Correlation Between Molecular Changes and Inflammatory Cascade in Deep Carious Lesions with Dental Pulp Exposure Using Superoxide Dismutase

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Abstract:

Objective: The objective of this study was to use Superoxide dismutase (SOD) as a diagnostic biomarker to correlate molecular changes in the inflammatory cascade in deep carious lesions with dental pulp exposure. **Materials and Methods:** A total of 40 cases with deep carious lesions with expected pulp exposure and signs of pulp vitality without signs of periapical inflammation were selected from the outpatient clinic, Faculty of Dentistry, Mansoura University. Another 6 control cases were selected from the orthodontics department clinic. **Results:** Our results show that there is a statistically significantly lower mean percent inhibition among cases than in the control group 63.31±24.61versus 94.52±2.97 (p=0.004). However, there appears to be no statistically significant difference between the different treatment modalities as regard mean percent inhibition with the highest mean percent of inhibition being among direct pulp capping cases 75.35±13.51 followed by partial pulpotomy 67.18±24.35, 60.66±28.15 for complete pulpotomy and 58.81±25.22 for RCT (P=0.708). **Conclusions:** Vital pulp therapy is an ultra-conservative treatment that can be applied with the proper tools and magnification with a high success rate based on clinical signs and symptoms. However, in terms of treatment decisions, a molecular diagnosis does not appear to be superior to direct tissue visualization.

Introduction:

For a long time, the ideal treatment for the irreversibly inflamed pulp was complete pulpectomy which sounds more like an overtreatment as the inflammation may not extend to the radicular pulp.[1] Also, the tooth structure is weakened as much of the dentin is removed. The irrigants that are used, such as sodium hypochlorite and EDTA cause dentinal erosion[2,3], leaving the tooth more brittle and more prone to vertical root fracture.[4,5]

By introducing bioactive capping materials and clinical protocols that more predictably take into account the microbiological aspects of failure, treatment therapies have been reimagined. Based on the idea that pulp tissue has an inherent active repair capability when microorganisms and their byproducts are effectively excluded, attention will be focused on the preservation of the pulpally involved permanent teeth.[6,7]

In 2019, Ricucci et al. [8] conducted a systematic review of the treatment of deep carious lesions with pulp exposure with treatment modalities ranging from direct pulp capping, partial pulpotomy , complete pulpotomy, and pulpectomy based only on clinical signs of the normal appearance of the pulp tissue under magnification with a dental operating microscope and bleeding control within 10 minutes .Moreover, the diagnosis of irreversible pulpitis was based only on the clinical signs and symptoms, which do not always match those of the histological changes of the pulp tissues.[9,10] So, it is more appropriate to diagnose the pulp according to the molecular changes taking place throughout the pulp tissues.[11,12] Different studies have been conducted to study the molecular changes that take place in the inflamed pulp, including a study that was conducted by

Rechenberg et al. [11], which used different locations to obtain samples for the identification of these molecular changes. The best of which was the dental pulp blood sample. Another study, which was conducted in 2012, stated the way to obtain a blood sample from the pulp and how to prepare and test it to evaluate the levels and concentrations of different inflammatory molecules.[13] Many studies were also conducted to study the different changes and accurateness of measurements of different molecules.[14-16]

There are different types of biomarkers including cytokines such as ILs, TNF, MIPs, and TGFs, proteases and other enzymes such as MMPs, SOD, Elastase, and Catalase, antimicrobial peptides such as HBDs, growth factors, and others.

In a systematic review conducted by Rechenberg et al.[16] data for 89 biomarkers were collected from different studies to test their significance in pulpal inflammation. Only 68 of these were found to be significant in pulpal inflammation. One of the studied biomarkers that showed significance in pulpal inflammation was SOD.

Superoxide dismutases (SODs) are a category of metalloenzymes that are present in all kingdoms of life. SODs are the first line of defense against injury caused by reactive oxygen species (ROS).[17] By catalyzing the conversion of superoxide anion free radical (O2-) into

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molecular oxygen and hydrogen peroxide (H2O2), these proteins lower the level of O2-, which when present in high concentrations destroys cells.[18] SODs are a crucial component of the body's antioxidant defense against oxidative damage.[19]

Materials and methods:

Ethical considerations: This study was approved by the ethical committee of the Faculty of Dentistry, Mansoura University (Reference Number: M02031120). The procedures were explained to the patients with the possible complications and their inclusion in the study, and written consent was taken from them.

Subjects: Sample size calculation was based on a percentage of success rate between different pulpotomy agents retrieved from a study conducted by Kumar et al.[20] Using G*power version 3.0.10 to calculate sample size based on a difference of 65% using a 2-tailed Z test, α error =0.05 and power = 80.0% then total sample size will be 40 in each group.

A total number of 40 cases with deep carious lesions with signs of vital pulp without signs of periapical pathosis were selected outpatient clinic, Faculty of Dentistry, Mansoura University. Another 6 control cases were selected from the orthodontics department clinic.

Case selection: Cases were selected based on the following inclusion and exclusion criteria. Inclusion criteria included age range from 15-45 years, patients of both sexes, patients with clinical symptoms of pulpitis with normal apical tissues supported by radiographs showing no periradicular changes, and positive results for cold and electric pulp testing. Exclusion criteria included patients over 45 years of age, patients with clinical symptoms of periradicular inflammation or any periradicular radiographic changes, and mnegative results for cold and electrical pulp testing.

Procedures: The patients were selected according to the previously mentioned criteria. A Full medical and dental history was taken from each patient. Clinical and radiographic examination (cone beam computed tomography) for the offending tooth. After anesthesia, a rubber dam was applied, and complete caries removal was done. Deep inside the cavity, the excavation was done using a hand spoon excavator. After the first exposure, a blood sample was taken from the first blood drops of the pulp using a micropipette. The blood sample was transferred to 1 mL saline in a Lithium Heparin coated tube (KEMICO, Cat. No. KV40021).
Pulp tissue at the exposure site was viewed under the dental operating microscope. If the pulp tissue does not appear well vascularized, pulp tissue was progressively removed using a high-speed round bur 1 mm at a time till obtaining a homeostatic, homogenous wound that is well attached to the surrounding dentin (well-established dentin-pulp complex) and free from any dentin chips. In cases where these criteria were not obtained after complete pulpotomy, the case was root canal treated.

The cavity was disinfected with 1.5% sodium hypochlorite and the pulp tissue was covered by calcium aluminosilicate paste (Putty type) by Well-Root™. The cavity was restored with glass ionomer restoration (Fuji II by GC™).

The decision for treating the cases with vital pulp therapy was based on several criteria through direct viewing of the pulp tissue under a dental operating microscope, which includes obtaining hemostasis within 10 minutes, the pulp tissue appearing well vascularized, the pulp wound appears homogenous, and obtaining a well-established dentin-pulp complex (pulp tissue attached to surrounding dentin). When the criteria were not met, the cases were treated with root canal treatment, Figure 1A.

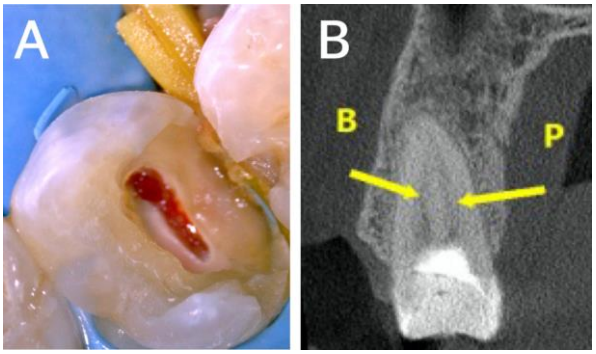


Figure 1: a, clinical picture showing ideal criteria for capping with bioceramic material . B,coronal cut showing a vpt treated tooth on the sixth month follow up.

Follow-ups were done on day one postoperative, one week postoperative, one month postoperative, three months postoperative, and six months postoperative, by clinical examination including cold testing, vertical percussion, and a traditional computed tomography (CBCT) radiograph on the sixth month follow up visit to detect any changes in the periradicular tissues and to look for evidence of calcific bridge formation beneath the bioceramic material.

After that, the glass ionomer restoration was changed with composite resin restorations. For the control cases, the blood samples were taken from teeth just before extraction for orthodontic treatment.

The blood samples were stored at -20 C. The blood samples were centrifuged at 6000 x for 10 minutes at 4 °C and tested for percentage of inhibition of Superoxide dismutase using a Superoxide dismutase kit from Biodiagnostic following the manufacturer's

recommendations.

Statistical analysis and data interpretation: Data were fed to the computer and analyzed using IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Qualitative data were described using numbers and percentages. Quantitative data were described using means and standard deviations for normally distributed data after testing normality using Shapiro-Wilk test. The significance of the obtained results was judged at the (0.05) level. Student t-test to compare cases & control groups and one-way ANOVA was used to compare different techniques.

Results:

Out of the 40 cases, 2 cases were treated with direct pulp capping, 17 cases were treated with a partial pulpotomy, 6 cases were treated with complete pulpotomy and 15 cases were treated with conventional root canal treatment, Table 1.

Table 1: Procedure of treatment among studied cases

Procedure	Cases (N)	%
Direct pulp capping	2	5.0
Partial pulpotomy	17	42.5
Complete pulpotomy	6	15.0
RCT	15	37.5
Total	40	100.0

On the follow-up visits, neither the patients treated with non-surgical root canal treatment nor the patients treated with vital pulp therapy reported any pain post-operatively except for one patient that was treated with a complete pulpotomy. The SOD test of this patient was omitted from the study. Pulp sensibility testing was done with cold testing on the patients treated with vital pulp therapy and they responded normally. The post-operative CBCT showed normal periapical tissues with no abnormal calcification in the pulp space, Figure 1B.

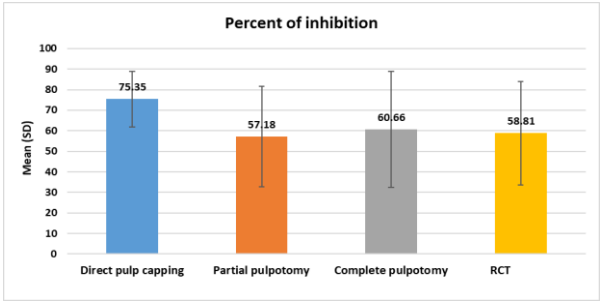
As for the SOD test, our results that there is a statistically significantly lower mean percent inhibition among cases than in the control group 63.31 versus 94.52 (p=0.004) as shown in Table 2 and Figure 2.

Table 2: Comparison of percent of inhibition between studied groups

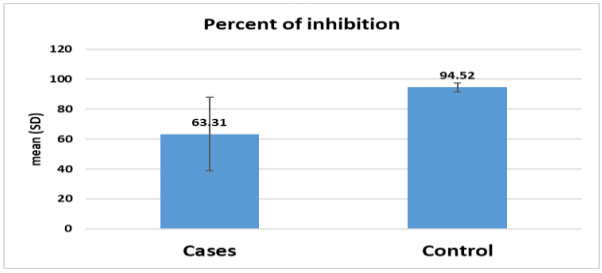
	Cases n=40	Control n=6	Test of significance
Percent of inhibition	63.31±24.61	94.52±2.97	t=3.07 p=0.004*

t:Student t test , * statistically significant

However, there appears to be no statistically significant difference between the different treatment modalities as regard mean percent inhibition with the highest mean percent of inhibition among direct pulp capping cases followed by partial pulpotomy, 60.66 for complete pulpotomy, and 58.81 for RCT as presented in Table 3 and Figure 2.



(A)



(B)

Discussion:

This study aimed to investigate the relationship between biomarkers level in the pulpal blood and the level of inflammation and to find a correlation between the extension of inflammation and the levels of Superoxide dismutase in the pulpal blood.

When neutrophils and macrophages are in pathological conditions, they produce highly reactive molecules called reactive oxygen species (ROS), which have a short life span. Superoxide anions rank among the most crucial of these compounds.[1] These molecules' primary function is to amplify the inflammatory response by raising the levels of cytokines, chemokines, and adhesion molecules. Larger doses, however, result in tissue damage and thrombosis. Endogenous enzymatic and non-enzymatic antioxidants are used by the body's intricate antioxidant defense grid. These chemicals work together to combat free radicals, preventing them from harming essential biomolecules and eventually body tissues. They can be divided into first, second, third, and even fourth-line defense antioxidants depending on how they react to a broad free radical assault. One of the first-line defense antioxidants is Superoxide dismutase (SOD). In a study conducted by Ge et al.[21] it was found that its activity was significantly increased in case of pulp inflammation in comparison to a normal pulp. So, SOD was chosen as the biomarker in this study our results showed that

Table 3: Comparison of percent of inhibition between studied subgroups

	Direct pulp capping n=2	Partial pulpotomy n=16	Complete pulpotomy n=7	RCT n=15	Test of significance
Percent of inhibition mean±SD	75.35±13.51	67.18±24.35	60.66±28.15	58.81±25.22	F=0.466 P=0.708

F: One Way ANOVA test

there was a statistically significant difference between the cases and the control in terms of the percentage of SOD inhibition. However, the statistical analysis does not show that there is a correlation between the extension of inflammation and the percentage of inhibition that we can rely on in terms of the treatment decision to be taken for each case. Although the theory of molecular diagnosis appears promising, it does not appear to be better than clinical observation and clinical signs in terms of the treatment decision, or at least for the studied biomarker. Direct observation and following the aforementioned criteria for visualizing healthy tissues appear more accurate in decision-making.

This may be attributed to the biomarker selected itself or to the site from where the blood sample was taken. This is further complicated by the fact that in multirooted teeth, the inflammation may involve one root canal and not the other. This was demonstrated in a study conducted by Koli. *et al*.[22] where mandibular teeth were treated with a combination of non-surgical endodontic treatment in one of the roots and vital pulp therapy in the other.

Conclusions:

- Vital pulp therapy is an ultraconservative treatment with a comparable success rate to root canal therapy.
-Molecular testing does not seem to be better than direct observation of pulp tissue using a dental operating microscope.
-Further research is needed to find a reliable biomarker for molecular diagnostics.

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