ORIGINAL ARTICLE

CHANGES IN SERUM LEVELS OF IL-6 IN THE EARLY POSTOPERATIVE PERIOD AFTER PREEMPTIVE ANALGESIA WITH NIMESULIDE, METAMIZOLE SODIUM AND PLACEBO IN REMOVAL OF IMPACTED MANDIBULAR THIRD MOLARS

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ABSTRACT

Objective: The aim of this study is to determine the change in IL-6 serum levels in patients undergoing preemptive analgesia and surgical removal of an impacted mandibular third molar. Study Design: This is a prospective, double-blind, placebo-controlled study in 80 patients who had an atypical extraction of an impacted mandibular third molar. Results: After surgical removal of impacted mandibular third molars, elevated levels of IL-6 in the early postoperative period were found, and the highest level was 14 pg/ml. Conclusion: Postoperative IL-6 levels rise regardless of the medication used for preemptive analgesia. In the group treated with nimesulide, a trend to reducing IL-6 levels was observed, but further study in a larger number of patients is needed.

KEYWORDS: interleukin-6, preemptive analgesia, third molar surgery

Introduction

Preemptive analgesia is an antinociceptive method formulated by Crile in 1907 and revived as a concept in a series of animal studies carried out by Woolf in 1983 [1]. It is based on the principle of preventing central sensitization [2]. Preemptive analgesia in surgery aimed at controlling the neuropathic and inflammatory components of postoperative wound pain. One of the key factors influencing and modulating pain is IL-6.

In recent years, numerous studies of various substances related to postoperative pain have been carried out. Certain cytokines are of particular concern, particularly IL-6, which has both proinflammatory and anti-inflammatory properties. The interest in IL-6 is determined primarily by the fact that it synthesized after nerve injury, both in the peripheral nerves and the spinal cord, and it can affect transduction, conduction, and transmission of the nociceptive signal to the cognitive centers in the central nervous system (CNS) [3]. Another important feature is that IL-6 plays an essential role in local pain due to its proinflammatory properties [4].

There are numerous reports on using IL-6 as a marker of pain and inflammation in various fields of surgery, as well as on the existence of a correlation between IL-6 concentration and the gravity of surgery [5, 6]. Studies of many authors show that IL-6 is an early and sensitive marker of tissue injury, which is particularly informative in the first 24 hours of the postoperative period [7, 8, 9, 11]. Some authors, in their studies, find a correlation between IL-6 concentrations and the development of postoperative complications [10, 11].

Copyright © 2016 by the Bulgarian Association of Young Surgeons
DOI:10.5455/jsm.mandibular-third-molar
First Received: April 20, 2016
Accepted: May 21, 2016
Manuscript Associate Editor: George Baitchev (BG)
Editor-in-Chief: Ivan Iinkov (BG)
Reviewers: Janayna Gomes Paiva-Oliveira Gomes (BR); Anuj Kumar (IN); Esra Yüksel (TR); Syed Sirajul Hassan Hassan (IN); F W G Costa Gurgel Costa (BR)
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Some studies have shown that changes in IL-6 levels could be seen in oral surgery procedures [12]. There are relatively few studies of the use of this cytokine as a marker in surgical removal of mandibular third molars [13, 14]. According to some authors, anti-inflammatory agents used also affect IL-6 concentration in the early postoperative period [15, 16]. Nimesulide is a commonly used drug with a rapid and pronounced analgesic effect. This medication reaches its peak plasma concentration in 1.22 to 3.16 hours without causing any changes in platelet activity and has less pronounced ulcerogenic effects compared to other NSAIDs. [17] Another commonly used medication is metamizole sodium, which introduced into clinical practice in 1922. It has a pronounced analgesic effect, without the side effects of other NSAIDs. Its metabolites provide analgesic and anti-inflammatory effects for 8 hours when a single dose of 500 mg is received [18].

**Objective**

The purpose of this study is to determine whether there are changes in IL-6 concentration after preemptive analgesia with nimesulide, metamizole sodium or placebo in surgical removal of impacted mandibular third molars.

**Patients and Methods**

The study conducted after obtaining approval by the Ethics Committee at the Medical University of Plovdiv and informed consent of all patients enrolled in the study. The study included 80 patients who visited the Department of Oral Surgery at the Faculty of Dental Medicine at the Medical University of Plovdiv in the period October 2013 - December 2014 for surgical removal of a mandibular third molar.

Laboratory equipment and materials used in the study: monovettes of “Sarstedt” type 2.6 ml; kits for IL-6 testing (“Human IL-6 QuantiKine ELISA Kit - R&D Systems”); micropipettes Thermo Scientific, 10 - 100 microliters; automatic pipettes Accumax, 5 - 50 microliters; sample storage chamber at -80°C; centrifuge T24D; ELISA reader “Sunrise - basic TECAN”.

**Inclusion Criteria:**

1. Clinically healthy patients aged between 16 and 45, with indications for extraction of a mandibular third molar;
2. No clinical symptoms related to the mandibular third molar to be extracted;
3. Extraction of mandibular third molars, Pell and Gregory classes A and B, subclasses 1 and 2.

**Exclusion criteria:**

1. Patients whose age is beyond the age range for the study;
2. Pregnancy;
3. Allergy to nimesulide, metamizole sodium or lactose – the main ingredient of placebo;
4. Acute inflammation of the tooth to be extracted;
5. Taking antibiotics or NSAIDs in the last seven days;
6. Patients needing antibiotic prophylaxis;
7. Patients with systemic diseases.

To avoid problems associated with the surgeon’s experience and the surgical technique used, all the surgeries were performed by the same operator.

**Surgical technique**

The surgical procedures for removal of mandibular third molars performed as per the standard procedure. The following analgesia with articaine hydrochloride 4% solution (Ubiestin), an incision was made, starting from the anterior ridge of the ramus of the mandible and reaching the distal end of the second molar.

Then the incision continued as vertical, by going sideways downward and forward, ending at the vestibular vault. Thus, a triangular mucoperiosteal flap was formed. After removal of the bone covering the tooth, the tooth crown or part thereof was separated from the root portion of the third molar, as appropriate. The next stage was luxation and extraction of the tooth. The final stage included smoothing the bone edges, irrigation of the surgical wound with physiological saline and placing sutures which should be removed on postoperative day 7. Postoperative instructions were carefully explained to the patients.

**Study design**

This is a randomized, double-blind, placebo-controlled study. Patients were randomized into three groups (simple randomization) by giving a numbered vial containing one of the two medications or placebo: The first group (30 patients) received oral nimesulide (Enetra - Actavis) 30 minutes prior to the intervention, at a dose of 100 mg, which the patients continued to take at every 12 hours for 5 days; The second group (30 patients) received oral metamizole (Algozone - Elder) 30 minutes prior to the intervention at a dose of 500 mg, and then at every 12 hours for 5 days; The third group (20 patients) received oral placebo 30 minutes prior to the intervention and then at every 12 hours for 5 days.

Venous blood samples were taken twice from all patients to separate the serum. The first sample was taken 1 hour before the surgery. The second sample was taken 24 hours after the surgery. The following algorithm used for the preliminary stage of IL-6 testing: One 2.6 ml monovette of blood made for separation of serum; Samples were allowed to clot for up to 30 minutes; they centrifuged for 15 minutes at 1000 x g; the clot removed from the serum, and the serum was analyzed without further processing or after being proportionally diluted with inactive substances (saline). The samples stored at ≤ -80°C until carrying out ELISA assay. LOQ (Limit of quantitation) = 2 pg/ml.

**Statistical methods**

The Kruskal-Wallis test was used to compare the age of the patients among the three investigated groups (treated with nimesulide, metamizole sodium, and placebo). Boxplot diagrams used for graphical visualization of the patients’ age (outliers distinguished according to the criteria 1.5 of the interquartile range). Fisher’s exact test applied for comparison of categorical variables and stacked bars (to 100%) used for graphical presentation of this data. Calculations made with MS Excel 2016.
Changes by gender, age and medication administered were analyzed using descriptive statistics methods. We found homogeneity among the three groups about sex, age, and side of extraction. Gender distribution showed that the ratio male : female patients by groups was as follows: for the nimesulide group - 70% : 30%; for the metamizole sodium group - 67% : 33%, and for the placebo group - 65% : 35% (Figure 1).

Using Fisher’s Exact Test (P = 0.95), we proved that proportions by gender were statistically identical in all three treatment groups.

We found that IL-6 concentrations in the blood samples taken 24 hours after the removal were statistically identical in all the groups treated with different medication (Fisher’s exact test, P = 0.54). Summary of the obtained IL-6 concentrations for each group presented in Figure 4. In Table 1 the obtained results for IL-6 are presented, stratified by gender. Further analysis
Table 1 Comparison between treatment groups of IL-6 levels (after the extraction) and stratification by gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Concentration pg/ml</th>
<th>Placebo</th>
<th>Algozone</th>
<th>Enetra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>&lt;2.00</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2.00-5.99</td>
<td>3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6.00-9.99</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;=10.00</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>7</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>&lt;2.00</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2.00-5.99</td>
<td>6</td>
<td>10</td>
<td>13</td>
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<tr>
<td></td>
<td>6.00-9.99</td>
<td>2</td>
<td>3</td>
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<td>1</td>
</tr>
<tr>
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<td></td>
<td>13</td>
<td>20</td>
<td>21</td>
</tr>
</tbody>
</table>

The comparison of IL-6 concentrations in the preoperative and postoperative samples showed that there was a statistically significant difference in each of the treatment groups. Table 1 presents the stratification of postoperative levels of IL-6 by gender.

Discussion

The objective of this study was to detect changes in IL-6 concentrations after surgical removal of impacted mandibular third molars after preemptive administration of nimesulide, metamizole sodium or placebo, as well as to detect an effect of the drugs used on the release of IL-6. To avoid problems associated with the surgeon’s experience and the surgical technique used, all the surgeries were performed by the same operator.

It is noteworthy that preoperative IL-6 levels were below the LOQ. This is in disagreement with studies by other authors [13, 14]. In our opinion, the reason for this is the inclusion and exclusion criteria of the study, which were defined so to eliminate the phenomenon of central sensitization. The results obtained at postoperative hour 24 showed a significant increase in IL-6 levels compared to the pre-operative results. These findings are identical to the results of studies of some authors, according to whom IL-6 levels are highest at postoperative hour 24, and then decrease [14, 19, 20]. The analysis of the data obtained by us showed that the rise in IL-6 levels after surgical removal of impacted third molars was not higher than 14 pg/ml. These findings are consistent with the results of studies by Singh et al. [14], where the peak of IL-6 concentration at hour 24 is 10.08 pg/ml. A significant effect on IL-6 levels in the nimesulide treatment group is noteworthy, which, in our opinion, is due to the preemptive effect of the medication. As in other procedures in oral surgery, because of the relatively small number of studies of IL-6 after extraction of third molars, extrapolation of data from surgical interventions in other fields was necessary. When performing surgery of a larger extent, IL-6 levels are significantly elevated [21]. This shows that we can use the levels of the cytokine studied as an early marker of tissue injury, as well as of the extent of the surgery performed. We are unable to answer whether the reason for the rise in IL-6 levels is tissue damage or development of inflammation after the surgical trauma. Some authors make a correlation between the increase in the IL-6 levels and the likelihood of post-operative complications; the level above which complications develop is found to be 400 pg/ml [10, 11]. Other factors influencing IL-6 serum levels are increased muscle activity, type 2 diabetes, as well as surgical stress (a factor accompanying each surgical procedure) [22, 23]. The gender of the patient is also necessary for the change in the serum levels of the studied cytokine, and the elevation was more pronounced in female patients. [24]

When analyzing the results, no significant difference in IL-6 levels between the three groups was found, regardless of the preemptive analgesia administered. These results are in agreement with the findings from other studies [25], as well as with a meta-analysis made on the issue of preemptive analgesia [26]. Other studies [27, 28] have shown a reduction in serum levels of IL-6 in the preemptive analgesia group.

Conclusion

A correlation between IL-6 serum levels and the gender of the patients was not found. Preemptive analgesia with nimesulide, metamizole sodium or placebo did not demonstrate a statistically significant effect on the expression of IL-6 in the treatment groups.

Stratification by gender of the postoperative values of IL-6 showed no correlation between the cytokine studied and sex of the patients. Regardless of the data obtained, there is a statistical trend toward reduction of IL-6 serum levels in the group treated with nimesulide, but to verify the relevance of this, it is necessary to conduct a study involving a significantly larger number of patients.
Authors’ Statements

Competing Interests
The authors declare no conflict of interest.

References

