



Effects of Hyperbaric Oxygen Therapy on MMP-8 Saliva Levels in Chronic Periodontitis: A Preliminary Research

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Abstract

Introduction: Chronic periodontitis is inflammation of the periodontal tissue due to the accumulation of bacteria in plaque which causes progressive attachment loss and bone loss. Scaling root planing (SRP) is the initial treatment for periodontitis and often required as an adjunctive therapy to enhance the improvement of the inflammatory response. This study aimed to determine the effect of hyperbaric oxygen therapy (HBOT) on MMP-8 saliva levels in chronic periodontitis.

Materials and Methods: This was an experimental research, non-randomized controlled trial with convenience sampling technique. The sample consisted of 9 patients with chronic periodontitis who were divided into 3 treatment groups, namely group 1 (SRP), group 2 (HBOT), group 3 (SRP+HBOT), each group consisted of 3 people. Measurement of MMP-8 levels in saliva was carried out before and after treatment on day 14. Data analysis to compare the difference in the mean levels of MMP-8 in the three treatment groups (before and after) was by using analysis of variance (F test); while to compare the decrease, the Kruskal-Wallis test was used. Comparison of MMP-8 levels between before and after treatment was by using paired t-test.

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Results: The difference in MMP-8 levels from the data before and after treatment in the combination of the three groups with analysis of variance (F test) did not show any significant difference ($p>0.05$); however, the difference in MMP-8 levels between before and after treatment in each group showed a significant difference ($p<0.05$) based on the Kruskal Wallis test; except in group 2 (HBOT) there was no significant difference ($p>0.05$).

Conclusion: Administration of hyperbaric oxygen therapy (HBOT) after scaling root planing in chronic periodontitis patients had an effect on decreasing salivary MMP-8 levels.

Keywords: Chronic periodontitis; hyperbaric oxygen therapy; MMP-8 levels.

1. Introduction

Periodontal disease is an oral health problem that has a high prevalence in the world. Global Burden of Disease research data from 1990-2010 showed that periodontitis was a disease with the sixth highest prevalence (11.2%) and affected about 743 million people in the world. Periodontitis has increased in prevalence by 57.3% in a period of 10 years [1,2]. In Indonesia, based on RISKESDAS data in 2018, the prevalence of periodontitis was about 74.1% [3].

Periodontitis is an inflammatory disease of the tooth supporting tissues caused by certain microorganisms, which results in progressive destruction of the periodontal ligament and alveolar bone with the formation of periodontal pockets and loss of attachment.[4] Invasion of bacteria and their endotoxins initiates a host response and an inflammatory process confined to the gingiva is called gingivitis. The tissue destruction stage is the transitional stage from gingivitis to periodontitis. Periodontal tissue destruction occurs when there is a disturbance in the balance of the number of bacteria with the host response, this can occur because the subject is very susceptible to periodontal infection or the subject is infected with large numbers of bacteria [5].

Bacteria will produce toxins, Lipopolysaccharides (LPS), antigens that will destruct the epithelium and periodontal structures [6]. Bacterial components will induce a host response that will activate immune cells. This will lead to increased production of inflammatory mediators such as cytokines, chemokines, prostaglandins, and proteolytic enzymes such as matrix metalloproteinases (MMPs) [5].

Matrix metalloproteinase is a protease enzyme from zinc and calcium endopeptidase which is secreted or released by various host cells such as polymorphonuclear leukocytes, macrophages, fibroblasts, bone, epithelial and endothelial cells. Matrix metalloproteinase has the capacity to degrade almost all extracellular matrix, basement membrane matrix, and its components including collagen which is the main structural protein in the connective tissue of the periodontium. The body naturally has an MMP inhibitor, namely Tissue Inhibitor Matrix Metalloproteinase (TIMP) to maintain balance in the body's physiological processes. Pathological conditions such as periodontitis cause an increase in the number of MMP so that there is an increase in the degradation of extracellular matrix components such as collagen [7,8].

One of the matrix metalloproteinase families, MMP-8 (collagenase-2) is synthesized by differentiated

granulocytes in the bone marrow and stored in specific granules of circulating neutrophils. It can be detected in saliva and gingival crevicular fluid. MMP-8 levels have been considered as a key marker in chronic periodontitis that can predict the development of periodontal disease. The increase in MMP-8 levels was found to be associated with an increase in the parameters of the periodontal clinical features. MMP-8 levels in periodontitis may decrease after periodontal treatment [8].

The gold standard in the management of periodontal disease can be done by means of Scaling Root Planing (SRP) which is a non-surgical initial therapy that must be carried out in periodontal treatment so as to restore the health of periodontal tissues by removing plaque, calculus, and endotoxins that cause gingival inflammation on tooth surface. SRP treatment sometimes can not achieve maximum results because of the complexity of the anatomy of the teeth and the depth of the pocket which makes it difficult for the instrument to reach the base of the pocket so that the effectiveness of root planing is limited [9,10,11,12].

Administration of systemic antimicrobials orally or locally after SRP treatment as adjunctive therapy in periodontal treatment, there is another option of adjunctive therapy in the form of hyperbaric oxygen therapy (HBOT). Hyperbaric oxygen therapy is a physical therapy method by inhaling 100% pure oxygen in a high pressure room of more than 1 ATA (Absolute Atmosphere). Hyperbaric oxygen therapy has been used successfully as a potential treatment for chronic periodontitis.^{15,16,17} Hyperbaric oxygen therapy has been used successfully in several general medical applications. Its therapeutic effect is associated with an increase in the partial pressure of oxygen in the tissues. The pressure itself increases the dissolved oxygen in the tissue fluid (Kindwall and his colleagues 1991). HBOT is commonly used in general medical practice, but is still rarely applied or known in dentistry [12].

Research on HBOT in chronic periodontitis patients has been investigated by Wandawa and his colleagues (2017) that with 8 sessions of HBOT proved to be an effective adjunctive therapy for chronic periodontitis after conventional SRP treatment. Gingival index decreased both in HBOT alone and HBOT with SRP could eliminate the signs of inflammation and persisted for two months after therapy [15].

To date, HBOT research in periodontal treatment is still limited to clinical examination, the number of effective HBOT sessions carried out in chronic periodontitis and examination of the number of bacteria in periodontitis, but there were no study of HBOT by using biomarkers including MMP-8. The authors are interested in knowing the effect of HBOT therapy after SRP on MMP-8 levels in chronic periodontitis.

2. Material and Methode

This study was conducted on Navy personnel who came to the Navy Ladokgi R.E Martadinata, Central Jakarta for SRP and saliva collection, at the Dr. Mintohardjo Naval Hospital to conduct HBOT, and the Laboratory of Molecular Genetics, Faculty of Medicine, UNPAD Bandung, to examine saliva samples. The period of the study began in August - December 2020 and participants were willing to take part in the study by signing an informed consent. This was an experimental research, Non-randomized controlled trial.

2.1 Ethical Approval

Ethical approval was obtained from the Research Ethics Commission of Padjadjaran University Bandung (Number: 784/UN6KEP/EC/2020). A similar approval was also obtained from the Health Research Commission of the Indonesian Naval Hospital, dr. Mintohardjo (Number: 006/EC/LKS/RSMTH/VIII/2020).

2.2 Sample Size

Sample size was determined by convenience sampling technique. The sample in this study amounted to 9 patients with chronic periodontitis, divided into 3 study groups and each group consisted of 3 patients. The determination of the number of samples was determined based on the consideration of the current COVID-19 situation and conditions as well as the limitations of the sample and time in carried out the research.

2.3 Inclusion and Exclusion Criteria

Inclusion criteria: Male and female patients, age from 30 to 50 years, patients with chronic periodontitis in at least 2 teeth with a pocket depth of 5 mm, willing to take part in this study and signed an informed consent, had done a rapid test with non-reactive results. Exclusion Criteria: Using fixed or removable prostheses and orthodontic appliances, consuming mouthwash and anti-inflammatory antibiotics in the last 3 months, having an infectious disease such as HIV or systemic disease such as diabetes, hepatitis, tuberculosis, hypertension, cardiovascular disease, undergoing periodontal treatment in the last 6 months, smokers, sufferers of Corona Virus Disease (COVID-19).

2.4 Data Analysis

Data analysis was performed by using SPSS version 25. To analyze the difference in the mean levels of MMP-8 before and after administration of hyperbaric oxygen therapy. The data processing step was first done by doing test of normality of the MMP-8 level data based on the Shapiro-Wilk test. Based on the data normality test, to compare the difference in the average levels of MMP-8 in the three treatment groups (before and after) analysis of variance (F test) was used, while to compare the decrease in MMP-8 levels, the Kruskal-Wallis test was used. Comparison of MMP-8 levels between before and after treatment was by using paired t-test.

2.5 Methods

- Navy personnel who came to Ladokgi TNI AL R.E Martadinata, Central Jakarta, who meet the criteria for an initial examination including screening of COVID-19 patients, recording general data, disease history, extraoral and intraoral.
- Periodontal pocket depth was measured by using a UNC 15 probe and recorded at least 2 teeth with a pocket depth of 5 mm.
- Saliva of subjects was taken by the researcher before the SRP was carried out as much as 3 ml with the spitting technique. Two hours before the saliva collection the patients did not eat and brush their teeth. The patient was instructed to rinse the mouth with water prior to saliva collection. Salivary collection with the

spitting technique was done by instructing the subject to collect 3 ml of unstimulated saliva on the floor of the mouth and spit it out in a saliva container and stored it in a coolbox.

- The saliva container was stored in the refrigerator (refrigerator) until all samples were collected and brought to the Laboratory of Molecular Genetics, Faculty of Medicine, University of Padjadjaran and tested by using the ELISA kit.
- The subjects were divided into 3 groups, namely group 1 which was administered SRP, group 2 was administered HBOT, and group 3 was administered SRP+HBOT.
- Research subjects included in the control group who were given SRP treatment were carried out at one time in all regions by the research assistant.
- After SRP was performed, patients who were in group 2 (HBOT) and group 3 (SRP+HBOT) were given hyperbaric oxygen therapy at Dr. Mintohardjo Naval Hospital, Central Jakarta. Hyperbaric oxygen therapy was administered by a hyperbaric chamber operator.
- On the 14th day, saliva samples were taken again as on day 0 in the same way by the researcher.

3. Results

Research on the effect of hyperbaric oxygen therapy (HBOT) after scaling root planing (SRP) on MMP-8 saliva levels in chronic periodontitis has been conducted in nine patients who were divided into three treatment groups, namely group 1 (SRP), group 2 (HBOT), and group 3 (SRP+HBOT) with three patients in each group.

Table 1: Demographic Characteristics of the Study Group.

Variable	Group			Percentage (%)
	SRP (n=3)	HBOT (n=3)	SRP+HBOT (n=3)	
Age				
Mean ± SD	42.33 ± 3.215	44.33 ± 2.517	43.33 ± 8.963	
Rentang	40-46	42-47	33-49	
Gender				
Man	3	3	2	88.9
Woman	0	0	1	11.1
Occupation				
Navy	2	0	2	44.4
Civil servant	1	2	0	33.3
Freelancer	0	1	1	22.2

Characteristics of patients aged between 33 to 49 years with a mean of 43.3 years, eight males and one female (table 1). There were 44.4% of patients who work as Indonesian Navy; as many as 33.3% as civil servants and 22.2% as a freelancer. Examination of MMP-8 levels was carried out twice, first when they came before treatment and second after 14 days after given treatment.

Table 2: Comparison of MMP-8 levels from the SRP, HBOT, and SRP+HBOT groups in chronic periodontitis.

MMP-8 Level (pg/ml)	Treatment			P Value ^{*)}
	Group 1 (SRP)	Group 2 (HBOT)	Group 3 (SRP+HBOT)	
Before:				
Mean (SD)	5588.86 (1291,42)	3514.45 (2501,00)	7800.19 (2427.76)	0.125 (NS)
Median	6248.01	2291.71	6522,12	
Range	4100.88-6417.69	1860.05-6391.59	6278.46-10600.00	
After:				
Mean (SD)	1193.97 (756.90)	1023.20 (433.10)	703.21 (324.70)	0.557 (NS)
Median	852.36	881.57	570.62	
Range	668.09 -2061.46	678.65 – 1509.38	465.79 – 1073.23	
Comparison between Before and After: (Paired T-Test)				
	p=0.033 (S)	p=0.088 (NS)	p=0.014 (S)	
Decreased:				
Mean (SD)	4394.89 (2039.91)	2491.25 (2094.80)	7096.98 (2105.40)	0.039 (S)
Median	5565.33	1613.06	5951.50	
Range	2039.42 -5579.92	978.48 – 4882.21	5812.67 – 9526.77	

Notes: *) For data before and after treatment with the F test (analysis of variance); while for the decrease in MMP-8 levels with the Kruskal-Wallis test, NS: Non significant, S: Significant p value ≤ 0.05.

The difference in MMP-8 levels in from data before and after treatment in the combination of the three groups did not show a significant difference ($p > 0.05$); but the difference in MMP-8 levels between before and after treatment in each group showed a significant difference ($p < 0.05$); except in group 2 (HBOT) there was no significant difference ($p > 0.05$) (table 2). The amount of decrease in MMP-8 levels in group 1 (SRP) was 4394.89 on average; in group 3 (SRP+HBOT) the average was 7096.98; and in group 2 (HBOT) the average was 2491.25.

Table 3: Comparison of decreased MMP-8 levels between SRP, HBOT, and SRP+HBOT groups after chronic periodontitis treatment.

Comparison of decreased MMP-8 (pg/ml)	P Value ^{*)}
SRP vs HBOT	0.127 (NS)
SRP vs SRP+HBOT	0.050 (S)
HBOT vs SRP+HBOT	0.050 (S)

Notes: *) based on the Wilcoxon test. NS: Non significant, S : Significant p value ≤ 0.05.

The difference in the decrease of MMP-8 levels between group 1 (SRP) and group 3 (SRP+HBOT), and between group 2 (HBOT) and group 3 (SRP+HBOT) showed a significant difference ($p = 0.05$); while between group 1 (SRP) and group 2 (HBOT) it was not significant ($p = 0.127$) (table 4.2). It could be concluded that the administration of hyperbaric oxygen therapy (HBOT) could have an adjunctive effect in reducing MMP-8 levels after treatment of chronic periodontitis with SRP. The decrease in MMP-8 levels in the three treatment groups is shown in the following figure.

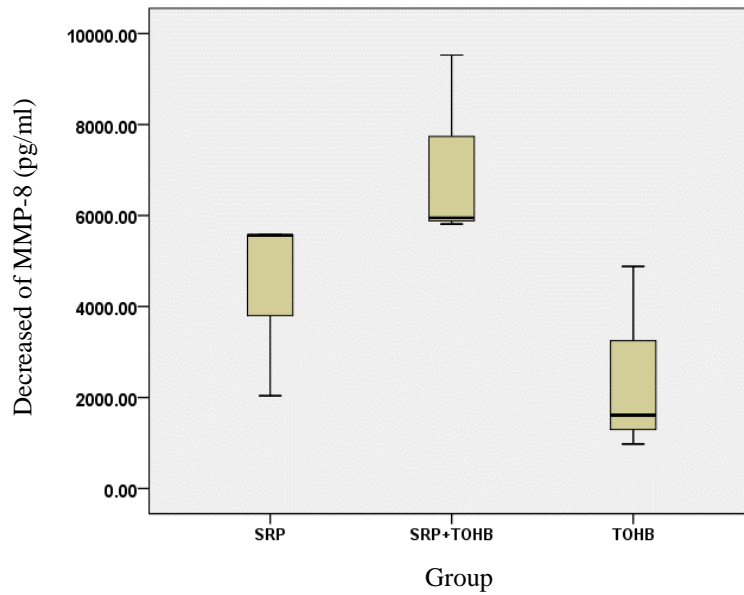


Figure 7: decreased MMP-8 levels of the SRP, HBOT, and SRP+HBOT groups after chronic periodontitis treatment.

Based on Figure 7, it could be explained that the median value (thick line in the box) in group 3 (SRP+HBOT) was the highest compared to the other treatment groups. From the graph it could also be seen that the distribution of data in the three treatment groups was not significantly different. This could be seen in the size of the box on the graph, which was almost the same for the three treatment groups. Based on the boxplot results, it also showed that the median value of group 1 (SRP) was close to its maximum value (top of the line). On the other hand, the median of group 2 (HBOT) and group 3 (SRP+HBOT) were close to their minimum values (peak bottom line). This showed that the treatment in group 2 and group 3 still had the opportunity to produce a higher average decrease in MMP-8 levels.

4. Discussion

This study aimed to determine the effect of HBOT on MMP-8 levels after treatment of chronic periodontitis from saliva which was carried out on nine patients divided into three treatment groups, namely group 1 (SRP), group 2 (HBOT) and group 3 (SRP+HBOT). The research was conducted at Navy Ladokgi R.E Martadinata, Central Jakarta for SRP treatment and saliva collection, followed by Dr. Mintohardjo Naval Hospital for HBOT, and examination of saliva samples at the Laboratory of Molecular Genetics, Faculty of Medicine, UNPAD Bandung. Examination of MMP-8 levels was carried out twice, first when they came before treatment and second after 14 days after given treatment. The difference in MMP-8 levels from data before the combined treatment of the three groups was 0.125 and the data after the treatment in the combined three groups was 0.557 (table 2). This showed that the data before and after treatment in the combination of the three groups did not statistically show a significant difference ($p > 0.05$); but the difference in MMP-8 levels between before and after treatment for group 1 (SRP) was 0.033; group 3 (SRP+HBOT) of 0.014 showed a significant difference ($p < 0.05$); except for group 2 (HBOT) which was 0.088 ($P > 0.05$). The difference in the decrease MMP-8 levels between group 1 (SRP) and group 3 (SRP+HBOT), and between group 3 (SRP+HBOT) and group 2 (HBOT)

showed that there was a significant difference in the comparison of the treatment groups ($p=0,05$); while between group 1 (SRP) and group 2 (HBOT) showed that the comparison of the treatment groups was not significantly different ($p=0.127$) (table 3). From these results it could be concluded that the administration of hyperbaric oxygen therapy (HBOT) could provide an adjunctive effect in decreasing MMP-8 levels after treatment of chronic periodontitis with SRP. MMP-8 level is one of the biomarker parameters in assessing the presence of inflammation in periodontal tissues according to research from Johnson N and his colleagues (2016) which showed the use of saliva biomarkers to distinguish periodontal disease from healthy ones. Al-Majid and his colleagues (2018) stated that MMP-8 as a biomarker of inflammation in periodontitis and periimplantitis. Active MMP-8 levels can also be used to predict and determine the progression of periodontitis. According to research by Gupta and his colleagues (2015) showed that there was an increase in MMP-8 concentrations in individuals with chronic periodontitis. Mauramo and his colleagues (2017) and Kasuma (2018) showed that levels of MMP-8 were higher in periodontitis patients compared to gingivitis patients as well as in healthy patients [8,16,17,18,19,20]. MMP-8 is present in serum, saliva, and gingival crevicular fluid. According to Gupta, N and his colleagues (2015) stated that detecting MMP-8 levels in saliva was very easy, non-invasive, and fast, its measurement did not require special equipment [21,22]. Examination of MMP-8 levels in this study was done by using ELISA examination, carried out examination on samples on day 0 and day 14. According to the study of Nardi GM and his colleagues (2020) that examining MMP-8 in saliva showed a change on day 14 after non-surgical periodontal treatment and a decrease in MMP-8 was increasingly visible after 1 month and 3 months [17]. Marcaccini A and his colleagues (2009) stated that the decrease in MMP-8 reached a very significant decrease on day 90 after SRP [18]. SRP therapy could reduce MMP-8 levels in periodontitis patients, this was in accordance with research by Marcaccini A and his colleagues (2009) and Konopka (2012). Scaling root planing can change the composition of bacteria in the pocket so that it will inhibit the activity of the collagenase enzyme in the periodontal tissues and reduce the levels of MMP-8. The effectiveness of SRP can be increased by several other adjunctive therapies for the treatment of chronic periodontitis, namely hyperbaric oxygen therapy (HBOT) [10,12,18,19]. Hyperbaric oxygen therapy is a physical therapy method by inhaling 100% pure oxygen in a high pressure room of more than 1 ATA (Absolute Atmosphere). Hyperbaric oxygen therapy has been used successfully as a potential treatment for chronic periodontitis [10,12,13]. According to Kindwall and his colleagues (1991) stated that hyperbaric oxygen pressure increased oxygen solution in tissue fluid [12].

Research on HBOT in chronic periodontitis patients has been done by Wandawa and his colleagues (2017) that with 8 sessions of HBOT proved to be an effective adjunctive therapy for chronic periodontitis after conventional SRP treatment [12]. The gingival index decreased with both HBOT alone and HBOT and SRP, and was able to eliminate signs of inflammation and persisted for two months after therapy [15]. Chen T and his colleagues (2002) study showed that HBOT increased oxygen distribution at the base of the periodontal pocket, it could inhibit the growth of anaerobic bacteria and also allowed ischemic tissue to receive adequate oxygen intake for rapid recovery of cellular metabolism [23].

To date, HBOT research in periodontal treatment is still limited to clinical examination, the number of effective HBOT sessions carried out in chronic periodontitis and examination of the number of bacteria in periodontitis, there were no study of HBOT by using biomarkers including MMP-8, but there was a research from Zhang Q

(2013) which showed that HBOT significantly decreased MMP1, MMP2, and MMP8 in ischemic wound tissue at day 7 [20].

The difference in the decrease MMP-8 levels before and after treatment which was not significant in this study indicated that SRP therapy was the gold standard for periodontitis treatment and would provide better results when combined with adjunctive therapy, namely HBOT. This was especially seen in the treatment group 3 (SRP+HBOT) where the levels of MMP-8 before and after treatment were more visible in the decrease. The author in conducting this research there were limitations that might affect the results of the study, because the author conducted research in the middle of the situation and conditions of the Corona Virus Disease 2019 (COVID-19) pandemic that occurred in Indonesia. As a result of this pandemic situation affecting research in a minimal number of samples and limited research time.

5. Conclusion

Administration of hyperbaric oxygen therapy (HBOT) after scaling root planing in chronic periodontitis might have an additional effect in reducing MMP-8 saliva levels.

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