Nitric oxide levels in periodontitis with relation to obesity

Pradnya Shree Dhotre[1,*], Rajashree Basawraj Bhogade[2], Abdul Kayyum Shaikh[3]

[1]Assistant Professor, [3]Professor and Head, Ashwini Rural Medical College,
Hospital and Research Centre, Kumbhari, Solapur
[2]Assistant Professor, Grant Medical College, Mumbai

*Corresponding Author:
E-mail: pradnyashree@yahoo.co.in

Abstract

Background: There is growing evidence that nitric oxide (NO) is critically involved in obesity and its clinical consequences like cardiovascular disease, hypertension and diabetes. However the role of nitric oxide in oral condition like periodontitis in relation to obesity is not clearly understood. Hence we have attempted to elucidate role of serum and salivary nitric oxide in obese and non-obese periodontitis patients.

Method: We investigated 100periodontitis patients; out of which 50 were obese (Group C) and 50 were non-obese patients (Group B) along with 50 normal weight, age matched healthy controls (Group A). Various dental and clinical parameters as well as body measurements were assessed.

Results: Group B and Group C showed a marked increase (P <0.001) in clinical attachment loss and probing depth when they are compared with Group A. Group B and Group C patients are also compared for body mass index and waist circumference with Group A.BMI and WC was found to be non significant in Group B when compared with Group A. Group C patients exhibited a highly significant increase in BMI and WC when compared with Group A. Group C patients also showed a highly significant increase in BMI and WC when compared with Group B.

The study also documented a significant increase in serum & salivary nitric oxide levels in Group B and C when compared to Group A. (P<0.001).Obese periodontitis patients (Group C) revealed highly significant increase (P <0.001) in nitric oxide levels than non-obese periodontitis patients (Group B).

Conclusion: Obesity can be considered as an important contributing factor in enhancing the severity of periodontitis as well as nitric oxide production in these patients.

Keywords: Nitric oxide, Obesity, Periodontitis.

Introduction

Periodontal diseases are a group of chronic inflammatory diseases, involving the soft tissue and bone surrounding the teeth in the jaws, or known as periodontium. Periodontal diseases including gingivitis and periodontitis are among the most common dental diseases after tooth decay in humans. Periodontal diseases are characterized by inflammation of tooth-supporting tissues caused by bacterial infection[1]. Gingivitis is a very common and reversible condition, which manifests as redness, gum swelling and bleeding during tooth-brushing and flossing. Gingivitis may progress into periodontitis which promote damage of periodontal tissues, ligament and alveolar bone if left without appropriate treatment. Teeth may become mobile and in due course exfoliates following the decline of periodontal supporting tissues[2]. This process is attributed to the release of toxic products from the pathogenic bacterial plaque in addition to the inflammation of gingival tissues elicited by the host response[3]. Nitric oxide (NO), a colorless inorganic free radical gas, is produced in all tissues[4,5] and plays a role in almost every biologic system[6].

Nitric oxide is synthesized from L-arginine by a family of enzymes called nitric oxide synthase. There are 3 forms i) Type 1-NO synthase-neuronal enzyme ii) Type 2-NO synthase-inducible enzymes(iNOS) found in macrophages iii) Type 3-NO synthase–endothelial cell enzymes (eNOS). Nitric oxide is a short living product of nitrogen metabolism, produced by many cells in the organism with much important physiological role. Macrophages and other inflammatory cells can induce its synthesis and release. The most important inductor of NO synthesis are bacterial products.[7,8]

Important functions of nitric oxide are i) it acts as an endothelial derived relaxer of vascular smooth muscle, an inhibitor of platelet aggregation and adhesion and neuronal messenger. ii) 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 on cellular proteins, DNA and lipids leading to periodontitis.[9]

Andrukhov O et al[10] studied the production of nitric oxide & lipid metabolism in periodontitis patients wherein they documented a significantly lower serum & salivary levels of nitric oxide metabolites in periodontitis patients than control group. On the other hand, a study[11] reported a significantly increased salivary nitric oxide levels in gingivitis & periodontitis patients as compared to controls. The reports of role of nitric oxide in periodontitis are inconsistent.

Obesity is a condition involving an excess accumulation of body fat, and the prevalence of it is rapidly increasing worldwide. Excessive weight and obesity are leading to nutrition related disorders of clinical and public health concern.[12,13]

It has been reported that obesity is related to the presence of risk factors for periodontal disease, such as biofilm and dental calculus[14]. There was a[15] report that periodontal disease was positively associated with body mass index (BMI) whereas another study[16] did not observe any association between obesity in early adulthood and severity of periodontitis at the age of 60–70 years. A systematic review of Suvan et al. observed heterogeneity among studies, but all of them have reported higher odds of periodontitis among obese individuals[17]. However, the effect of obesity on nitric oxide in periodontitis patients has not been studied extensively. Recognizing this lack of information, we attempted to expose the probable role of obesity on nitric oxide in periodontitis patients.

Materials and Methods

This was a case-control study comprising of 150 subjects visiting to the dental outpatient department of Ashwini Rural Medical College, Hospital and Research Centre, Kumbhari, Solapur, Maharashtra. Out of them 50 were volunteers (Group A) and 100 were periodontitis patients.100 periodontitis patients were further grouped into non-obese (Group B)& obese (Group C). Dental examination was done by a periodontist using William's periodontal probe. The study was approved by institutional ethical committee.

The purpose of our study was explained to all subjects and their consent was taken. All the subjects were in the age range of 25-55 years irrespective of the gender. Clinical Examination: The patients were screened for obesity based upon BMI and WC. The currently recommended cut-offs of BMI recommended by World Health Organization include 18.5 - 24.9 kg/m2 for normal, 25.0 - 29.9 for overweight and >30 kg/m2 for obesity.[18] Common surrogate measure of abdominal obesity is WC. Waist circumference is a simple, easily obtainable anthropometric parameter, which can be assessed in the outpatient setting. The currently recommended cut-offs of WC >102 cm in men and >88 cm in women. BMI was obtained by measuring the heights of all individuals. They were also accurately weighed with digital balance.

BMI is defined as the individuals body weight divided by the square of their height and measured in Kg/m2. (BMI=weight (kg) /height (m^2).

Waist circumference was measured in centimeters at the level of umbilicus. The measurements were taken after participants exhaled.

Inclusion criteria:
A) Healthy controls: 50 healthy, normal weight and age matched volunteers were enrolled.
i) They were without periodontitis.
ii) They were not obese.
iii) They were not suffering from any type of systemic disease/ses.

B) Study group subjects:
I) 50 non-obese periodontitis patients were included who had
i) BMI ≤ 25 kg/m^2
ii) Waist circumference: ≤ 88cm.
iii) Clinical attachment loss of ≥ 4mm.
iv) Periodontal pocket depth ≥ 4mm.
v) Bleeding on gentle probing.
vi) Not undergone any periodontal treatment for at least six months prior to sampling.

i) 50 obese periodontitis patients were included who had-
ii) BMI ≥ 30Kg/m^2
iii) Waist circumference:≥ 88cm.
iv) Clinical attachment loss of ≥ 4mm.
v) Periodontal pocket depth ≥ 4mm.
vi) Bleeding on gentle probing.
vii) Not undergone any periodontal treatment for at least six months prior to sampling.

Exclusion criteria:
i) Subjects suffering from any systemic disease/s.
ii) Subjects with hypertension.
iii) Requiring antibiotic or anti-inflammatory drug therapy.
iv) Having history of alcoholism, smoking.
v) Subjects regularly using mouth washes.eg. chlorhexidine mouth wash.

2 ml unstimulated whole saliva was collected in disposable tubes and centrifuged at 3000rpm for 10 minutes. Supernatant was used for analysis of salivary nitric oxide. 3 ml blood was collected in sterile heparinised bulb and was allowed to clot. Serum was separated by centrifugation at 3000 rpm for 10 minutes at room temperature. Both the samples were analyzed on the same day of collection.

Serum & salivary nitric oxide was estimated by the method of Cortas NK and Wakid NW [19] in which nitrate is reduced to nitrite by copper coated cadmium granules. This nitrite produced is determined by diazotization of sulphanilamide coupling to naphthy-lethylenediamine to form purple complex. The intensity

of colored complex is measured at 540 nm using colorimeter of kanad vidyut(HANS 253).

Statistical Analysis

Descriptive statistics such mean and SD was used. Comparison between three groups was done by using ANOVA test followed by post hoc Bonferroni Multiple Comparisons Test. A p-value less than 0.05 was considered as significant.

Observations and results:

Table 1: Shows the levels of clinical attachment loss (CAL) & periodontal pocket depth (PD), Body Mass Index (BMI) and waist circumference (WC) in healthy controls and periodontitis patients

Parameters	Healthy controls (n=50) Group A	Non-obese periodontitis patients(n=50) Group B	Obese-periodontitis patients (n=50) Group C
CAL (mm)	2.22 ± 0.53	4.6 ± 0.52 *	6.8 ± 1.03 * #
PD (mm)	1.45 ± 0.37	4.3 ± 0.44 *	7.31 ± 0.72 * #
BMI (Kg/m^2)	22.92 ± 5.7	25.3 ± 5.4	35.58 ± 6.9 * #
WC (cm)	83.52 ± 6.9	84.4 ± 3.9	105.34 ± 6.3 * #

Compared to healthy controls * P < 0.001
Compared Non-obese & Obese # P< 0.001

Table 2: Depicts the levels of serum and salivary nitric oxide in healthy controls and periodontitis patients

Parameters	Healthy controls (n=50) Group A	Non-obese periodontitis patients(n=50) Group B	Obese-periodontitis patients (n=50) Group C
Salivary nitric oxide (µmol/L)	27.50 ± 2.23	53.93 ± 9.54 *	78.02 ± 4.82 * #
Serum nitric oxide (µmol/L)	31.64 ± 4.45	54.58 ± 6.43 *	96.5 ± 7.42 * #
Correlation coefficient (r) value	0.003	-0.011	-0.04

Compared to healthy controls *P<0.001
Compared Non-obese & Obese # P< 0.001

Table no. 1 shows the levels of dental parameters like clinical attachment loss and periodontal pocket depth along with obesity markers like body mass index and waist circumference in healthy controls and periodontitis patients. Significantly elevated (p<0.001) levels of clinical attachment loss, periodontal pocket depth were observed in Group B and Group C when they are compared with Group A whereas there was no change observed in case of body mass index and waist circumference in Group B when compared with Group A. Group C exhibited significantly increased body mass index, waist circumference, clinical attachment loss and periodontal pocket depth when compared with Group B patients (p<0.001). Our results are consistent with Buhlin K. et al[20]and Saito T et al.[21] Obesity has been postulated to reduce blood flow to the periodontal tissues, promoting the development of periodontal disease.[22]

Table no. 2 depicts the levels of nitric oxide in serum and saliva. The levels of nitric oxide in serum and saliva were significantly increased in Group B and

C when compared with Group A (P<0.001). The study also showed a significant increase (p<0.001) in serum and salivary nitric oxide levels in Group C than Group B.

Discussion

Periodontitis, mainly caused by bacteria is characterized by inflammation and destruction of attachment apparatus of the teeth. Inflammation and infections are the hallmarks of periodontitis.

In the present study, periodontitis is affected by the elevated obesity markers like BMI and WC. Obesity has been found to be significantly associated with alveolar bone loss among adults, with a stronger association in females[23]. It has been postulated that obesity reduces blood flow to the periodontal tissues, promoting the development of periodontal disease[22].

Furthermore, obesity may enhance immunological or inflammatory disorders, which might be the reason why the obese subjects, tend to exhibit escalating poor periodontal status relative to non-obese individuals[24].

Nitric oxide is produced in many different cells and is involved in the regulation of physiological and pathophysiological processes as inflammation, vasodilatation, and metabolism. Present study documented increased nitric oxide levels in Group B and Group C as compared with Group A (P < 0.001).

NO levels may be increased due to increased activity of iNOS. LPS of the dominant Gram negative bacteria in periodontitis may directly trigger resident or immigrant cell populations for expression of iNOS or provoke an immune response that result in release of pro-inflammatory cytokines that subsequently may induce iNOS[25,26]

Being a free radical, it also participates in the suppression of proteoglycan and collagen synthesis and this may be one of the mechanisms by which NO contributes to the reflective early loss of collagen in gingival lesions.

Our results are in consistent with Menaka KB[27] et al who assessed the levels of nitric oxide in serum in chronic periodontitis patients and correlated these levels with severity of periodontitis. Periodontitis patients showed significantly elevated nitrite levels than healthy subjects. These findings help to understand role of nitric oxide in progression of disease.

Further, a high increase in nitric oxide level was observed in Group C as compared with Group B. Obese periodontitis patients exhibited a high increase in nitric oxide level in serum as well as saliva than non-obese periodontitis patients. Increased NO levels in Group C could be due to increase in NO production through cytokine-induced iNOS induction. In addition, it has also been shown that obese subjects have a capacity for increased NO production.[28]

Our results are in accordance with a study documenting that NOS activity is present in human adipose tissue and produces NO through inducible NOS from a source of NO, adipose tissue per se[29]. Another study[30] also reported that NOS activity and inducible NOS protein were present in human subcutaneous adipose tissue.

There are reports of increased serum NOx levels in a group of diseases and the study suggested that it may be due to increase in NO production through cytokine-induced iNOS induction.[29,30,31] Thus, there are reports about increased nitric oxide and it's metabolites in normal study population as well as disease conditions; but very few reports are available documenting increased nitric oxide levels in periodontitis in relation to obesity. Present study results revealed a positive involvement of obesity in aggravating periodontitis as well as increased nitric oxide production in the disease.

Conclusion

Obesity not only aggravates periodontitis but also contributes to increased nitric oxide production in these patients.

Conflict of Interest: None
Source of Support: Nil

References

1. Jin LJ, Chiu GKC, and Corbet EF. Are periodontal diseases risk factors for certain systemic disorders—what matters to medical practitioners? Hong Kong Medical Journal.2003;9(1):31–7.
2. Tonetti MS and Van Dyke TE. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases: Journal of Periodontology.2013; 84(4): supplement, S24– 9.
3. K.S.Kornman, R.C.Page, M.S.Tonetti. The host response to the microbial challenge in periodontitis: assembling the players: Periodontology 2000;1997;14: 33–53.
4. Higashino H, Tabuchi M, Yamagata S, Kurita T, Miya H, Mukai H, et al. Serum nitric oxide metabolite levels in groups of patients with various diseases in comparison of healthy control subjects. Journal of Medical Sciences. 2010; 10: 1 – 11.
5. Knowles RG, Moncada S. Nitric oxide synthases in mammals. Biochem J. 1994; 298 (Pt 2): 249 – 258.
6. Yoon S, Moon J, Shin C, Kim E, Jo SA, Jo I. Smoking status-dependent association of the 27-bp repeat polymorphism in intron 4 of endothelial nitric oxide synthase gene with plasma nitric oxide concentrations. Clinica Chimica Acta. 2002; 324: 113 – 120.
7. Tsikas D. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L- arginine/nitric oxide area of research. J Chromatogr B Analyt Technol Biomed Life Sci. 2007; 851: 51 – 70.
8. Michael T, Feron O. Nitric oxide synthesis: which, where, how and why? J.Clin.Invest. 1997;100 (9): 2146-52.
9. Sunita M, S. Shanmugham. Evaluation of salivary nitric oxide levels in oral mucosal diseases: a controlled clinical trials. Ind. J. Dent Res 2006. 17 (3): 117-120.
10. Andrukhov O et al. Nitric oxide production, systemic inflammation and lipid metabolism in periodontitis patients: possible gender aspect. J.Clin.periodontol 2013;40(10): 916-923.
11. Parwani SR, Chitnis PJ, Parwani RN. Salivary nitric oxide levels in inflammatory periodontal disease-A case-control and interventional study. Int J Dent Hygiene. 2012; 10:67-73.
12. Morley JE, Flood JF. Effect of competitive antagonism of NO synthetase on weight and food intake in obese and diabetic mice. Am J Physiol.1994; 266: R164–8.
13. Morley JE, Kumar VB, Mattammal M, Villareal DT. Measurement of nitric oxide synthase and its mRNA in genetically obese (ob/ob) mice. Life Sci. 1995;57:1327–31.
14. de Castilhos ED,et al. Association between obesity and periodontal disease in young adults: a population-based birthcohort. J Clin Periodontol. 2012;39: 717–724.
15. Morita I et al. Five-year incidence of periodontal disease is related to body mass index. Journal of Dental Research. 2011; 90:199–202.
16. Linden G., Patterson C., Evans A., Kee F. Obesity and periodontitis in 60–70-year- old men. Journal of Clinical Periodontology. 2007; 34:461–466.
17. Suvan J., D'aiuto, F., Moles D. R., Petrie A., Donos, N. Association between over- weight/obesity and periodontitis in adults. A systematic review. 2011: Obesity Reviews 12, e381– e404.

18. Anoop Misra et al. Consensus Statement for Diagnosis of Obesity, Abdominal Obesity and the Metabolic Syndrome for Asian Indians and Recommendations for Physical Activity, Medical and Surgical Management. JAPI. Feb 2009; 57:163-170.

19. Cortas N, Wakid N. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem. 1990; 36(8):1440-1443.

20. Kare Buhlin , Anders Gustafsson, A. Graham Pockley, Johan Frostegard Bjorm Klinge 2003 Risk factors for cardiovascular disease in patients with periodontitis Eur. Heart J.2003; 24:2099 –2107.

21. Saito T et al. Relationship between obesity, glucose tolerance, and periodontal disease in Japanese women: the Hisayama study. J Periodontal Res. 2005;40(4):346-53.

22. Shuldiner A, Yang R, Gong D. Resistin, Obesity and insulin resistance-the emerging role of the adipocytes as an endocrine organ. New England journal of medicine 2001; 345:1345–46.

23. Al abdulkarim M et al. Alveolar bone loss in obese subjects. Journal of the International Academy of Periodontology 2005; 7(2):34–8.

24. Nishida N, Tanaka M, Hayashi N. Determination of smoking and obesity as periodontitis risks using the classification and regression tree method. Journal of Periodontology 2005;76: 923–8.

25. H. El-Sayed Amin. Relationship between overall and abdominal obesity and periodontal disease among young adults. EMHJ. 2010; 16(4):429-33.

26. AC Batista, TA Silva, JH Chun, VS Lara. Periodontal Diseases: Pathogenesis Nitric oxide synthesis and severity of human periodontal disease. Oral Diseases. 2002;8: 254–60.

27. Menaka K. B. Estimation of nitric oxide as an inflammatory marker in periodontitis. Journal ofIndian Society of Periodontology. 2009 May-Aug;13(2):75-78.

28. Mather KJ, Lteif A, Steinberg HO, Baron AD. Interactions between endothelin and nitric oxide in the regulation of vascular tone in obesity and diabetes. Diabetes. 2004; 53: 2060 – 2066.

29. Jong Weon Choi Soo Hwan Pai, Soon Ki Kim, Masafumi Ito, Chang Shin Park and Young Nam Cha. Increases in Nitric Oxide Concentrations Correlate Strongly with Body Fat in Obese Humans. Clinical chemistry. 2001;47(6):1106-1109.

30. Elizalde M et al. Expression of nitric oxide synthase in subcutaneous adipose tissue of nonobese and obese humans. J Lipid Res 2000;41:1244–1251.

31. Higashino H et al. Serum nitric oxide metabolite levels in groups of patients with various diseases in comparison of healthy control subjects. Journal of Medical Sciences. 2010;10: 1 – 11.