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% H2O2 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 H2O2 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% H2O2 application site. The number of polymorphonuclear leukocytes (PMN) had increased significantly 1 hour after application of 38% H2O2 (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 of 38% H2O2. Conclusion: Application of 38% H2O2 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

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ABSTRAK


Kata kunci: Perawatan pemutihan gigi vital, hidrogen peroksida 38%, inflamasi
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.3 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% H₂O₂ 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 (H₂O₂). 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% H₂O₂ 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% H₂O₂. In control group, the teeth were extracted without application of 38% H₂O₂. 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 H₂O₂ 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.
The result of ANOVA test showed a statistical significant difference of inflammatory cells number among groups, indicating that application of 38% H$_2$O$_2$ 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$_2$O$_2$ (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$_2$O$_2$.

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$_2$O$_2$ as a vital tooth bleaching agent induced inflammatory cells infiltration in human dental pulp. This finding supported previous study by Costa et al. They showed that vital tooth bleaching with 38% H$_2$O$_2$ induced pulp inflammation, but they only observed the pulp 2 days after the treatment.

H$_2$O$_2$ 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. In order to promote the tooth lightening effect, H$_2$O$_2$ have to penetrate into enamel and dentin. 14% H$_2$O$_2$ have the ability to penetrate into enamel and dentin; therefore, it can enter the pulp chamber through the dentinal tubules. The higher H$_2$O$_2$ concentration of bleaching agent results in the higher pulpal peroxide penetration.

Enamel and dentin have a high permeability to H$_2$O$_2$ and free radical. Penetration of H$_2$O$_2$ and free radical through the tooth structure occur mainly because of their low molecular weight which increases the ion movement. Dental enamel contains 0.6% of organic material. H$_2$O$_2$ increases the porosity and loss of substances of enamel matrix as a result of free radical oxidation, thus increases

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**Figure 1.** Dental pulp tissue in control group (A) and after application of 38% H$_2$O$_2$ (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).
H₂O₂ penetration. H₂O₂ also has ability to increase enamel porosity by denaturation of enamel’s protein. Dentinal tubules connect dentin with pulp chamber. This morphology encourages physical passage of H₂O₂ and free radical to enter pulp chamber.

Odontoblasts are cells located in the peripheral of the pulp chamber, being the first defense against irritation of the pulp. Penetration of H₂O₂ into the pulp chamber causes changes in the odontoblast cell layer. The present study revealed vacuolation of odontoblast cell layer in all treated groups. Vacuolation of odontoblast cell layer on the pulp tissue is the first response of odontoblast cells to injury and usually occurs before the pulp is inflamed.

H₂O₂ and perhydroxyl radicals induced the expression of interleukin-8 (IL-8). H₂O₂ and radical perhydroxyl 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.

In this study, the number of PMN increased immediately (1 hour) after application of 38% H₂O₂, while the number of macrophages were started to increase 5 days after the application of 38% H₂O₂. 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. 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.

Inflammation continues unless the irritating material can be successfully removed. 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 H₂O₂ from the pulp chamber, by producing catalase and peroxidase enzymes. Catalase breaks down hydrogen peroxide into water and oxygen; while peroxidase uses H₂O₂ to oxidize some other substrates. In conclusion, application of 38% H₂O₂ as a vital tooth bleaching agent induces acute inflammation in human dental pulp; however, the inflammation will decrease 8 days after the application.

REFERENCES