotransmitters (NANC)[11]. Nitric oxide (NO) is a gaseous molecule that is generated during the conversion of the amino acid L-arginine into L-citrulline by NOS, which consists of two constitutive isoforms, including both endothelial (eNOS) and neuronal NOS (nNOS), as well as an isoform generated by different stimuli: the inducible NOS (iNOS). NO is a labile reactive molecule that quickly oxidizes into nitrate and nitrite end products. The scientific evidence points to its participation in the pathophysiological mechanisms of pain and PD[1-8].

The inducible isoform (iNOS) produces substantial amounts of NO when stimulated by many proinflammatory cytokines and is expressed in several types of inflammatory cells[12]. The isoform of eNOS releases NO from the endothelium causing smooth muscle relaxation[13], and nNOS is expressed by neurons and is involved in neurotransmission and neuroendocrine functions[14].

Despite many evidence of the participation of this enzyme and its products in pain and PD, there are few association studies that investigate its expression in both conditions and that allow to develop a critical reasoning about the biological plausibility of the interrelationship between them.

CONTENTS

This systematic review addressed the following question: "What is the relation of nitric oxide synthase enzyme to Periodontal Disease and Orofacial Pain"? The PECO question (P = Population, E = Exposure, C = Control Group and O = Outcome) was: patients with PD and/or DOF, expression of nitric oxide synthase in patients with PD and OP, patients without PD and OP and higher expression of the enzyme nitric oxide synthase in PD and OP respectively.

The inclusion criteria were case-control, cohort, clinical studies, or cross-sectional studies; adult population; correlation between NOS in patients with PD and/or OP.

Exclusion criteria were case reports, literature reviews, studies without statistical analyses, studies with children, studies with other diseases and letter to the reader. The search was conducted on seven electronic databases (PubMed, Web of Science, Scopus, Cochrane Library, BBO, LILACS and Clinical Trial) until April 2021, using the keywords: periodontal disease, nitric oxide synthase, nitric oxide and orofacial pain. A search in the gray literature was done through the Google Scholar search engine. No restrictions were placed on the year of publication and on languages.

In addition, a manual search was carried out on the reference list of included studies. 1,960 articles were obtained in total. After the duplicates were removed, 991 titles and abstracts were submitted to the first calibration, completed by two independent reviewers who read a sample of 10% of the studies.

After obtaining a substantial agreement level (Kappa) of 1, the independent reviewers read all remaining studies. After this first selection, 74 studies were selected to be thoroughly read. Out of these, 63 were excluded because they did not meet the defined inclusion criteria. Disagreements were resolved by consensus and discussion with a third reviewer.

The study was registered in the International Prospective Registry for Systematic Review (PROSPERO) under registration # CRD42018093246. This report conforms to the Preferred Report Items for Systematic Reviews and Meta-analyzes (PRISMA).

Quality assessment

Two independent reviewers evaluated the selected articles through the quality evaluation tool of the Public Health Practice Project (EPHPP), since this assessment instrument is created to address articles in a wide range of health-related topics and include parameters that could be approached with scientific purpose and universally applicable to any health topic[15]. The following parameters were evaluated: selection bias, study design, confounding factors, blinding, methods of data collection and withdrawals. Each item can be evaluated by the following rates: strong, moderate, weak and not applicable to some cases.

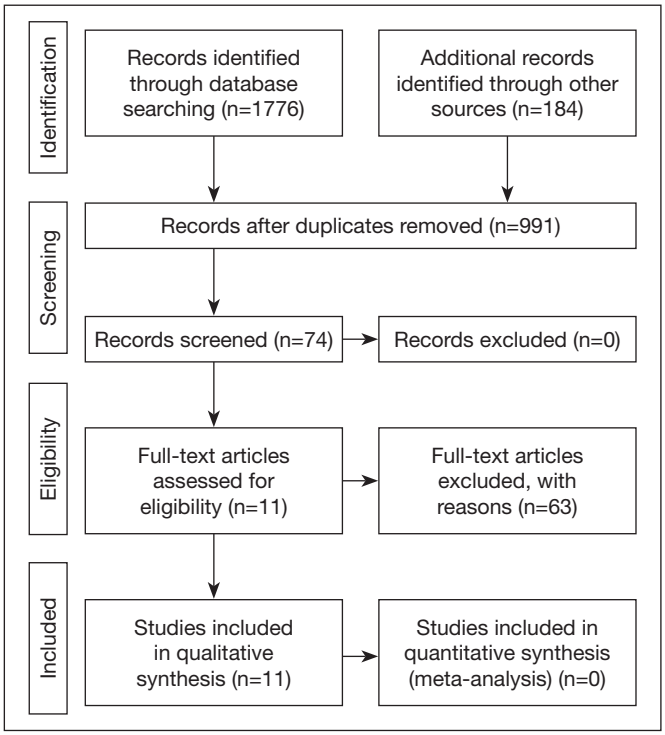

Figure 1. Study data related to the pattern of Preferred Items for the analysis of Systematic and Meta-Analyzes (PRISMA) 2009

RESULTS

At the end of the selection process, 11 articles were obtained. Nine studies observed the expression of NOS and/or NO through the clinical and laboratory studies[7,14,16-22].

The methodological parameters used to evaluate the periodontal condition were the following: PI = plaque index (%), CEJ = cementoenamel junction, GI = gingival index, BP= bleeding on probing (%), CAL = clinical attachment level (mm), and probing depth .About laboratory studies, the incisional gingival biopsies and laboratory tests by immunohistochemistry were described. In this way, the presence of some isoform of NOS in PD were analyzed.

On the other hand, the parameters for assessing OP followed the criteria of the International Headache Society (IHS), the visual analog scale (VAS) and the MIDAS (headache deficiency assessment). In addition, the studies included an analysis of endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS) in serum of patients with OP.

Among the 11 articles included in this systematic review, 5 studies looked at the expression of NOS and/or NO and their metabolites in PD[3,7,19-21] and other 5 studies quantified the expression of NOS, NO and metabolites in OP[14,17,18,22,24]. Only one article revealed the expression of NOS simultaneously in patients with PD and OP[16].

Outcomes about NOS expression in periodontal disease

The data revealed a greater expression of iNOS[19,21], eNOS[7] in the gingiva and soluble NOS in serum[20] of patients with PD when compared to healthy patients.

Additionally, patients with periodontitis had higher iNOS expression before periodontal treatment. Furthermore, the expression of iNOS is greater in sites subjected to scaling and root planning procedures than in those submitted to periodontal surgical procedures (modified Widman flap)[23]. Arginase, which reduces NO production by decreasing intracellular arginine concentration, increased after periodontal treatment[23]. Table 1 shows the studies about the expression of the NOS enzyme related to PD.

Findings about nitric oxide in OP

The findings disclosed a potential participation of NO in painful orofacial processes. Studies about oxidative stress associated with migraine showed that there was increased nitric oxide stress when compared to the control group[17]. Furthermore, there are evidence about dysfunction of eNOS, an endothelium-derived vasodilator that cause changes on blood flow, in migraine patients[22].

On the other hand, through genetic analysis in a study with 337 patients with migraine and 341 healthy subjects, the distribution of the eNOS promoter genes was not different among the clinical groups[18]. In addition, another genetic experiment on NOS alleles has shown that the genetic variations of the three NOS genes do not contribute to the susceptibility to cluster headache[14]. However, it warns of the complexity of factors involved between gene expression and the presence of the final metabolite, since there are a range of NO donors that could constitute a common pathophysiological feature for several forms of primary headaches[14].

Table 1. Expression of nitric oxide synthase in PD

Authors	Country	Study	Investigation	Sample	Results
Gullu et al.[23]	Turkey	Clinical and laboratory	Comparison of the expression of iNOS before and after the two therapeutic modalities (surgery x scraping) and evaluation of the arginase before and after the periodontal treatment.	Sites with clinical attachment loss ≥7mm from 13 patients ($p < 0.05$)	The patients with PD had a higher expression of iNOS and a lower concentration of arginase before treatment ($p<0.05$).
Batista et al.[19]	Brazil	Laboratory	Quantification of iNOS positive.	19 cases of gingivitis 19 cases of periodontitis 13 healthy cases.	Increased iNOS labeling in patients with gingivitis and PD compared to control ($p<0.05$).
Shibata et al.[20]	England	Laboratory	Activity of NOS was rated by the radiolabeled L-arginine to L-citrulline conversion assay. Membrane associated-NOS (MA-NOS) and soluble NOS (S-NOS) were extracted from cells.	Neutrophils isolated from 10 patients with PD.	Greater number of NOS in periodontitis compared to inhibition of neutrophil chemotherapy ($p<0.05$).
Kendall et al.[7]	Australia	Laboratory	The sharing of eNOS was analyzed in inflamed and non-inflamed samples of human gingiva using a monoclonal antibody against. eNOS	6 gingival samples from patients with PD and 3 samples from healthy controls.	Higher eNOS expression in inflamed compared to non-inflamed gingival tissue. Cells within the inflamed connective tissue expressed eNOS.
Lappin et al.[21]	Scotland	Clinical and laboratory	INOS expression in gums of patients with PD x control.	16 patients with PD and 5 control patients.	Increased iNOS expression in PD patients.

INOS = inducible nitric oxide synthase; PD = periodontal disease; MA-NOS = Membrane associated-NOS; S-NOS = soluble NOS; NOS = nitric oxide synthase; eNOS = nitric oxide endothelial synthase; p = significance value.

There are results suggesting that pain, which can be an orofacial manifestation, can be associated to changes in NO concentrations in patients with this syndrome[25].

The expression of NOS has also been investigated in temporomandibular dysfunction (TMD). The iNOS investigated by using immunohistochemistry, and compared with clinical, arthroscopic and histological findings of TMD specimens, obtained arthroscopically from diseased TMJ, was correlated significantly with arthroscopic evidence of synovitis (r=0.406, p≤ 0.05)[24]. Table 2 highlights the particularities of these investigations.

Analyzing simultaneously NOS expression in PD and OP

Throughout the literature search, only one study simultaneously investigated the immunohistochemical expression of NOS in PD and OP. The results showed a greater expression of the enzyme in patients with the two associated conditions compared to patients presenting only PD[16] (Table 3).

Table 2. Expression of nitric oxide synthase in oral periodontal

Authors	Country	Study	Investigation	Total of sample	Results
Aguilar et al.[25]	Spain	Clinical and laboratorial	The relationships between serum NO levels (chemiluminescence--based assay), oxytocinase activity and enkephalin-degrading aminopeptidase (EDA) activity (fluorometrically determined) and pain-related clinical manifestations in women with fibromyalgia were evaluated.	Fifty-eight women diagnosed with fibromyalgia.	Significant relations were observed between levels of NO and dominant occiput pressure pain thresholds, non-dominant occiput pressure pain thresholds, and fibromyalgia effects.
Van der Schueren et al.[22]	Belgium	Clinical and laboratorial	Each individual received L-arginine infusion or placebo and the nasal and exhaled NO and NO metabolites were evaluated.	20 patients with migraine and 20 healthy subjects.	Increased nasal and exhaled NO of patients with migraine in the initial evaluation compared to controls and lower production of metabolites after l-arginine infusion in patients with migraine, indicating eNOS dysfunction (p = 0.81).
Gruber et al.[17]	Austria	Clinical and laboratorial	Several parameters of the NO pathway, such as nitrate, nitrite, arginine, citrulline, nitrosylated proteins, asymmetric dimethylarginine, symmetrical dimethylarginine, expression of eNOS and iNOS and two eNOS polymorphisms were investigated.	130 patients with migraine and 76 control subjects.	Migraine patients suffer under sustained increased nitrosative stress in the headache-free period, which is associated with a 3.6-fold higher risk for migraine.
Toriello et al.[18]	Spain	Laboratorial	Genetic study of eNOS alleles.	337 patients with migraine and 341 controls.	The distribution of the alleles was not different between the groups (p <0.01).
Takahashi et al.[24]	Japan	Clinical and laboratorial	Analysis of iNOS expression in synovial biopsies of patients with TMD.	15 patients with symptomatic internal derangement (ID) or osteoarthritis (OA) and 8 control patients (3 with habitual luxation of the mandible, one with ID with clicking, and 4 with mandibular condyle fractures).	Intense iNOS immunoreactivity in both synovial lining cells and TMJ endothelial cells with internal derangement and osteoarthritis and correlation of iNOS with synovitis and not with cartilage degeneration.
Sjöstrand et al.[14]	Sweden	Laboratorial	Study of genetic variation of NOS genes in patients with migraine headache.	91 patients with migraine headache and 111 control subjects.	The phenotype and allele frequencies were similarly distributed in patients with migraine and controls, except for the iNOS allele that was significantly more common in controls. It's unlikely that variation in NOS genetics is associated with susceptibility to headache in salvage (p<0.05).

DI = symptomatic internal derangement; iNOS = inducible nitric oxide synthase; NO = nitric oxide; eNOS = endothelial nitric oxide synthase; TMD = temporomandibular disease; OA = osteoarthrosis; p = significance value.

Table 3. Expression of nitric oxide synthase in periodontal disease and orofacial pain

Authors	Country	Type of study	Investigation	Total of sample	Results of NOS in PD
Fabri et al[16]	Brazil	Clinical and laboratory	Evaluation of iNOS and eNOS expression in the gingiva samples of patients with OP and PD compared to patients with PD without chronic pain	38 (20 patients with PD and OP and 18 patients with PD)	High expression of iNOS and eNOS in patients with PD and OP compared to patients with PD without OP ($p < 0.001$)

PD = periodontal disease; OP = orofacial pain; p = significance value; INOS = inducible nitric oxide synthase; ENOS = endothelial nitric oxide synthase.

DISCUSSION

This review summarizes the findings of the scientific literature on the relation of higher NOS expression and its metabolites in OP and PD. The relevance of this review is in the observation of aspects not yet clearly understood about the participation of this enzyme and its products in two conditions prevalent in the world population[2,26-28] and their correlation[5].

Both have important impacts on quality of life and systemic repercussions[4,28-31]. Experimental animal studies have shown that ligand-induced periodontitis increased local NO production, and that mercaptoethylguanidine (MEG) treatment, a selective inhibitor of iNOS and a peroxynitrite purifier, protected against bone destruction. These findings already suggested that NO and peroxynitrite played a significant role in the pathogenesis of periodontitis[32].

Clinical studies investigating the expression of NOS in periodontitis strongly reinforced this pathological mechanism of NO. Higher numbers of NOS, mainly iNOS, were observed in sick patients compared to healthy ones, and there was a reduction in NOS in treated sites[7,19,20,21,23].

Macrophages and endothelial cells produce iNOS[21-33] and the cytokines interleukin-1beta (IL-1), tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) stimulate iNOS production in inflammatory cells and a combination of the three cytokines has a synergistic effect on the induction of iNOS[34]. As these cytokines are increased in PD[35], iNOS expression was also increased as expected.

The participation of NO in periodontitis is remarkable. Increased levels of NO production via the iNOS enzyme in periodontal tissues may lead to the activation of matrix metalloproteinases, a decrease in the level of their inhibitors and tissue destruction[23]. Although, NO is necessary for bacterial killing, the main factor for initiation of PD[36].

On the other hand, the hypothesis that NOS is related to OP[37] has been tested by investigating the expression of the enzyme, its metabolites and the genes that encode it[14,17,18,22,24]. This assumption came from the observation that administration of exogenous NO donors, such as stimulation of endogenous NO production by histamine, induced late headaches in subjects with migraine. In addition, a NOS and NG-monomethyl-L-arginine (L-NMMA) inhibitors provided pain relief in patients suffering from migraine[37].

Genes involved in the production of NO have been suggested as genetic factors for migraine because NO plays a major role in neurotransmission, inflammatory response and vasodilation[38]. eNOS releases NO from the endothelium causing smooth muscle relaxation[13.] Exogenous NO administration worsens headache pain[39] and increases peptide activity related to the calcitonin gene (CGRP)[40]. An eNOS gene mutation was associated with a reduction in basal NO[41-42] production.

In this review, clinical studies on NOS in migraine indicate the involvement of NOS in pain[17,22,24]. However, genetic studies on eNOS allele genes found no evidence of their participation in migraine susceptibility and cluster headache[14,18].

While genetically there was no different distribution in NO-coding genes in patients with OP, there was an increase in nitrate production (associated with oxidative stress) and a reduction in nitrite production (related to NO interaction with free nitrogen) in patients with migraine when compared to the control group[17]. Nitrate is generated from NO by interaction with oxygen free radical species and nitrite is constructed from NO by interaction with nitrogen free species.

Therefore, the study indicates additional oxidative stress in migraineurs, corroborating other studies which affirm that oxidative stress is involved in the pathophysiology of migraine[43]. In this sense, the evidence for the participation of NO in migraine motivates studies on the use of NOS inhibitors and other molecules within the NO signaling pathway for the promising treatment of this disorder[44].

In addition, there was no generalized increase in NOS activity in patients with migraine out of the pain crisis. However, after infusion of L-arginine (NO generator from NOS), there was a small increase in plasma levels of L-citrulline and urinary excretion of nitrite/nitrate and cGMP (metabolites and end products of NO) in patients with migraine compared to healthy volunteers.

There appears to be a decrease in NOS activity in those patients compared to healthy volunteers. The plasma level of L-citrulline and the urinary excretion of nitrite/nitrate depend largely on the vascular end of eNOS[45,46]. Therefore, the authors conclude that the modest increase in plasma excretion of L-citrulline and urinary nitrite/nitrate and cGMP after treatment with L-arginine may indicate eNOS dysfunction in patients with migraine[22].

Interestingly, NO is produced locally in the synovial lining of TMJ in the processes of internal derangement and osteoarthritis[24]. NO is produced by iNOS, which is activated by proinflammatory cytokines[47] detectable in TMD[48]. Therefore, locally produced NO may play a significant role in the pathophysiology of TMD. It has been shown that NO is a potent vasodilator[49]and angiogenic[50]. Moreover, it inhibits collagen synthesis[51] and is involved in tissue damage[52]. Furthermore, the previous studies indicated that modulation of the NO pathway could be a therapeutic strategy based on mechanisms of peripheral NO signaling that promote peripheral analgesia[53].

With all this evidence demonstrating the participation of NOS in the pathogenesis of PD and OP, there is a shortage of studies on the association of these two conditions. An earlier study by the present team demonstrated that patients with chronic OP and PD had greater expression of iNOS and eNOS when compared to patients who had only PD[16]. The exact mechanism is not yet fully understood. It can be assumed that either the patients have a greater susceptibility to pain because they have a greater expression of NOS or they have a greater expression of NOS since they are patients with chronic pain, or else they present a more diffuse pain because they have a greater expression of NOS. It may still be an association of all these aspects.

It should be noted that patients with refractory chronic pain and PD when under periodontal treatment complained significantly less about OP[5]. Thinking about the reduction in NOS in treated periodontitis sites[23], it's possible to suggest that the local reduction of NOS with less NO production could explain this pain relief, however, additional experimental studies with this purpose are necessary to comprise this idea.

CONCLUSION

Although there is sufficient evidence that NOS plays a significant role in the pathogenesis of PD and OP, genetic studies on NOS gene mutations in patients with migraine and cluster headache have not shown the expected susceptibility to pain. The association between NOS expression in PD and OP occurring simultaneously is scarce. However, there is evidence of involvement of PD aggravating OP. This review highlighted new targets and strategies for future research on NOS in two very common correlated diseases such as PD and OP.

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