Malondialdehyde levels and total antioxidant capacity in the dental follicles of the asymptomatic impacted third molars

Umut Tekin, DDS, PhD, a Ucler Kisa, Dr, b Fethi Atıl, DDS, PhD, a Meltem Karsiyaka Hendek, DDS, PhD, a Ozlem Dogan, Dr, d Safa Gurcan, Dr e

a Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Kirikkale University, Kirikkale, Turkey
b Department of Biochemistry, Faculty of Medicine, Kirikkale University, Kirikkale, Turkey
c Department of Periodontology, Kirikkale University, Faculty of Dentistry, Kirikkale, Turkey
d Department of Biochemistry, Keçiören Education and Research Hospital, Ankara, Turkey
e Department of Statistics, Faculty of Veterinary, Ankara University, Ankara, Turkey

ABSTRACT

Objectives: Malondialdehyde (MDA), is one of many low molecular weight end products of lipid peroxidation (LPO), increases in oxidative stress. Antioxidants such as total antioxidant capacity (TAC) have a protective effect against reactive oxygen species. The aim of this study is to examine the development of the antioxidant defense mechanism in dental follicles (DFs) of radiologically asymptomatic impacted third molars (ITMs) by using MDA and TAC.

Materials and Methods: This study involved 40 DFs of 40 patients referred for clinically and radiographically asymptomatic ITMs. 40 healthy gingival tissues in the same patients obtained during surgical removal of teeth as a control group. This study involved DFs widths on periapical radiographs narrower than 2.5 mm were included in the study. All of tissues samples were analyzed for MDA and TAC.

Results: Levels of the MDA and TAC in DFs were significantly higher than the levels of MDA and TAC provided from healthy gingival tissues of the same patients (p<0.05).

Conclusions: The results of our study showed that an important antioxidant defense mechanism may also occur in DFs of asymptomatic ITMs. In the light of these preliminary findings of the presented study, supplementary studies should be undertaken to establish the differences between inflammation affecting the DFs and clinical outcomes.

Keywords: Oral mucosa, Dental follicle, Malondialdehyde, Total antioxidant capacity

ARTICLE INFO

Article history:
Received: 12-Sep-2014
Accepted: 01-Feb-2015

Corresponding author at: Meltem Karsiyaka Hendek, Kirikkale University, Faculty of Dentistry, Department of Periodontology, Kirikkale/TURKEY,
Tel: +90 318 224 49 27, Fax: +90 318 225 06 85, e-mail: mltksysk@yahoo.com
INTRODUCTION

The surgical removal of impacted third molars (ITMs) is one of the most frequent operations in oral surgery. However, there is still no general agreement about the need for surgical removal of asymptomatic ITMs.1-4 Despite the recommendations of a National Institute of Health (NIH), Consensus Development Conference on the removal of ITMs,5 there is still no general agreement about the need for prophylactic surgical removal of asymptomatic ITMs.6,7

Previous studies have supported that epithelial tumors and odontogenic cysts are derived from epithelial cells in oral tissues such as rests of odontogenic epithelium found in dental follicle (DF) of an ITMs and surface epithelial lining of the oral mucosa.4 It is accepted that pericoronal radiolucency of < 2.5 mm in a follicular width is non-pathologic in DF of asymptomatic ITMs.8

Reactive oxygen species (ROS) are chemically-reactive molecules containing oxygen. ROS are highly reactive due to the presence of unpaired electrons. Oxidative stress reflects an increase in the production of ROS and/or decrease in protective antioxidants.9 ROS are capable of initiating lipid peroxidation (LPO) and damaging DNA and cell membranes. All mammalian cells contain antioxidants that prevent or limit oxidative tissue damage.10 Cells have developed several antioxidant defense mechanisms to neutralize this harmful ROS.11-13 However, if scavenging capacity of affected tissues is exceeded by an overwhelming production of free radicals, significant tissue damage could occur. The defense mechanism against ROS includes three antioxidant pathway, intracellular, extracellular and membrane antioxidants.10

ROS can induce LPO having effects on cells. When ROS interact with the polyunsaturated fatty acids in membranes or lipoproteins, the process of LPO begins. In the resulting LPO, the fatty acids are transformed to the primary product of lipid peroxides.14 Overproduction and/or decreased clearance of ROS by scavenging mechanisms may result in oxidative stress and damage.10,15 Because LPO is an indicator of oxidative stress, numerous markers have been used to observe this process. Malondialdehyde (MDA) is one of many low molecular weight end-products of LPO and increased MDA levels can be an indicator of oxidative stress. In recent years, more attention has been focused on the role of ROS, antioxidants mechanisms, oxidative stress, and LPO products in several oral diseases.16-18

Antioxidants can provide protection against ROS. In healthy organisms, the balance is maintained by the interaction of oxidants and antioxidants. Oxidative stress has been variably determined by the measurement of a decrease in total antioxidant capacity (TAC) or by estimation of the products of oxidative damage. TAC may be the most relevant parameter for assessing defense capabilities.19

The risk of developing pathology at third molar sites might be associated with endogenous free radical scavenging mechanisms (antioxidant enzymes) prevented free radical accumulation. If the scavenging capacity of DF tissues is exceeded by an overwhelming production of free radicals, significant tissue damage could occur. We hypothesized that antioxidant defense mechanisms may be developed against free radical accumulation in DF of radiologically asymptomatic ITMs. Therefore, we aimed to determine the level of MDA and TAC in DF of radiologically asymptomatic ITMs.

MATERIALS AND METHODS

40 DFs in 40 patients having impacted mandibular third molar, completely
covered by bone were included in the study. Since ITMs, partially covered by bone can be affected by bacterial contamination and infection, antioxidant and free radical levels can be affected due to the possible infection. ITMs, which were partially covered by bone were excluded in this study. Patients’ ages ranged from 14 to 33 years (mean age of male and female patients: 20.05 ± 4.90; 19.09 ± 2.52, respectively). 18 male and 22 female patients were selected within the scope of the study. All of the patients were having a healthy gingival with a probing depth (PD) of ≤ 3 mm and a clinical attachment level (CAL) ≤ 1 mm; with no clinical sign of gingival inflammation and also maintaining good oral hygiene and no periodontal disease history.

Healthy gingiva usually has a color that has been described as “coral pink, a smooth arcuate or scalloped appearance around each tooth, a firm texture that is resistant to movement, and the surface texture often exhibits surface stippling (Orange peel appearance). All patients were required to meet the criteria of ‘healthy’ at all sites. 40 healthy gingival tissues were obtained from these patients during surgical removal of teeth covered by bone as control group. The exclusion criteria included a history or sign of infection, smoking cigarettes, or enlarged tissues surrounding impacted third molars (ITMs). Approval for the study was obtained from the Ethics Committee of the Kirikkale University (2010/B062) and informed consent was obtained from all participating patients. The Helsinki Declaration was read and the guidelines were followed by the authors in this study.

Follicular spaces of patients were measured by panoramic radiographs. The DFs with a follicular width of 2.5 mm or lower were included in the study. Surgical removal of the ITMs was performed under local anesthesia by conventional third molar surgery. Bone removal and tooth resection were performed so as not to injure the pericoronal tissues of the ITMs. Following removal of the ITMs, the remnants of pericoronal tissue were curetted from the bony socket and a small piece of gingival tissue was excised from the edge of the mucogingival flap.

All of the samples were taken carefully and kept in a deep-freezer (-80°C) promptly. After washing with 0.9% NaCl, tissue was homogenized (Labor Technique, Germany) with 1 ml 0.9% NaCl solution in ice. Homogenized tissue was centrifuged at 1,500 g for 10 min at 4°C. Supernatants were used for protein and MDA determination. Protein level was measured using Lowry’s method. MDA levels, indicating LPO, were measured by the method described by Armstrong and Al-Awadi, which was modified from the Yagi method. Measurement of TAC level was determined using a novel automated colorimetric measurement method developed by Erel. The calibration curve was prepared with 1, 1, 3, 3-tetramethoxypropane (Sigma, USA) standards of 1–25 nM dilutions. The results were measured as nmol/g protein.

STATISTICAL ANALYSIS

The normality of the data distribution was examined using the Kolmogorov-Smirnov test. Power analysis was used to calculate the minimum sample size, as alpha = 0.05 significance level, and the power of test 0.85, the calculated sample size was n= 38 by using G power ver.3.1.3 (Gpower, Franz Faul, Universität Keil, Germany). The differences between the DF and gingival tissues were investigated with independent sample t-test. The correlations between MDA and TAC levels were examined using the Pearson’s correlation coefficient. A p<0.05 value was accepted as being statistically significant.
RESULTS

The distribution of patients according to age and gender are listed in Table 1. Increased MDA and TAC levels were found in DF tissues. Levels of the MDA and TAC in DF were significantly higher than the levels of MDA and TAC obtained from healthy gingival tissues of the same patients (p<0.05) (Table 2). Correlations between MDA and TAC levels in DF and gingival tissues were investigated for all patients. On analysis, strong and positive correlations were observed between MDA and TAC levels in DF, statistically significant correlations were observed between MDA and TAC levels in gingival tissues (p< 0.05). Correlations between the MDA and TAC levels in DF were found to be stronger than those between the MDA and TAC levels in gingival tissue (Table 3).

DISCUSSION

Removal of ITMs is a common procedure in oral surgery. In order to give an idea about the incidence of pathology, radiological and histopathological studies on ITMs follicles have been done. Some approaches were demonstrated the involvement of oxidative stress in the pathophysiologic mechanisms in several diseases. ROS-related tissue destruction can be measured by the final product of LPO, such as MDA. MDA is the principal and most studied product of polyunsaturated fatty acid peroxidation. In the present study, MDA levels in DFs displayed significant increase according to healthy gingival tissues. Our findings in this study were consistent with our previous study indicating MDA levels in DF of asymptomatic ITMs. As our knowledge, there is no study comparing ROS and antioxidant levels in DF of asymptomatic ITMs. Therefore, we aimed to investigate both ROS and antioxidant in DF.

Investigation of the TAC of dental follicles could provide valuable information about biological protection against ROS. The collaboration among the different antioxidants obtains greater protection against ROS, than any one compound alone. The capacity of known and unknown antioxidants, their cumulative action and the synergistic interaction of all the antioxidants present in body fluids or tissues are all of considerable

Table 1. Demographic characteristics of the study group

<table>
<thead>
<tr>
<th>Age (mean (sd))</th>
<th>Male</th>
<th>20.05 (4.90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>19.09 (2.52)</td>
<td></td>
</tr>
<tr>
<td>Gender n (%)</td>
<td>Male</td>
<td>18 (45)</td>
</tr>
<tr>
<td>Gender n (%)</td>
<td>Female</td>
<td>22 (55)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of tissue levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) between the dental follicular tissues and healthy gingival tissues (all values expressed in mean (sd))

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/g protein)</th>
<th>TAC (nmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental follicular tissues (DF)</td>
<td>19.11 (2.52)</td>
<td>0.83 (0.08)</td>
</tr>
<tr>
<td>Healthy gingival tissues (HG)</td>
<td>8.86 (1.06)</td>
<td>0.59 (0.06)</td>
</tr>
<tr>
<td>p value</td>
<td>0.001*</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

*Significant difference between test and control groups
importance to understand. Thus, the total antioxidant potential may give more biologically relevant information than that obtained by measuring concentrations of individual antioxidants. 27 Studies are also limited with regard to ROS and/or antioxidants mechanisms in dental follicles. To our knowledge, this was the first study to investigate antioxidant defense in DF of asymptomatic ITMs. The results showed that TAC level increased in DFs of asymptomatic ITMs. However, in the present study, both MDA and TAC levels were significantly higher in DF of asymptomatic ITMs than in healthy gingival tissue. We suppose that there should be a low-level balance between oxygen free radicals and antioxidant enzymes in DF of asymptomatic ITMs. It may be a reasonable explanation that the over-activity of oxygen free radicals leads to over-generation of antioxidant enzymes. These results may be explained as a result of tissue protective and adaptive mechanisms. TAC level in DF might be displayed as an increase in the antioxidant activity in response to ROS.

Highly LPO are implicated in the pathogenesis of oral cavity cancer. Several investigators have revealed that the cellular responses are mediated by LPO and these have been implicated in tumor development. 28 The incidence of malignant tumors around ITMs is very low. High levels of MDA and TAC in dental follicular tissues of asymptomatic ITMs provided in the present study might indicate that DF of asymptomatic ITMs developed antioxidant defense mechanisms.

Panjamurthy et al. 29 reported that disturbance in the antioxidant defense system due to overproduction of LPO products at inflammatory sites could be related to a higher level of oxidative stress in periodontitis patients. Sculley and Langley-Evans 30 reported that periodontal disease is associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity. Over production of LPO products at inflammatory sites could be related to a higher level of oxidative stress in periodontitis patients. The results of our study revealed that a significant antioxidant defense mechanism may occur in non-inflamed dental follicular tissue.

The increase in the mean LPO in smokers was caused by an increase in the free radical production. The increase in free radicals might be attributed to cigarette smoking. For this reason, smokers have been excluded in the study group. There are a few studies investigating the proliferative potential of DFs of asymptomatic ITMs. 1,4,8,22,23 Some authors suggested that proliferative potential of DFs of asymptomatic ITMs higher than proliferative potential of oral epithelium. Therefore, the prophylactic removal of ITMs is necessary. 1,8 On the other hand, Saracoglu et al. 4 reported that MIB-1 (A monoclonal antibody, which recognizes the epitope of Ki-67 antigen) positive cells are not seen in asymptomatic DFs, these teeth should not be extracted for prophylactic purposes. Due to the lack of data, this subject is still a source of controversy. The results obtained from presented study revealed that MDA levels and TAC were significantly elevated in DFs.

Table 3. Correlations between malondialdehyde (MDA) and total antioxidant capacity (TAC) levels in all subjects

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental follicular tissues (DF)</td>
<td>0.84</td>
<td>0.001</td>
</tr>
<tr>
<td>Healthy gingival tissues (HG)</td>
<td>0.60</td>
<td>0.001</td>
</tr>
</tbody>
</table>

r, Pearson’s correlation coefficient
CONCLUSION

In conclusion, the results of our study showed that an important antioxidant defense mechanism may occur in DFs of asymptomatic ITMs. In this study, the scavenging capacity of dental follicular tissues is not exceeded by an overwhelming production of free radicals, significant tissue damage could not occur. The findings also suggest that significant relations exist between oxidant status and dental follicular pathologies, and that oxidative stress and antioxidant defense mechanism may play an important role in the development of cyst and tumors from DFs. Dental follicular tissue TAC levels are considered as the markers of antioxidant defense mechanism for follicular tissues. MDA and TAC levels are higher in the DF as compared to the healthy gingival tissues. Therefore, investigation of the role of oxidative stress in DF might not only be very useful in clarifying the mechanism of dental follicular pathologies, but might also determine the role of antioxidants that scavenge the free radicals in DFs. In the light of the these preliminary findings of the presented study, further investigations and comprehensive studies are required to determine the differences between inflammation affecting the DF and clinical outcomes.

ACKNOWLEDGEMENTS

The authors declare that they have no conflict of interests. This study was self-funded by the authors and their institutions.

REFERENCES

11. Chapple IL, Mason GI, Garner


27. Serafini M, Del Rio D. Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity the right tool? Redox Rep 2004;9:145-152.


How to cite this article: Umut Tekin, Ucler Kisa, Fethi Atil, Meltem Karsiyaka Hendek, Ozlem Dogan, Safa Gurcan. Malondialdehyde levels and total antioxidant capacity in the dental follicles of the asymptomatic impacted third molars. Cumhuriyet Dent J 2015;18(2):108-115.