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ESTIMATION OF NITRIC OXIDE AS AN INFLAMMATORY MARKER IN PERIODONTITIS

Dental Science

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ABSTRACT

INTRODUCTION: In periodontal diesases,inflammatory stimuli increase production of nitric oxide which causes detrimental as well as beneficial role.

AIM: 1) To assess the level of NO(nitric oxide) in serum in chronic periodontitis and healthy patients
2) To correlate these levels with the severity of periodontitis.

MATERIALAND METHOD: Two group selected: Group I Healthy subjects without chronic periodontitis and other systemic diseases .Group II Chronic periodontitis patients. Blood sample was collected. Concentration of nitric oxide measured in serum by griess reagent.

RESULT: The results of our study showed a higher value in the serum levels of NO(nitric oxide) in group II when compared to group I with mean values (1.23±0.04) µM/50 µl, (0.013±0.004) µM/50µl respectively.

CONCLUSION: Nitric oxide level in serum is incresed in chronic periodontitis patients than healthy patients.

KEYWORDS

Inflammatory marker, nitric oxide, periodontitis

INTRODUCTION:

Periodontal disease, encompassing both gingivitis and periodontitis, is a host- mediated inflammatory process initiated by oral bacterial insult which may result in significant alterations in the normal structure and/or function of the supporting tissues of the dentition.The innate host response is directed by gingival epithelial cells and polymorphonuclear leukocytes (PMNs).If this process is ineffective then various immune and inflammatory cells are recruited to the area, a multitude of cytotoxic and inflammatory mediators are released,resulting in enhanced inflammation and tissue destruction.

Thus, recent studies have primarily been directed at modification of the host's contribution to this disease process. The inhibition of a variety of key mediators like nitric oxide, is currently receiving much attention due to its association with periodontal disease.

Nitric oxide (NO) is a free radical produced from L-arginine through the action of isoenzymes, globally named NO synthases (NOS).NO act as both beneficial role as well as detrimental role . When NO is locally produced in high concentrations, it can act as a cytotoxic molecule, against cells infected by fungus, bacteria, protozoa, as well as tumoral cells and cells close to the production site, possibly resulting in tissue destruction.It is currently known that NO has an important participation in bone metabolism regulation, directly acting over clastic cells **.** The presence of this gas in periodontal disease may reflect the participation of an additional mediator of bone resorption responsible for disease progression.(**Ralston SH, Grabowski PS ,1996)**

This NO-mediated cytotoxicity probably occurs in combination with the action of metalloproteinases and collagenases, liberated by activated macrophages, polymorphonuclear cells and resident fibroblasts.

MATERIALS AND METHODS:

Study design:

The study group comprised of 60 patients , of age group ranging from 20-60 years ,reporting to department of priodontia ,GDCH ahemdabad .They are divided into 2 groups.

Group 1: I Healthy subjects without chronic periodontitis and other systemic diseases
Group II Chronic periodontitis patients.

INCLUSION CRITERIA

- Age group: 20-60 years of either sex.
- Subjects with a diagnosis of chronic generalized periodontitis based on the clinical criteria proposed by the 1999 world workshop for classification of periodontal diseases and conditions. Patients diagnosed with chronic periodontitis, more than 30%sites with clinical attachment loss ≥4mm and probing pocket depth ≥ 5mm at baseline.
- Healthy subjects without chronic periodontitis and other systemic diseases as control group.
- Subjects who have not received periodontal therapy within the last 6 months.

- **Exclusion criteria**
- Subjects with any other systemic disease
- Subjects who have used anti-inflammatory drugs or antimicrobial drugs within a 3-month period before the study began
- Subjects who have used any antibiotic within previous 3 months
- Pregnant or lactating females
- Subjects who are smokers

The following clinical parameters were assessed at six sites of all teeth (Mesiobuccally, mid- buccal, disto buccal, mesio-lingual, mid lingual and disto-lingual) using William's graduated periodontal probe.

Clinical parameters were assessed:

1)Plaque index (silness and loe ,1964)
2)Gingival index (loe and silness 1964)
3)Probing pocket depth (PPD)
4)Clinical attachment level (CAL)

Collection of sample

A blood sample (2ml of venous blood) was collected from the antecubital fossa by venipuncture using a 24 guage needle with 2ml syringe, after periodontal examination. Serum was separated from blood by centrifuging the sample at 3000rpm for 10 min. and stored till the assay procedures.

Biochemical assay

NO(nitric oxide) is a highly reactive free radical gas and remains stored in serum as nitrates (NO_3-) or nitrites (NO_2-). Thus, NO concentration can be estimated by measuring concentrations of nitrates (NO_3-) and nitrite (NO_2^-) in combination. Nitrates can be estimated by Griessreagents(1% sulphanilamide, 0.1% naphthylethylenediamine dihydrochloride and 2.5% hydrochloric acid) colorimetric reaction can be used to measure the nitrite levels (No_2-).

The concentrations of nitrite were calculated from a standard curve established with serial dilutions of sodium nitrite.

Formula for measurement of nitrite levels

OD of sampl × Concentration of standard (µM) × total volume (µl)
---------- =Concentration of sample(µM)
OD of standard × 50µl serum

OD - Optical density

RESULTS:

All the 60 subjects completed study. Table no.1 showed comparison of nitric oxide level (µm/50µl) between case and control groups

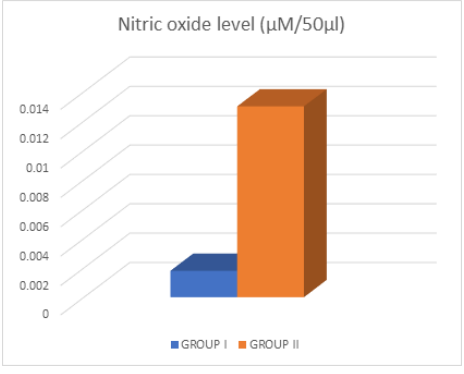
Table no.1 comparison of nitric oxide level (µm/50µl) between case and control groups:

Nitric oxide uM/50µl	Group	N	Mean	St. Deviation	t value	p value
	Group I (control)	30	0.00182	0.000381	12.790	0.001
	Group II (case)	30	0.01309	0.004744		

This table shows the t-test for equality of means. P < 0.05, on comparing between the two group which was statistically significant increase in NO expression in periodontal disease.

Graph:

Comparision of nitric oxide level (um/50µl) (mean) in group i (control) and group ii(case):



Graph shows a bar graph comparing nitric oxide levels in the case and control groups.

DISCUSSION:

Periodontitis is a inflammation and infectious disoder of periodontal supporting tissue.Many factors are e, are initiated and sustained by the subgingivalmicroflora.Some of these substances can directly damage host cells and tissues, while others activate endogenous cellular and humoral inflammatory systems which secondarily affect the integrity of the periodontium.

Many studies have been conducted to elucidate the role of nitric oxide in the immuno-inflammatory response and characterize its potential involvement in the pathogenesis of periodontal disease. Numerous reports have established the presence of inducible nitric oxide synthase in gingival tissues and have demonstrated its subsequent upregulation in humans and animals with periodontal disease.(Matejka et al 1998)
NO(nitric oxide) is generated within biologic tissues via the enzymatic conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS). This enzyme exists in the body as three distinct isoforms: neuronal (nNOS or NOS-I), inducible (iNOS or NOS-II) and endothelial NOS (eNOS or NOS-III).**Lohinai and coworkers** studied that several groups that the gingival epithelium expresses iNOS with most activity being localized to the basal keratinocytes and intraepithelial macrophages in periodontitis patients .Low levels of enzyme expression have even been observed in the tissues of periodontally healthy patients, leading to the presumption that iNOS may be constitutively expressed or continually induced in the gingival epithelium**.Gowen et al** demonstrated that NO could either stimulate or inhibit the osteoclast activity of resorption in a dose-dependent pattern of NO(nitric oxide) concentration .

NO(nitric oxide) has been considered an important molecular signal in a wide variety of tissues and may play a significant role as a cytotoxic mediator of the nonspecific immune response, with beneficial and harmful effects. Inducible NO synthase (iNOS) is expressed in response to inflammatory stimuli, resulting in higher amounts of NO production has been demonstrated in periodontal disease tissues. Because of the reactivity of NO and its short life direct measurement of NO from body fluids has been thought hard to be perform. Thus nitrite, a end product of NO oxidation has been measured.

The results of our study showed a higher value in the serum levels of NO(nitric oxide) in group II when compared to group I with mean values (1.23±0.04)µM/50 µl, (0.013±0.004) µM/50µl respectively. This was in accordance to the previous study where increased NO(nitric oxide) levels were found in saliva and GCF of subjects with chronic periodontitis as compared to healthy subjects.(Cope P Andrew 2008).NO(nitric oxide) is known to potentiate matrix degradation which includes suppression of proteoglycan and collagen synthesis and upregulation of metalloproteinases activity Currently an alternative approach to reduce periodontal inflammation using NOS inhibitors has been proposed. Selective inhibitors of iNOS - mercaptoethylguanidine, guanidoethyldisulfide, aminoguanidine- are shown to reduce peroxynitrite formation as well as inhibiting prostaglandin production via inhibition of COX. It is therefore logical that modulation of this biological messenger might be useful for the treatment of chronic inflammatory diseases such as periodontal disease.

Further studies must be undertaken to assess nitrite levels in other forms of periodontitis and gingivitis and compared within subgroups to reveal expression of NO during different stages of periodontal disease progression.

CONCLUSION:

Analyzing our study we concluded that NO act as a protective role in normal physiologic condition but it may also act as a detrimental role in excess inflammatory condition and causes tissue destruction.

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