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Antioxidant Enzymes in Serum of Patients with Painful Acute Periradicular Abscess

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The purpose of this study was to compare the pre-treatment and post-treatment serum antioxidants levels in acute periradicular abscess, with pain. Thirty-two patients (18 males, 14 females) with acute and painful endodontic abscesses were included in this study. Before treatment, patients had severe symptoms of inflammation, but at the end of treatment no symptoms of inflammation were observed. Glutathione reductase and catalase activities concentrations were measured in serum of patients with acute periradicular abscess, before and after treatment. There was no statistically significant effect on levels of catalase activity and glutathione reductase concentrations ($p > 0.05$).

Key Words: Acute periradicalar abscess, Glutathione reductase, Catalase, Pain, Antioxidant.

INTRODUCTION

Acute periradicular abscess (APA) is often a sequel to and a progression from reversible pulpitis. Severe pulpal damage from extensive dentin removal during operative procedures or impairment of pulpal blood flow caused by trauma or orthodontic movement of teeth may also cause APA. This is a severe inflammation that will not resolve even if the cause is removed. The pulp slowly or rapidly progresses to necrosis. APA may also be associated with intermittent or continuous episodes of spontaneous pain. Pain of APA is usually sharp, dull, localized or diffuse and may last from minutes to hours[1].

Pulpal inflammation is a subject of interest not only to endodontists, but to all dental disciplines. We understand pulpal inflammation clinically as a toothache and histologically as an accummulation of polymorpholeukocytes around the site of insult in a pulp section. In addition to this evidence, however, are many complicated dynamic mechanisms, some of which are yet unknown[2].

Inflammation in the dental pulp is accompanied by release of a wide variety of highly oxidative molecules know as reactive oxygen species (ROS), ROS concentrations are controlled *in vivo* by an antioxidant enzyme scavenger system that may be overwhelmed by the increases in ROS production seen during inflammation.

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Supplementation of the antioxidant defense system, therefore, may limit the severity of the inflammatory response to injury due to this component[3]. Inflammation can result from mechanical, chemical or bacterial insult. Release of a wide variety of chemical mediators is involved in inflammation in this process, where in ROS are either important intermediaries or end-products in and of themselves[3].

Natural antioxidant enzymes manufactured in the body provide an important defense against free radicals. Glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase are among the most important antioxidant enzymes.

Recent data from several reports indicate that free radicals are involved in aetiopathogenesis of much human pathology, including inflammatory disorders. Increased reactive oxygen species (ROS) and other free radicals that play an important role in the inflammatory process and contribute to tissue destruction can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death, if the antioxidant system is impaired[4,5].

Reactive oxygen species (ROS) are generated as a result of normal metabolism, but a deleterious condition, termed oxidative stress, can occur when their production is accelerated or when the mechanisms involved in maintaining the normal reductive celular milieu are impaired. ROS include radical species such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) and reactive nitrogen species such as nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$)[4,5].

Catalase is a widely distributed enzyme that catalyzes the breakdown of H O a strong biological oxidizing agent[6,7], by dismutation, yielding oxygen and water Moreover, catalase also reduces H O *via* oxidation of low-molecular-weight alcohols[8].

Catalase (CAT, EC 1.11.1.6) catalyzes the reaction $2H_2O_2 \rightarrow 2H_2O + O_2$. Each aerobic cell contains this enzyme. CAT exist in 80 % perox and 20 % cytozoles. CAT enzyme is a protein of 240000 dalton moleculer weight composed of 4 subunits and that has a heme group [Fe(III)-prothoporphyrin] in each subunit[9].

GSH-Reductase (E.C 1.6.4.2) catalyses the reaction $GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$. Its molecular weight is 120000 dalton. It is a protein that has two subunits[10,11].

The reaction of catalase in the decomposition of hydrogen peroxide is[12]:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

While complete mechanism of catalase is not currently known, the reaction is believed to occur in two stages[13]:

$$H_2O_2 + Fe(III)\text{-}E \rightarrow H_2O + O = Fe(IV)\text{-}E$$

$$H_2O_2 + O = Fe(IV)\text{-}E \rightarrow H_2O + Fe(III)\text{-}E + O_2$$

(where Fe()-E represents the iron centre of the heme group attached to the enzyme).

To circumvent the damage caused by the ROS, multiple defense systems, collectively called antioxidants are present in human serum as well as erythrocytes. Most of the antioxidant ability of serum has been attributed to the presence of ascorbate, transferin and ceruloplasmin (CP), an acute phase protein[14-16]. Erythrocytes are

excellently equipped to handle intracellular oxidative stress through the combined activities of hexose monosphosphate shunt (HMPS), catalase (CAT), glutathione peroxidase (GPx) and glutathione[9]. Glutathione (GSH) is an important member of the antioxidant team as it has been shown to destroy ROS and other free radicals by enzymatic as well as non-enzymatic mechanisms[17]. The extracellularly generated O_2^- and H_2O_2 have been shown to traverse erythrocyte membranes[18].

Glutathione reductase (GRD) utilizes reduced nicotinamide adenine dinucleotide phosphate (NADPH) to reduce oxidized glutathione[15].

To our best of knowledge, there are no data available on serum catalase (CAT) activities, glutathione peroxidase (GRD) activities concentrations in patients with APA. In the present study, we measured serum catalase (CAT) activities, glutathione peroxidase (GRD) activities concentrations in patients with APA.

EXPERIMENTAL

Patients were selected from emergency services of the University of Atatürk Dental School. Thirty-two patients (18 males, 14 females) with APA were introduced into this study. Their ages ranged from 18 to 41 years (mean, 24 years). Consent was obtained from all participants. The patients had localized swelling with moderate to severe pain. No fistula or fever was present. Patients with periodontal disease associated with the periapical infection were excluded from the study. A detailed medical history was taken. None of the subjects had intestinal absorption defects or showed any clinical or laboratory signs of liver disease, diabetes mellitus, thyroid disease, infection disease or coronary artery disease. Subjects did not use alcohol, antioxidants and/or vitamins for at least 30 days before this study. The smokers were excluded because of the probability of accelerated production of ROS by activating phagocytes in these subjects[20]. Many studies have reported the influence of smoke on antioxidant levels[21]. The following procedure was employed in each patient.

At the first visit, after local anaesthesia, a rubber-dam was inserted and access was achieved. The root canal was prepared by a crown-down method using rotary HERO 642 (Micro-Mega, France) NiTi instruments.

The tooth was allowed to drain until the discharge stopped and then the canal was irrigated gently with 0.12 % chlorhexidine gluconate. The canal was dried with absorbent points and a dry, sterile cotton pellet was placed in the chamber. The access opening was sealed with cavit (3M ESPE AG, Germany).

When all of the symptoms had disappeared, root canal treatment was completed. The canal was rinsed with 0.12 % chlorhexidine gluconate solution, dried with sterile paper point and obturated with laterally condensed guttapercha and sealapex (Kerr, Romulus, MI USA). With this treatment inflammation disappeared. Blood samples were taken before and after treatment. Venous blood (3 mL) was collected in vacutainers without additive, allowed to clot for 0.5h at room temperature and centrifuged at 3000 g for 5 min to separate serum. The serum aliquots were stored at -80 ºC until analysis.

Catalase (CAT) activity was measured in serum at 20 ºC, according to the method of Aebi[22]. Using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}, the rate of the first 30 s was used to calculatethe activity. Catalase activity was expressed as U mL^{-1} (U/mL).

Glutathione reductase (GRD) activity was assayed by using oxide glutathione as substrate[23] and based on the absorbance changes at 340 nm due to oxidation/ reduction of reduced nicotinamide adenine dinucleotide phosphate/oxidized nicotinamide adenine dinucleotide phosphate (NADPH/NADP).

The findings were expressed as mean ± SD. Statistical analysis was carried out using Wilcoxon Signed Ranks test (nonparametric) and Spearman's rank correlation coefficient test. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL).

RESULTS AND DISCUSSION

Mean, SD lowest and highest values of, CAT,GRD before and after therapy are shown in Table-1.

TABLE-1
MEAN ± SD, CAT, GRD, ACTIVITIES, CONCENTRATIONS IN SERUM OF PATIENTS WITH ACUTE PERIRADICULAR ABSCESS (DESCRIPTIVE STATISTICS)

	N	Mean	SD	Min	Max
Before					
CAT	32	2.0629	1.65576	0.24	6.99
GRD	32	0.0778	0.11923	0.02	0.55
After					
CAT	32	2.0435	1.82800	0.20	6.06
GRD	32	0.0584	0.08816	0.01	0.53

CAT (U/mL) = Catalase; GRD (U/mL) = Glutathione reductase.

Serum catalase concentrations were not statistically significant between before and after treatment ($p > 0.05$) (Fig. 1). Also, the levels of serum GRD activity did not significantly change ($p > 0.05$) (Fig. 2). However, a decrease in average of catalase and GRD concentrations were observed.

In healthy individuals the plasma levels of antioxidants may be affected by many factors other than the nutrient intake, such as the degree of absorption, intake of other nutrients, homeostatic regulation, *etc.*[24].

Inflammatory cells including polymorphonuclear leukocytes, monocytes and macrophages can produce ROS when activated[25]. The small vessels are the target for neutrophils and for immune complexes to precipitate at vasculitis. Increase in the ROSs generation or decrease in the antioxidants will result in a shift in the oxidant/antioxidant balance toward increased oxidative stress that may produce damage in cell metabolism[26,27].

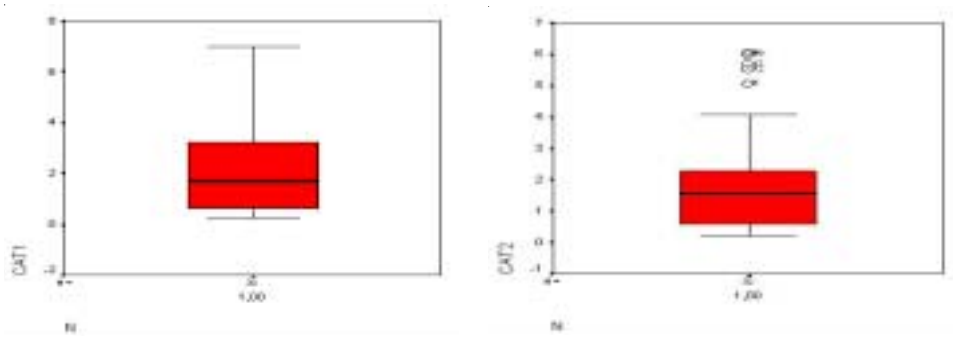

Fig. 1. Serum CAT (U/mL) concentrations before and after the treatment. (CAT1- Before, CAT2-After)

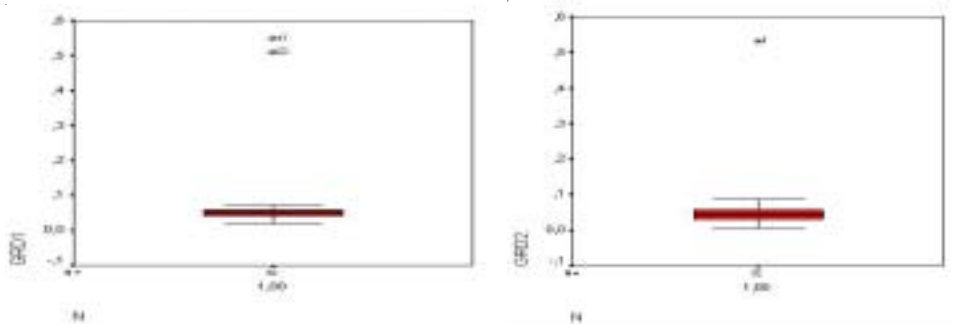

Fig. 2. The comparison of levels of serum GRD (U/mL) activity before and after the treatment. (GRD1-Before, GRD2-After)

Recently it has been reported that ROS are associated with the pathogenesis of some diseases[28]. ROS are capable of reversibly or irreversibly damaging all biochemical substrates of cells, including nucleic acids, protein, free amino acids, lipids, lipoproteins, carbohydrates and connective tissue macromolecules. These species may impair cell activities, such as membrane function, metabolism and gene expression. This chain reaction spreads[29].

It has been claimed that the imbalances in levels of free radicals and ROS with antioxidants may play an important role in the onset and development of several inflammatory oral pathologies[30]. Physiologically, free radicals/ROS in the mouth are derived mainly from polymorphonuclear neutrophils (PMN), which may also help control bacterial growth by the well-known 'respiratory burst'. Such physiological processes are usually efficiently counteracted by intrinsic antioxidant systems: if such systems fail, tissue damage can result[31].

Esposito *et al.*[32] results confirm that a significant increase in catalase activity is detected in the dental inflamed pulp tissues in comparison to healthy controls.

To our best of knowledge, there are no data available on serum catalase activities and GRD activities concentrations in patients with APA. However, many studies have been conducted on various inflammatory diseases (*e.g.*, rheumatoid arthritis).

In some of these studies, it is reported that there is an increase in the activity of both enzymes, but other studies have found a decrease[33]. In addition, there are also some studies indicating that while one of the enzymes is increasing, the other is decreasing[33,34].

Changes were observed both in catalase and GRD before and after APA. However, these changes were not significant. This may be due to different sensitivities of the methods used. Further studies on the subject are needed.

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