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The antioxidant and anti-inflammatory efficiency of hyaluronic acid after third molar extraction

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ABSTRACT

Purpose: Hyaluronic acid (HA) has a number of clinical applications in current practice. Therefore, correlation of HA with free radicals and inflammatory cells is clinically important. The purpose of this study is to measure the efficacy of high molecular weight HA on the oxidative stress of oral wounds (glutathione (GSH) and lipid peroxidation (LPO) levels), the inflammatory reaction (leucocytes, collagen and angiogenesis content), pain (visual analogue scale (VAS) records) and trismus (maximum interincisal opening (MIO) records) after third molar (M3) extraction.

Patients and methods: 40 patients were included in this study. 0.2 ml 0.8% HA was applied immediately after surgery within the HA group ($n = 20$). Nothing was applied to the control group ($n = 20$). The primary outcome variables were the changes in the inflammatory reaction (leucocyte, angiogenesis and collagen content), oxidative stress (GSH, LPO) and clinical parameters (VAS, MIO). Results were compared immediately after extraction (T0) and 1 week after surgery (T1). Bivariate analyses were used to assess the differences between the HA and control groups for each study variable.

Results: There was a statistically significant difference of leucocyte infiltration and angiogenesis between the groups at T1. The HA group showed less leucocyte infiltration and more angiogenesis than the control group. There was no statistically significant difference in oxidative stress, VAS or MIO levels between the groups.

Conclusion: Our results confirm the hypothesis that HA has an anti-inflammatory effect following M3 extraction. However, the oxidative stress levels and clinical outcomes were similar after one week. Further studies examining these parameters at different times are necessary.

1. Introduction

Hyaluronic acid (HA) is a high molecular weight glycosaminoglycan. It is one of the major components of the extracellular matrix. It has various functions including: maintenance of the elastoviscosity of joint synovial fluid; control of tissue hydration; and many receptor-mediated roles in cell detachment, such as mitosis, migration, tumour metastasis and inflammation (Chen and Abatangelo, 1999; Teh et al., 2012).

The unique viscoelastic nature of exogenous HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications including: supplementation of joint fluid, assisting wound regeneration and dermal filling (Price et al., 2005; Bannuru et al., 2011). However, there is insufficient evidence about its application for oral wound healing.

Wound healing consists of highly integrated and overlapping phases (Jiang et al., 2007). HA, can be involved at any stage of these phases or be indirectly associated with accompanying processes including: migration of inflammatory cells, interaction with remaining inflammatory elements, and the scavenging of free radicals (Prosdocimi and Bevilacqua, 2012). These potential interactions raise the question of whether there is a correlation between the presence of HA and the clinical outcomes of inflammatory reaction.

Extraction of mandibular third molars (M3), still the most common oral surgical procedure, often results in swelling, pain and trismus. Therefore, it affects the patient's quality of life (Majid and Mahmood, 2011). Corticosteroids are often used to reduce the inflammatory reaction. Correspondingly, exogenous HA application

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may offer similar outcomes; decrease the inflammatory response and prevent oxygen free-radical damage after tooth extraction (Foschi et al., 1990; Brown, 2004).

The purpose of this study is to measure the efficacy of high molecular weight HA on wound healing considering biochemical and histological parameters after M3 extractions. We compared glutathione (GSH) and lipid peroxidation (LPO) levels biochemically; pain and trismus levels clinically; and leucocytes, collagen and angiogenesis content histologically between control and HA groups.

2. Materials and methods

2.1. Study design and sample

We implemented a double blind, randomized, and controlled clinical trial. The tissue samples were obtained from 40 patients (female patients, $n = 20$), who were referred to the Department of Oral and Maxillofacial Surgery at Marmara University, Istanbul, Turkey, for evaluation and management of M3 between January 2011 and January 2012. Patients included in the study had M3s which had erupted, or were half impacted but without bone retention, and were vertically positioned. All patients were healthy and classified as ASA I–II (American Society of Anesthesiologists I–II). The mean age was 26.6 ± 6.3 years. Patients were excluded from the study if they had: signs of pericoronitis or pain before surgery; an extraction time greater than 30 min; antibiotics or any other medication therapies during the preceding 2 weeks; or active carious lesions and/or periodontal diseases.

The extractions, application of 0.8% HA gel (GENGIGEL PROF, Milano, Italy), sampling and follow-up were performed by the same operator. Marmara University Department of Biochemistry implemented the biochemical processing and made the analyses and evaluation. The Department of Histology and Embryology completed the histological evaluation of the fixed samples. The clinical research ethics committee of Marmara University approved the study protocol (Protocol number: 2011-1).

2.2. Variables

The primary predictor variable, HA exposure, was coded as a binary variable: the patients in the HA group ($n = 20$) had HA applied following M3 removal; the patients in the control group ($n = 20$) received no other application following extraction. Inflammatory response, oxidative stress, pain and trismus, as the primary comparisons, were recorded immediately after surgery (T0) and compared with the outcomes after one week (T1). The primary outcome variables of oxidative stress were tissue GSH as an important antioxidant, and LPO levels as an indicator of oxidative damage. The primary outcome variables of inflammatory reaction were leucocyte, angiogenesis and collagen content. A visual analogue scale (VAS) was used to record pain, and maximum interincisal opening (MIO) was noted to evaluate trismus as the primary clinical outcome variables.

2.3. Data collection, management, and analyses

Tissue samples, about 2 mm^3 in volume, were obtained following extraction. All samples were taken from the buccal wound edge of extraction socket. Wound closures were made with 3.0 silk sutures. Subjects were randomly assigned to receive HA application. In the HA group ($n = 20$), 0.2 ml 0.8% HA was applied immediately after M3 removal to the edge of extraction socket. In the control group ($n = 20$), nothing was applied. Tissue samples were taken from the same region after one week. They were divided and stored separately at −24 °C for biochemical and histological evaluation.

Samples were thawed and kept at 4 °C for biochemical evaluation. They were homogenized for determination of LPO and GSH levels. Afterwards, they were centrifuged and supernatants taken for analysis. LPO levels were determined by Ledwozyw's method (Ledwozyw et al., 1986). In brief, this method involves boiling the supernatant with thiobarbituric acid and extracting the adducts formed with n-butanol. Its absorbance at 532 nm was measured in terms of the tissue malonedialdhyde (MDA) content, which is taken as an index of LPO. The result was expressed in nmol MDA/g tissue.

GSH measurements were performed using a modification of the Ellman procedure (*Beutler,* 1975). Briefly, 0.5 ml of supernatant was added to 2 ml of 0.3 M $Na_2HPO_4.2H_2O$ solution after centrifugation at 3000 g for 10 min. Next, 0.2 ml of dithiobisnitrobenzoate solution (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of 1.36×10^4 $M^{-1}cm^{-1}$. Results are expressed in mg/g in tissue.

The specimens obtained for histological evaluation were embedded in paraffin blocks, sectioned and stained with hematoxylin and eosin and Masson's trichrome stain for examination under light microscope. The grading of the inflammatory infiltration was established by randomly selecting and counting fields for leucocyte infiltration, angiogenesis and fibrosis (collagen deposition) content at ×400 magnification. Scoring was made in the following manner: '0', when none of the fields show parameters; 'slight', when at least five fields contain the parameter that occupy <50% of the field; 'mild', when at least five fields show the parameter that occupy >50% of the field; and 'intense', when all 10 fields evaluated show parameters that occupy >90% of the field.

Trismus was evaluated by measuring the distance between the edges of the upper and lower right central incisors at maximum opening of the jaws preoperatively and 7 days after surgery. Pain intensity was assessed using a 10-point VAS, with the patient placing a mark on the scale to indicate an intensity range from no pain, 0, to severe/unbearable pain, 10. The severity of the pain was evaluated on the operation day and on postoperative day 7.

The results were analysed using the Statistical Package for the Social Sciences (SPSS version 12.0; SPSS, Chicago, IL). Descriptive statistics was computed for all variables. A paired sample *t*-test, chi-square test and Mann–Whitney U test were used to assess the differences between the HA and control groups for each study variable. The level of statistical significance was set at $p < 0.05$.

3. Results

There was no statistically significant difference between groups regarding distribution of the parameters: leucocytes, angiogenesis, collagen content, GSH and LPO at T0; and also GSH, LPO and collagen content were the same at T1 (Tables 1–3), (Figs. 1 and 2). There was a statistically significant difference in leucocyte infiltration and angiogenesis between the groups at T1. Evaluation of leucocyte infiltration in the HA group found fewer cases of 'mild' and 'intensive' and more 'slight' infiltration than the control group (Table 4). Angiogenesis in the control group was found to be more 'mild' and 'slight' with fewer 'intensive' changes then the HA group (Table 5).

There were no statistically significant differences in VAS between the two groups on the day of the operation or on the postoperative 7th day. There was a significant decrease in mean VAS for both groups after 1 week ($p < 0.05$) (Table 6). On the 7th postoperative day, almost all of the patients had regained their preoperative MIO and there was no statistically significant difference between the two groups (Table 7).