Original Article - Evaluative Study

Salivary Levels of Malondialdehyde and Total Antioxidant Capacity Following Third Molar Surgery - An Evaluative Study

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Abstract

Introduction: Impacted and semi-impacted third molar surgery can lead to considerable distress and discomfort in patients. **Aims:** The present study aimed to assess the changes in salivary levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) after third molar surgery. **Materials and Methods:** In this study, 210 patients scheduled for third molar surgery were selected. Unstimulated whole saliva samples were collected from patients preoperatively and one week after the surgery, via the 'spitting method'. The collected samples were immediately centrifuged and the supernatants were stored at −20°C until testing. The levels of MDA and TAC were measured with a spectrophotometer. **Results:** The results were presented as mean ± standard deviation and paired *t*-test was used to statistically analyse the changes in pre-operative and post-operative values of MDA and TAC. A decrease of mean salivary TAC ($P = 0.031$) and an increase of mean salivary MDA ($P = 0.034$) were detected postoperatively. **Discussion:** Following third molar surgery, the increase of mean salivary MDA and the decrease of mean salivary TAC were detected; however, these changes were not statistically significant.

Keywords: Malondialdehyde, oxidative stress, salivary biomarkers, third molar, total antioxidant capacity

Introduction

The surgical extraction of impacted or partially impacted third molars is a procedure performed in dental offices and can give rise to post-operative stress and numerous complications. Post-operative inflammation, infection and delayed healing are among the most common complications associated with third molar surgery.[1] In addition, post-operative bleeding, swelling, pain and trismus can often be noticed in patients who have undergone third molar surgery.[2] Despite rare prevalence, general impact of such complications should not be left unnoticed due to the notable number of patients undergoing third molar surgery.[1] Therefore, a thorough understanding of the pathophysiological changes in response to third molar surgery may have major significance for reducing post-operative pain and discomfort in patients.

Acute soft tissue inflammatory response after surgery leads to the infiltration of multinucleated white blood cells to the site of inflammation.[3] Leucocytes release free radicals, such as reactive oxygen species (ROS) to fight pathogens.[4] ROS could cause cell damage by lipid, protein and DNA oxidation.[5] Even though natural enzymatic and non-enzymatic antioxidant defence mechanisms exist in human body, excessive ROS production could lead to predominance of antioxidants and disruption of the oxidant/antioxidant balance, which is defined as oxidative stress (OS).[5] Lipid peroxidation is a result of OS and refers to the chain reaction of ROS with unsaturated fatty acids or with lipoproteins.[6]

Malondialdehyde (MDA) is one of the top-end lipid peroxidation products and directly indicates OS and therefore can be used as an OS biomarker.[4] The high diversity of antioxidants in biological fluids and synergistic effects make measuring all the antioxidant components complicated and time-consuming. Hence, total antioxidant capacity (TAC)

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in biological samples could be an appropriate indicator of antioxidant defence.[7]

Saliva is believed to be the first defence barrier against OS, comprising crucial mechanisms such as uric acid, albumin, ascorbic acid and glutathione.[8] It has been established that saliva plays an essential role in the early stages of wound healing following tooth extraction, by modulating the inflammatory mediators.[9] Furthermore, a broad variety of hormonal, infectious, immunological and toxicological biomarkers can be measured through saliva, consequently making it a diagnostic tool for numerous oral and systemic diseases.[10] In addition, collecting salivary samples is rather simple, low-cost and non-invasive in comparison to blood and biopsy samples, which proves to be highly significant.[10]

In previous studies, a higher level of OS biomarkers, including MDA and TAC, has been associated with periodontitis,[11] dental caries,[12] impacted wisdom tooth follicles[13] as well as multiple oral diseases such as lichen planus[14] and aphthous stomatitis.[15] Refahee *et al*.,[16] demonstrated a significant increase in serum OS biomarkers after extraction of premolars and canines of dogs. Few studies conducted on human subjects, some with rather contrary results regarding MDA levels, have reported an increase of serum OS markers after third molar extraction.[17,18] There is a scarce evidence on OS changes in salivary samples.[19] Therefore, extensive investigation is demanded for further confirmation of previous findings. This study aimed to investigate the changes in OS biomarkers in patients undergoing third molar surgery by the assessment of salivary MDA and TAC levels.

Subjects and Methods

This study included patients (120 males and 90 females) who were referred to the Department of Oral Surgery of Urmia University of Medical Sciences (in Urmia, Iran) for surgical extraction of an impacted or partially impacted third molar between 2014 and 2016. Subjects of the study were generally healthy individuals aged 18 years or older. The exclusion criteria included history of systemic diseases affecting periodontal health (e.g., diabetes mellitus, immunocompromised status, cardiovascular diseases or rheumatologic disorders), chronic oral mucosal diseases (e.g., oral lichen planus, pemphigus vulgaris and chronic aphthous stomatitis), history of drug allergies, smoking or alcohol use, recent history of antioxidant medication consumption (e.g., vitamin A) and pregnant or post-menopausal females. The present study was designed in accordance with the guidelines issued and approved by the Ethics Committee of Urmia University of Medical Sciences (code: IR. UMSU. REC.1395.137. Date: 2016/07/19). All participants were informed about the objectives of the study and were requested to fill in a written consent form. The study protocols were conducted according to the Declaration of Helsinki for medical research.

Panoramic radiographs were examined preoperatively. All participants were instructed to abstain from eating, drinking and brushing their teeth 90 min before sample collection. All surgeries were performed by the same oral and maxillofacial surgeon with 15 years of experience. Nerve block technique was implemented using one carpule of 2% lidocaine with 1:100,000 epinephrine as a local anaesthetic. Most surgeries lasted 30–60 min. Horizontal and releasing incisions were made to have sufficient access. Next, the flap was carefully reflected and the alveolar bone was partially removed using a round bur in a surgical handpiece. Following the tooth extraction, the socket was curetted in all patients and irrigated with sterile saline solution and sutures were performed consequently. In addition to standard post-operative instructions, all patients were prescribed 500 mg amoxicillin for three days (in allergic cases, 500 mg erythromycin was prescribed b.i.d. x4 days) and 200 mg ibuprofen t.i.d. x2 days. The patients were advised to avoid consumption of any other drugs, and in case of post-operative complications, to only seek help from the conductors of the study.

Saliva samples were collected on two separate occasions: the initial samples were taken preoperatively, prior to local anaesthetic injection; and the second samples were taken seven days after the operation, before the suture removal.

The subjects were instructed to rinse their mouths thoroughly with deionised water and then were seated upright and asked to rest for 5 min before sample collection. To reduce possible circadian interference, all samples were collected between 10 and 12 a.m. Unstimulated whole saliva was collected by asking the subjects to lean forward and spit into sterile 5 mL test tubes (Falcon, Falcon Co, China) every 60 s;[20] the lids of the tubes were tightly closed with parafilm (Jinhua Hisure Scientific Co., Ltd, Zhejiang, China) and labelled accordingly. The collected tubes were immediately coded and sent to the biochemistry laboratory of Urmia University of Medical Sciences to be centrifuged at 2000 rpm for 10 min to remove debris. The supernatants were transferred to microtubes, coded and stored at −20°C until testing.

The MDA measurement method was based on its reaction with thiobarbituric acid (TBA), (Merck AG, Darmstadt, Germany), to form MDA-TBA; absorption measurement was obtained by spectrophotometry at 532 nm wavelength and was compared with a standard curve.[4] To determine the TAC level in salivary samples, ferric reducing acid antioxidant power assay was performed, based on the reduction of Fe3+ to Fe2+ with 2,4,6-Tris (2-pyridyl)-S-triazine using a spectrophotometer at 539 nm, compared against the $FeSO_4$ standard curve.[21] The salivary MDA and TAC concentrations were reported in µmol/ml.

Sample size was calculated using Sample1 Software (Stoccu Inc. RaoSoft Co, Georgia, USA) with confidence interval of 95% and statistical power of 80%. For statistical analysis, the results were presented as mean ± standard deviation (SD) for quantitative variables. The paired *t*-test or Kruskal–Wallis *H*-test was used to assess the change in quantitative parameters for statistical analysis. $P \leq 0.05$ was considered statistically significant. The statistical software SPSS version 23.0 for Windows (IBM, Armonk, New York) was used for the statistical analysis.

Results

The mean age of the subjects of the study was 26.95 ± 5.45, ranged from 18 to 36 years. Regarding the type of tooth impaction, 38.1% had impacted, and 61.9% had semi-impacted status. The one-sample Kolmogorov–Smirnov test showed a normal distribution of data ($P > 0.05$) before and after the surgery; therefore, parametric tests were used to evaluate the objectives of the research. The mean saliva MDA level before the operation was 0.77 ± 0.32 μmol/ml which reached 1.01 ± 0.42 μmol/ml, indicating a post-operative increase with a medium to large effect size, which was statistically insignificant (Cohen's d = 0.62, $P = 0.074$). Furthermore, the pre- and post-operative values of TAC were 0.65 ± 0.31 μmol/ml and 0.59 ± 0.38 μmol/ml, respectively, suggesting a slight decrease (Cohen's d = 0.179, $P = 0.071$) [Table 1].

Discussion

This study was designed to investigate the changes in OS biomarkers seven days following third molar surgery. The present study suggests that surgical extraction of third molars could be associated with a post-operative increase in OS indicated by an increase in mean salivary MDA and a decrease in mean TAC; however, there is not enough evidence in this study to support this hypothesis. In this study, the post-operative changes in TAC value remained statistically insignificant despite a decrease in mean value of this marker. Previous studies have reported a significant decrease in serum and salivary levels of TAC, during the 1st week following the third molar surgery.[17-19] Similarly, the increase in MDA levels remained statistically insignificant; nevertheless, evaluation of standardised mean difference between pre- and post-operative data indicated that the effect size (Cohen's d) was considerable, therefore suggesting that the statistical insignificance of the results might be due to small sample size. Kindler *et al*.,[18] and Dias *et al*.,[19] revealed that MDA levels did not exhibit statistically significant changes 7 days after surgery, which is consistent with the present study; however, significantly higher MDA levels were observed 1 day post-surgery in previous studies.[16,19]

Interestingly, Graziani *et al*.,[17] reported a statistically significant reduction in serum MDA levels, which is in contrast with previous findings.

The existence of rather contrary results may be related to several factors, including the type of samples under study (salivary/serum), sample collection method (stimulated/unstimulated) and sample collection intervals. In general, the unstimulated method is reasonable for measuring biomarkers because saliva is easily collected, no interfering factor is present and the results are reproducible.[20] Moreover, such discrepancies in research results have been attributed to study samples in terms of size and diversity, which corroborates the need for further research to reach statistically definitive results.

Another limitation of the present study is the lack of post-operative samples on multiple additional intervals (e.g., hours/days/months following the extraction), as it could be argued that the time of sample collection attributes to the salivary levels of biomarkers. Similarly, this could explain the rather differing results of previous studies. Furthermore, in our study, no correlation between MDA level and post-operative complications were investigated, expected to be investigated further in future studies.

Table 1: Mean±standard deviation values of the study parameters

Variable (μmol/mL)	Baseline	7 days	*P*
MDA	0.77±0.32	1.01±0.42	0.034
TAC	0.65±0.31	0.59±0.38	0.031

MDA: Malondialdehyde, TAC: Total antioxidant capacity

Conclusions

The present study suggests that following third molar surgery, an increase in OS could be detected; however, the statistical analysis did not provide a reasonable level of significance. Future research is needed to further explore this issue and to investigate these findings in other samples. In addition, the possible advantage of prescribing supplementary antioxidants (e.g., Vitamin A, C and E) for patients with a higher risk of post-operative complications might prove an interesting topic for future research.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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