Effect of oxygen hyperbaric therapy on malondialdehyde levels in saliva of periodontitis patients with type 2 diabetes mellitus

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ABSTRACT

Background: Lipid peroxidation (LPO) has implication in pathogenesis of several pathological disorders including periodontitis. Malondialdehyde (MDA) is end products of lipid peroxidation. Hyperbaric Oxygen Therapy (HBOT) involves the administration of 100% oxygen under atmosphere pressure and has been used as an adjuvant therapy, while saliva is a diagnostic tool for many oral and systemic diseases. Purpose: The aim of this study was to examine the effect of HBOT on malondialdehyde in saliva to measure lipid peroxidation in periodontitis patients with type 2 Diabetes Mellitus (DM). Methods: Eight regulated type 2 DM subjects were compared to ten unregulated periodontitis patients type 2 DM (n = 18). Pre HBOT and after 10 days HBOT with 2.4 ATA dose, unstimulated whole saliva samples from study subjects were collected, centrifuged at 3000 g for 15 minutes and were then stored at −80°C until analyzed. The MDA level was determined with 2-thiobarbituric acid by a colorimetric method at 532 nm. Results: Data showed that regulated type 2 DM had lower level of MDA (3.08 ± 0.62 ug/mol) compared with unregulated periodontally type 2 DM (5.88 ± 1.04 ug/mol) (p>0.05). MDA levels were significantly lower after HBOT in regulated DM (2.30 ± 0.46 ug/mol) compared with unregulated periodontally type 2 DM (4.09 ± 0.77ug/mol) (p < 0.05). The regulated DM subjects and post HBOT showed MDA levels lower than the periodontally-unregulated group significantly. Conclusion: The saliva of periodontitis patients with unregulated type 2 DM showed more lipid peroxidation than regulated DM type 2. HBOT decreased MDA levels in regulated and unregulated type 2 DM with periodontitis.

Key words: hyperbaric oxygen, malondialdehyde, saliva

INTRODUCTION

Periodontal disease has been reported as the sixth complication of diabetes, along with neuropathy, nephropathy, retinopathy, and micro- and macrovascular diseases. Periodontal infection and gingival inflammation, a number of other oral complications have often been reported in patients with diabetes. These include xerostomia, dental caries, candida infection, burning mouth syndrome, lichen planus, and poor wound healing. Many studies have been published describing the bidirectional interrelationship exhibited by diabetes and periodontal disease. Studies have provided evidence that control of periodontal infection has an impact on improvement of glycemic control evidenced by a decrease in demand for insulin and decreased hemoglobin A1c levels.

Diabetes increases the risk of developing periodontitis. Epidemiologic research supports an increased prevalence and severity of attachment loss and bone loss in adults with diabetes. Subjects with type 2 diabetes had approximately threefold increased odds of having periodontitis compared with subjects without diabetes. Diabetes also may increase the risk of experiencing continued periodontal destruction.

Hyperbaric Oxygen Therapy (HBOT) is a mode of medical treatment in which the patient is entirely enclosed...
in a pressure chamber breathing 100% oxygen at a pressure greater than one atmosphere.\textsuperscript{4,5} Hyperbaric Oxygen Therapy (HBOT) has been successfully used for the treatment of a variety of clinical conditions related to hypoxia, including acute carbon monoxide intoxication, air embolism, soft tissue infections, radiation necrosis and impaired wound healing,\textsuperscript{5} but the effects of HBOT on oxidant/antioxidant metabolism are controversial and its effects on periodontitis with diabetes are not known.

Reactive Oxygen Species (ROS) are derived from a variety of sources, such as the xanthine oxidase system, activated neutrophils, the electron transport chain of mitochondria, and the arachidonic acid pathway. Since free radicals have very short half-life, the clinical assessment of oxidative stress in vivo is based on the measurement of different stable oxidized products of modified lipids, proteins, carbohydrates and nucleic acids.\textsuperscript{6,7} Free radicals can be defined as molecules or molecular fragments with an unpaired electron which imparts certain characteristics to the free radicals such as reactivity.\textsuperscript{4,8} Reactive free radicals are able to produce chemical modifications and to damage proteins, lipids, carbohydrates and nucleotides in the tissues.\textsuperscript{5,9} It’s known that free oxygen radicals are probably mediators for tissue damage in periodontal disease.\textsuperscript{3} Reactive free radicals may damage cells by initiation of lipid peroxidation that causes profound alteration in the structural integrity and functions of cell membranes. Free radical induced lipid peroxidation has been implicated in the pathogenesis of several pathological disorder.\textsuperscript{5,6,9}

Malondialdehyde (MDA), one of the most widely used biomarkers of oxidative stress, is produced enzymatically by the breakdown of unstable hydroperoxides during peroxidation of unsaturated fatty acyl moieties and used as a stable index of free radical attack on membrane phospholipids.\textsuperscript{6} The concentration of lipid peroxidation product, malondialdehyde (MDA), is most widely used.\textsuperscript{7} Saliva is a diagnostic tool for many oral and systemic disease. The detection of salivary MDA level may provide additional advantages in elucidating the pathogenesis of periodontal disease.\textsuperscript{2}

There is increasing evidence about the ability of HBOT to induce cellular protection in a similar manner with other protective oxidative stress mechanisms.\textsuperscript{7} Repeated HBOT exposure significantly attenuated the immunomediators, free radicals, and mortality in endotoxic rats.\textsuperscript{10} These protective effects of HBOT may be related to the fact that reactive oxygen species can trigger a wide variety of cellular mechanisms by functioning as signal molecules.\textsuperscript{3,10}

The aim of this study was to examine the effects of HBOT on the levels of MDA in noninsulin dependent diabetic patients with periodontitis who were exposed to hyperbaric oxygen for the treatment of periodontitis.

**MATERIALS AND METHODS**

Lipid peroxidation products malondialdehyde (MDA) was analyzed in patients of periodontitis with diabetic. Eighteen type 2 DM patients with periodontitis who received HBOT were included in the study. Eight periodontitis with regulated type 2 DM patients (HbA1c < 6,5) were compared to ten periodontitis with unregulated type 2 DM patients (HbA1c ≥ 7 ) who received HBOT. Local ethics committee approved the study protocol and all study subjects gave their informed consents. Patients were followed by the same physician responsible for diabetes control, wound care and antibiotic therapy according to the clinical and laboratory findings, and were given a diet depending on their metabolic needs without vitamin supplementation. All diagnostic tests were evaluated for diagnosing periodontitis disease.

Hyperbaric Oxygen Therapy (HBOT) was carried out in a multiphase hyperbaric chamber once a day for ten days. The treatment protocol was inhalation of 3 × 30 min periods of 100% oxygen at a pressure of 2,4 ATA, interspersed with 5 min periods of air breathing.\textsuperscript{11}

Samples were taken before HBOT with dose 2,4 ATA 3 × 30 minutes and after the exit from the chamber, on the day of 10th HBO sessions. Unstimulated whole saliva samples (2,5 ml) were collected from each patient in standard sterile vacuum tubes. Saliva samples were immediately centrifuged at 3000 g for 15 min and were then stored at −70°C until analyzed. The measurement lipid peroxidation products of malondialdehyde (MDA) levels were analyzed by modification Thiobarbiturat Acid Substance (TBARS) condense with two equivalents of thiobarbituric acid to give a fluorescent red derivative that can be assayed spectrophotometrically.\textsuperscript{2}

The pair t-test was used to compare both of groups. All hypothesis tests were two-tailed with statistical significance assessed at the p value < 0.05 level with 95% confidence intervals (CI). The data was expressed as the mean ± SEM. Statistical computations were calculated using SPSS 14 for windows software (SPSS Inc, Chicago, IL, USA).

**RESULT**

Statistic analysis showed that the regulated type 2 DM had lower level of MDA (4,09 ± 0,77ug/mol) compared with unregulated periodontally- type 2 DM subjects (5,88 ± 1,04 ug/mol) (p > 0.05). MDA levels were significantly lower after HBOT in regulated DM (2,30 ± 0,46 ug/mol) compared with unregulated periodontally-type 2 DM (3,08 ± 0,62 ug/mol) with p < 0.05. The regulated DM subjects and post HBOT showed MDA lower levels than the periodontally-unregulated group significantly.
DISCUSSION

Hyperglycemia stimulates the production of advanced glycosylated end products, enhances the polyol pathway, and activates protein kinase C, which may lead to increased oxidative stress.\(^2\) In the pathogenesis of diabetic complications, there is an increasing evidence for the role of oxidative stress, which is manifested by enhancing lipid peroxidation, superoxides,\(^9\) nitric oxide,\(^{12}\) increased protein, and DNA damage.\(^9\) Abnormal nitric oxide (NO) synthesis has been implicated in the pathogenesis of both periodontal disease and diabetes mellitus. In diabetic patients, increased inducible NO synthase in inflammed gingiva correlated with NO in gingival crevicular fluid.\(^{12}\)

Nitric oxide, a toxic free radical with multiple biological functions, including inhibition of neutrophil chemotaxis, adhesion to endothelium, and upregulation of tumor necrosis factor alpha, is generated by oxidative deamination of L-arginine by nitric oxide synthase (NOS).\(^{12,13}\) The inducible form of NOS (iNOS) is rapidly and durably expressed by inflammatory cells in response to bacteria or their products, such as lipopolysaccharide (LPS). Small amounts of NO induced by constitutive NOS are considered beneficial, whereas excess iNOS-induced NO can mediate cell and tissue injury. Periodontal diseases are chronic inflammatory infections associated with gram-negative bacteria, including *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans*, which stimulate macrophages to generate NO. Moreover, NO is increased in inflammed gingival tissue, and a selective iNOS inhibitor can prevent bone destruction in ligature-induced rodent periodontitis.\(^{12}\)

Inducible NOS, independent of calcium, catalyzes generation of large amounts of NO (nanomolar concentrations) over extended periods (hours or days) in response to inflammatory stimuli such as cytokines and lipopolysaccharides. Nitric Oxide Synthase (NOS) inhibition stabilizes oxygen to stimulate and prevent peroxidation during NO generation.\(^{13}\) During oxidant stress superoxide is readily generated. Under basal condition, nitric oxide undergoes a rapid biradical reaction with superoxide anions to form peroxynitrite. This reaction, and hence the formation of peroxynitrite is augmented in inflammatory conditions. NO is the only currently known biological molecule produced in high enough concentrations to react fast enough with superoxide to outcompete endogenous superoxide dismutase.\(^{8,13}\)

### Table 1. The salivary MDA levels of patients with regulated and unregulated periodontally type 2 DM

<table>
<thead>
<tr>
<th>Subjects</th>
<th>MDA levels</th>
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<tbody>
<tr>
<td></td>
<td>Pre HBOT</td>
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<tr>
<td>Regulated DM</td>
<td>4.09 ± 0.77 ug/mol</td>
</tr>
<tr>
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<td>5.88 ± 1.04 ug/mol</td>
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Peroxynitrite interacts with a number of biotargets, such as heme containing proteins where the iron is in its ferrous state, peroxidases, seleno-proteins such as glutathione DNA-binding transcription factors. In contrast to mostly beneficial and cytoprotective effects of NO, the generation of peroxynitrite has mainly been attributed with cytotoxic effects. In vivo, when thiol-containing agents (glutathione, albumin, cysteine) are available to convert the peroxynitrite anion to nitrosothiols and related products, it may exhibit protective properties.\(^{8,12,13}\)

Radicals attack other biomolecules such as DNA, protein, and most commonly lipids and in doing so generate new radicals. In the presence of metal ions the interaction between lipid peroxides and hydrogen peroxide can lead to a metal catalyzed Fenton reaction and this could form strong oxidizing agents capable of propagating lipid peroxidation. This leads to the production of toxic metabolites like aldehydes malondialdehydes (MDA). These products of peroxidation can increase vascular permeability, produce edema inflammation, promote cell death and can alter the functions of membrane proteins like receptors, ion channels and enzymes.\(^{2,11}\)

Lipid peroxidation has been shown to cause a cell damage and profound alteration in structural integrity.\(^5\) MDA, one of the most widely used biomarkers of oxidative stress. Elevated MDA level have been shown in periodontitis.\(^2\)

Hyperbaric Oxygen (HBO) treatment in human can caused DNA damaged in lymphocyte, but DNA damage was found only after the first HBO exposure and not after further treatment. Speit et al.\(^{14}\) demonstrate increased levels of heme oxygenase-1 (HO-1) in lymphocyte after HBO. Rothfuss et al.\(^{15}\) studies also provided evidence for a functional involvement of the inducible enzyme heme oxygenase-1 (HO-1) in this adaptive protection. The induction of HO-1 and increased sequestration of iron could explain why the cells are protected after HBO. Its suggest the increased of heme oxygenase-1 might be involved in the adaptive protection after HBO. The activity of HO-1 leads to degradation of the pro-oxidant heme and to accumulation of the antioxidant bilirubin. Bilirubin, a metabolite of heme degradation is in it self a potent antioxidant. Induction of ferritin synthesis as a result of iron removal from the degradation of heme by HO-1. The deleterious effects of reactive oxygen species such as H\(_2\)O\(_2\) are dependent on the presence of iron. Intracellular free iron can react with H\(_2\)O\(_2\) and increase the toxic hydroxyl radical via Fenton reaction. Due to the release of free iron during the catalysis of heme by HO-1, ferritin may be released and restrict iron from participation of the Fenton reaction. Various in vivo studies with animals and in vitro studies with mammalian cell lines have indicated the involvement of HO-1 in the resistance to oxygen toxicity.\(^{14,15}\)

Hyperbaric Oxygen (HBO) induced cellular protection in a similar manner with other protective oxidative stress mechanisms. These protective effects of HBO may be
related to the fact that reactive oxygen species can trigger a wide variety of cellular mechanisms by functioning as signal molecules.\textsuperscript{9,14} HBO treatment in human leads to the induction of adaptive response that protects cells against the induction of DNA damage by the second HBOT.\textsuperscript{4,10,15} The reactive oxygen species generated by Hyperbaric Oxygen (HBO) triggers the increase of antioxidant enzyme activities regulation, thereby induces tolerance against ischemia in the tissues.\textsuperscript{4,14} The body contains a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants or to repair cell damage caused by reactive oxygen species.\textsuperscript{15,16} The increase in concentration and partial pressure of oxygen during HBO therapy provides more oxygenation in the whole body.\textsuperscript{5} The increased tissue oxygen enhances the growth of fibroblast, formation of collagen, angiogenesis, and phagocytic capabilities of the hypoxic leukocytes, so it has beneficial effects on wound healing.\textsuperscript{5,17}

Diabetic patients have significant defects of antioxidant defense elements, and the generation of reactive oxygen species is one of the major determinants of diabetic complications.\textsuperscript{8} The current findings in diabetic patients with periodontitis indicated the upregulation of antioxidant enzymes by HBO therapy.\textsuperscript{18,19} Although the activities of antioxidant defense enzymes were not measured in this study, there were evidences to confirm this hypothesis. HBO improves the oxygen delivery to the tissues, accelerates the rate of healing, and also has anti-infectious properties against various microorganisms.\textsuperscript{5,14,16,20} This research showed MDA level decrease after HBOT. It has been proved by randomized, controlled clinical trials that HBOT is effective in diabetic periodontitis.

It is concluded that HBO 2.4 ATA with 3 x 30 minutes dose triggers and upregulates the defense mechanisms against oxidative stress. HBO decreased MDA levels in regulated and unregulated type 2 DM with periodontitis. Increased oxygenation of tissues due to HBO therapy may also activate other endogenous factors that prevent hazardous effects of the disease itself. Saliva of periodontitis patients with unregulated type 2 DM showed more lipid peroxidation than regulated type 2 DM. The findings of our study suggest that HBO 2.4 ATA 3 x 30 minutes for 10 days has beneficial effects on the treatment of periodontitis in diabetes and this effect may occur through the antioxidant systems.

REFERENCES


