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Research Report

Pulpal inflammation after vital tooth bleaching with 38% hydrogen peroxide

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ABSTRACT

Background: *In-office vital tooth bleaching is a treatment to remove tooth stains. Tooth sensitivity is one of side effect commonly complained by patients receiving this treatment.* ***Purpose:*** *The aim of this study was to examine histological inflammatory cells infiltration of dental pulp after application of 38% H_2O_2 as a vital tooth bleaching agent.* ***Methods:*** *Under informed consent, a total of 15 premolars from 8 healthy subjects scheduled for orthodontic extraction were used in this study. Thirty eight percent H_2O_2 was applied on the buccal surface of the treated group. The treated teeth were extracted after 1 hour, 5, 8, and 15 days. All specimens were embedded in paraffin wax, sectioned serially and stained with Hematoxyllin Eosin. Histological specimens were then observed under a light microscope.* ***Results:*** *All treated groups showed a slight disorganization of odontoblasts layer and slight inflammation in the pulp tissue adjacent to the 38% H_2O_2 application site. The number of polymorphonuclear leukocytes (PMN) had increased significantly 1 hour after application of 38% H_2O_2 ($p<0.05$), while macrophages had significantly increased 5 days after the application ($p<0.05$). The most intense PMN and macrophages infiltration was found 5 days after the application and gradually decreased 8 days after application of38% H_2O_2.* ***Conclusion:*** *Application of 38% H_2O_2 as a vital tooth bleaching agent induces acute inflammation in human dental pulp; however, the inflammation will decrease 8 days after the application.*

Key words: *Vital tooth bleaching, 38% hydrogen peroxide, inflammation*

ABSTRAK

Latar belakang: *Perawatan pemutihan gigi vital metode in-office merupakan tindakan untuk menghilangkan pewarnaan pada gigi. Salah satu efek samping yang sering dikeluhkan oleh pasien yang menjalani perawatan ini adalah sensitivitas gigi.* ***Tujuan:*** *Penelitian ini bertujuan untuk mengamati infiltrasi sel inflamasi pada pulpa gigi setelah aplikasi H_2O_2 38% sebagai bahan pemutih gigi.* ***Metode:*** *Sampel penelitian ini berupa 15 gigi premolar yang berasal dari 8 subjek sehat yang akan melakukan pencabutan gigi untuk perawatan ortodontik. Seluruh subjek telah menandatangani informed consent. Hidrogen peroksida 38% diaplikasikan pada permukaan bukal gigi kelompok perlakuan. Gigi kemudian dicabut 1 jam, 5, 8, dan 15 hari setelah aplikasi H_2O_2 38%. Seluruh spesimen kemudian ditanam dalam parafin, dipotong secara serial dan diwarnai dengan Hematoxillin Eosin. Pengamatan preparat histologis dilakukan dengan menggunakan mikroskop cahaya.* ***Hasil:*** *Hasil penelitian ini menunjukkan gangguan pada lapisan odontoblas dan peradangan pada jaringan pulpa di bawah daerah aplikasi H_2O_2. Jumlah PMN meningkat secara signifikan ($p<0,05$) 1 jam setelah aplikasi H_2O_2 38% sedangkan jumlah makrofag meningkat secara signifikan 5 hari setelah aplikasi hidrogen peroksida 38%. Infiltrasi PMN dan makrofag paling banyak ditemukan 5 hari setelah aplikasi dan menurun secara bertahap 5 dan 8 hari setelah aplikasi H_2O_2 38%.* ***Kesimpulan:*** *Aplikasi H_2O_2 38% sebagai bahan pemutih gigi vital dapat menginduksi inflamasi akut pada pulpa gigi manusia, namun, inflamasi akan mereda 8 hari setelah aplikasi.*

Kata kunci: *Perawatan pemutihan gigi vital, hidrogen peroksida 38%, inflamasi*

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INTRODUCTION

Many patients nowadays are interested in aesthetic dental treatments for a variety of reasons, from personal satisfaction to work-related needs. The goal of dental aesthetics is determined by various factors and one of the greatest causes of dissatisfaction is tooth discoloration that does not meet patient's expectations.[1]

Vital tooth bleaching is a therapeutic procedure that enables dental discoloration to be removed, thus giving the shade that meet the patient's expectation. The treatment is therefore, presented as non-invasive and conservative procedures that do not alter the natural shape of the teeth. This procedure is suitable for the vital teeth affected by shade problems which present no other dental or periodontal pathology and retain a correct anatomy and appropriate position in the arch.[1,2]

Vital tooth bleaching techniques include night guard and in-office techniques. These techniques may be used separately or in combination with one another. Night guard technique can be performed by the patients using low concentration of hydrogen peroxide all night for 1–2 weeks.[2] The in-office bleaching technique is performed by dentist by using high concentration of hydrogen peroxide. This treatment needs 1–2 visits. Although it has more potential for causing gingival irritation, this technique is often chosen because of the shorter treatment time.[3]

The active agent for the vital tooth bleaching commonly used today is hydrogen peroxide. This agent has to be applied directly on the tooth surface.[4] Hydrogen peroxide acts as a strong oxidizing agent through the formation of free radicals. The reactive molecule attacks the long-chained, dark-colored chromophore molecules and split them into smaller, lighter color, and more diffusible molecules.[5]

The common side effect of vital tooth bleaching using high concentration of hydrogen peroxide is tooth sensitivity.[6,7] This side effect normally persists for up to 4 days after the cessation of bleaching treatment.[7] In vitro and in vivo experiments have shown that the peroxide had ability to penetrate enamel and dentinal tubule and therefore, enter the pulp chamber.[8-10]

Little is known about the effects of hydrogen peroxide on dental pulp tissues. Many controversies arise related to the safety of vital tooth bleaching procedure although peroxide-based products had been accepted by the American Dental Association (ADA) as save and effective agents.[11] The aim of the study was to examine inflammatory cells infiltration of dental pulp after application of 38% H_2O_2 as a vital tooth bleaching agent.

MATERIALS AND METHODS

This research was approved by Ethical Commission of the Faculty of Dentistry Universitas Gadjah Mada. All patients who agreed to be a part of the study signed a consent form. The consent form for the patients under 18 years of age were signed by the parents. Patients were recruited from dental clinics in Sleman and Makassar. All of them were healthy, age 12–26 years, and have never done tooth bleaching treatment before.

The subject of this study consisted of 15 premolars which were scheduled for extraction for orthodontic treatment. Subject were divided into 2 group, control and treated group. Treated group were then divided into 4 subtreated group based on the extraction time, which were 1 hour, 5, 8, and 15 days after the application of 38% hydrogen peroxide (H_2O_2). Each subtreated group and control group were consisted of 3 premolars. The application of Opalescence Xtra Boost (Ultradent, Utah) which consisted of 38% hydrogen peroxide was performed according to the manufacturer's protocol. In brief, the tooth was drained from saliva and buccal retractor was mounted on the patient's mouth. Gingival barrier (OpalDam, Ultradent, Utah) was applied on interdental gingiva and gingiva around the treated tooth and activated by using a light curing unit. Opalescence Xtra Boost was mixed with activator (Ultradent, Utah) and applied 0.5–1 mm thick layer on the buccal surface of the treated tooth. After 45 minutes, 38% H_2O_2 was cleaned from the tooth surface by using the suction. Patients were then asked to rinse and the gingival barrier were cleaned by using an explorer.

The treated teeth were extracted 1 hour, 5, 8, and 15 days after the application of 38% H_2O_2. In control group, the teeth were extracted without application of 38% H_2O_2. Immediately after extraction, the most apical 4 mm of the root was sectioned off by using fissure bur, and fixed with 10% buffered formalin. The teeth were then decalcified in Morse solution (mixture of 50% formic acid and 20% sodium citrate with the same ratio) for 45 days.

After decalcification completed, teeth were embedded in paraffin, serially sectioned and stained with Hematoxylin and Eosin. The whole condition of the pulp tissue in the pulp chamber was examined. The number of polymorphonuclear leukocytes (PMN) and macrophages at the area of the pulp which encompassed the dentinal tubules corresponding to H_2O_2 application site were counted at 3 different fields using a light microscope at 400× magnification.

Results of each number of PMN and macrophages are presented as mean ± standard deviation. Difference among means of PMN and macrophages cells number were then analyzed separately using analysis of variance (ANOVA) and followed by Least Significant Difference (LSD) test at 5% level of significance.

RESULTS

All teeth in control group showed a normal dental pulp tissue organization. Odontoblast cell layer, cell free zone, cell rich zone, and pulp core were observed in all specimens (Figure 1A). A small number of inflammatory cells (PMN and macrophages) were seen in cell rich zone.

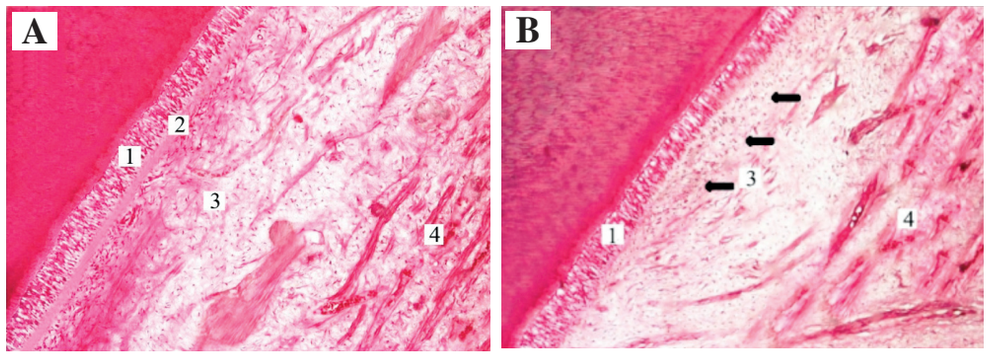

Figure 1. Dental pulp tissue in control group (A) and after application of 38% H_2O_2 (B). Four zones of the dental pulp tissue can be observed in the control group, while in the treated group, cell free zone can not be observed since it is occupied by PMNs and macrophages (black arrows). 1 = odontoblast layer, 2 = cell free zone, 3 = cell rich zone, 4 = pulp core.

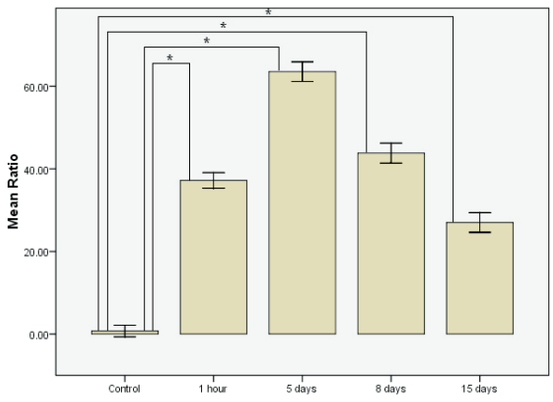

Figure 2. The mean ratio of pmn cells in dental pulp of control group and treated groups (*p < 0.05).

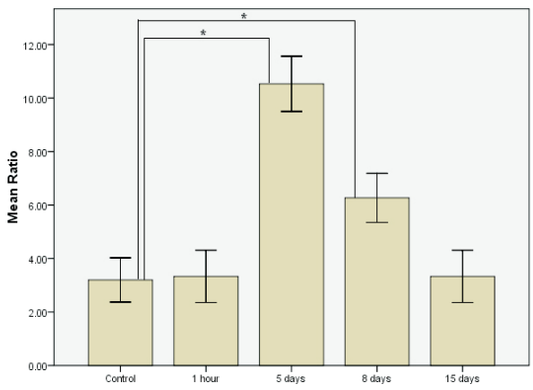

Figure 3. The mean ratio of macrophages in dental pulp of control group and treated groups (*p < 0.05).

The result of ANOVA test showed a statistical significant difference of inflammatory cells number among groups, indicating that application of 38% H_2O_2 increased the number of PMN and macrophages. In all specimens of the treated groups, cell rich zone could not be observed because infiltration of the inflammatory cells. Vacuolization and inflammatory cells infiltration were also found in odontoblast cell layer (Figure 1B). Lymphocytes were not found in both treated and control group.

The number of PMN was increased significantly immediately (1 hour) after application of 38% H_2O_2 (Figure 2). The most intense PMN infiltration were found in the specimens 5 days after application and gradually decreased 8 and 15 days after application of 38% H_2O_2.

The number of macrophages was significantly increased 5 days after application and decreased gradually 8 days after application (Figure 3). On extended time observation (15 days) macrophages cells number became fewer and had no significant differences compared to the control group.

DISCUSSION

Present study showed that application of 38% H_2O_2 as a vital tooth bleaching agent induced inflammatory cells infiltration in human dental pulp. This finding supported previous study by Costa *et al.*[12] They showed that vital tooth bleaching with 38% H_2O_2 induced pulp inflammation, but they only observed the pulp 2 days after the treatment.

H_2O_2 is a strong oxidizing agent that produces free radicals with unpaired electron, such as perhydroxyl radical and superoxyde anion. Free radicals break down large pigmented molecules in enamel and dentin into smaller and less pigmented molecules.[13,14] In order to promote the tooth lightening effect, H_2O_2 have to penetrate into enamel and dentin.[15,16] 14% H_2O_2 have the ability to penetrate into enamel and dentin; therefore, it can enter the pulp chamber through the dentinal tubules. The higher H_2O_2 concentration of bleaching agent results in the higher pulpal peroxide penetration.[16]

Enamel and dentin have a high permeability to H_2O_2 and free radical.[17] Penetration of H_2O_2 and free radical through the tooth structure occur mainly because of their low molecular weight which increases the ion movement.[18] Dental enamel contains 0.6% of organic material. H_2O_2 increases the porosity and loss of substances of enamel matrix as a result of free radical oxidation, thus increases

H_2O_2 penetration.[19] H_2O_2 also has ability to increase enamel porosity by denaturation of enamel's protein.[20] Dentinal tubules connect dentin with pulp chamber. This morphology encourages physical passage of H_2O_2 and free radical to enter pulp chamber.[15]

Odontoblasts are cells located in the peripheral of the pulp chamber, being the first defense against irritation of the pulp. Penetration of H_2O_2 into the pulp chamber causes changes in the odontoblast cell layer.[21] The present study revealed vacuolization of odontoblast cell layer in all treated groups. Vacuolization of odontoblast cell layer on the pulp tissue is the first response of odontoblast cells to injury[22] and usually occurs before the pulp is inflamed.[23]

H_2O_2 and perhydroxyl radicals induced the expression of interleukin-8 (IL-8).[24] H_2O_2 and radical perhidroksil which penetrate into the pulp may induce the odontoblast to produce IL-8. Interleukin-8 is a proinflammatory cytokine which has ability to stimulate chemotaxis of PMN and macrophages into the exposed area.[21,25]

In this study, the number of PMN increased immediately (1 hour) after application of 38% H_2O_2, while the number of macrophages were started to increase 5 days after the application of 38% H_2O_2. Inflammation is a local protective response generated by tissue damage, which serves to eliminate the irritating material or damaged tissue. Acute inflammation occurs a few minutes or hours after tissue damage and usually lasts for 1–2 weeks.[26] PMN and macrophages play an important role in the inflammation. They migrate from blood vessels into the tissue at the beginning of inflammation. PMN migration into inflamed areas occurs only in a few minutes after inflammation.[27]

Inflammation continues unless the irritating material can be successfully removed.[28] Eight days after the application, the number of PMN and macrophages were decreased, indicating that the irritating materials were begun to be eliminated from the pulp chamber. Dental pulp has defense mechanisms to eliminate H_2O_2 from the pulp chamber, by producing catalase and peroxidase enzymes. Catalase breaks down hydrogen peroxide into water and oxygen; while peroxidase uses H_2O_2 to oxidize some other substrates.[17] In conclusion, application of 38% H_2O_2 as a vital tooth bleaching agent induces acute inflammation in human dental pulp; however, the inflammation will decrease 8 days after the application.

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