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Salivary cortisol as a biomarker of surgical stress and clinical outcomes following third molar surgery

Submission date 07/05/2026	**Recruitment status** No longer recruiting	[_] Prospectively registered [_] Protocol [_] Statistical analysis plan [_] Results [_] Individual participant data [X] Record updated in last year
Registration date 11/05/2026	**Overall study status** Completed	
Last Edited 11/05/2026	**Condition category** Oral Health	

Plain English summary of protocol

Background and study aims
This study was designed to compare the effects of long-acting local anesthetic ropivacaine combined with dexamethasone on salivary cortisol levels as a measure of surgical stress, pain control, and recovery following surgical removal of impacted
mandibular third molars. The main aim was to evaluate differences in salivary cortisol levels, anxiety, duration of anesthesia, and postoperative analgesia among three treatment regimens

Who can participate?
Healthy patients who were requiring surgical extraction of an impacted mandibular third molar and who met eligibility criteria for oral surgical treatment.

What does the study involve?
The patients were randomly assigned to one of three groups and received a single dose of mandibular conduction anesthesia using the Gow-Gates technique:
• 4 mL of 0.5% ropivacaine + 1 ml dexamethasone
• 5 mL of 0.5% ropivacaine alone
• 5 mL of 0.5% bupivacaine alone
All patients underwent standardized surgical removal of the impacted tooth. Saliva samples were collected at three time points (15 minutes before application of local anesthesia on the day of the surgery, at 8h to 9h am) , 15 minutes after the surgery, and 24 h after the surgery, at 8h to 9h a.m. on the first follow-up) to measure cortisol levels as a marker of stress. Patients also completed an anxiety questionnaire before surgery and recorded postoperative anesthesia and analgesia, as well as the number of analgesic used.

What are the possible benefits and risks of participating?
The possible benefits and risks for participants can be: the benefit from improved pain control and a better understanding of stress responses during dental surgery. Risks were minimal and related to standard surgical procedures and local anesthesia,
including temporary pain, swelling, or discomfort.

Where is the study run from?
The study was conducted at the Faculty of Medicine, University of Niš, Serbia, including the Department of Oral Surgery and associated research laboratories.

When is the study starting and how long is it expected to run for?
July 2019 to July 2022.

Who is funding the study?
The study was performed under the Institutional academic support (Faculty of Medicine, University of Niš).

Who is the main contact?
Principal Investigator: Assistant Professor Dr Simona Stojanović, DDS, PhD, simona.stojanovic@medfak.ni.ac.rs

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Additional identifiers

Study information

Scientific Title
Evaluation of salivary cortisol as a biomarker of surgical stress and clinical outcomes following surgical removal of impacted mandibular third molars

Acronym
SCORPION-3M

Study objectives
This study aimed to assess salivary cortisol levels as a biomarker of the surgical stress response during the surgical extraction of impacted mandibular third molars, performed under local anesthesia with the long-acting anesthetic ropivacaine combined with dexamethasone.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 01/07/2019, Ethics committee, Faculty of medicine, University of Niš (Dr Zorana Đinđića 81 Blvd, Niš, 18000, Serbia; +381 (0)184226544; info@medfak.ni.ac.rs), ref: 12-7476-2/2

Primary study design
Interventional

Allocation
Randomized controlled trial

Masking
Blinded (masking used)

Control
Active

Assignment
Crossover

Purpose
Health services research, Supportive care, Treatment

Study type(s)

Health condition(s) or problem(s) studied
Surgical extraction of impacted mandibular third molars

Interventions
I Group: I Group – 30 patients received one dose of local anesthetic solutions: 4 ml of local anesthetic 0.5% ropivacaine chloride (ROPIvacaine 5 mg/mL, B|Braun Melsungen AG, 34209 Melsungen, Germany), and 1 ml/4 mg dexamethasone (Dexason®, solution for injection, 4 mg/1 mL, GALENIKA a.d., 11080 Belgrade).

II Group – 30 patients received one dose of local anesthetic solutions: 5 mL of local anaesthetic 0.5% ropivacaine chloride (ROPIvacaine 5 mg/mL, B|Braun Melsungen AG, 34209 Melsungen, Germany);

III Group – 30 patients received one dose of local anesthetic solutions: 5 mL of local anesthetic 0.5% bupivacaine (Marcaine®, 0.5% Astra Zeneca, Sodertalje, Sweden);

Sterile disposable plastic syringes of 5 mL capacity were used in all groups as containers for the local anesthetics, with the expiration dates checked beforehand. A sterile 21 G × 1½” (0.8 × 40 mm) needle was used to inject the test solution at the target site.

The method and frequency of administration
The following anesthetic solutions were administered once for mandibular conduction anesthesia:

4 mL of plain 0.5% ropivacaine combined with 1 mL (4 mg) of dexamethasone, in the same syringe
5 mL of plain 0.5% ropivacaine
5 mL of plain 0.5% bupivacaine

Mandibular conduction anesthesia was performed in the same manner for all groups using the Gow-Gates technique. The needle tip was directed toward the condylar neck at the beginning of the sulcus coli mandibulae, and an aspiration maneuver was always carried out before injecting the full anesthetic solution to ensure the needle was not positioned within a blood vessel. Gow-Gates technique is a mandibular conduction anesthesia method used to achieve a broad sensory block of the mandibular nerve. The injection is administered with the patient’s mouth fully open, directing the needle toward the neck of the mandibular condyle. After negative aspiration, the anesthetic solution is deposited near the mandibular nerve trunk, resulting in anesthesia of the inferior alveolar, lingual, buccal, and mylohyoid nerves. This technique is commonly used in oral and maxillofacial procedures because of its high success rate and reduced incidence of positive aspiration compared with conventional inferior alveolar nerve block techniques.
In case of sufficient anesthesia (i.e. absence of lower lip numbness, or the patients experienced pain during blunt stimulation of the lip or mandibular vestibular mucosa on the operated side, an additional 2 mL dose of the same local anesthetic (ropivacaine or bupivacaine plain) was administered according to the study group.

Impacted mandibular third molar surgery was performed using the same standardized surgical protocol in all patients. Preoperative antisepsis included extraoral disinfection with 10% iodine solution and intraoral rinsing with 0.12% chlorhexidine gluconate. A buccal mucoperiosteal flap was raised through a linear incision extending from the first molar to the anterior border of the ramus. Bone removal around the impacted tooth was carried out using a sterile round bur under continuous irrigation with cold sterile saline solution.
Tooth sectioning and extraction were then performed, followed by irrigation of the surgical site and repositioning of the flap with 4–0 resorbable sutures. Sutures were removed on the seventh postoperative day. Postoperatively, patients received antibiotic therapy with amoxicillin /clavulanic acid or clindamycin in cases of penicillin allergy. For pain control, ibuprofen 400 mg twice daily was prescribed as needed. Patients were instructed to record the onset and intensity of postoperative pain, as well as analgesic intake, using an NRS pain scale. Follow-up and suture removal were completed on the seventh postoperative day.

Saliva sampling and stress assessment
Saliva sampling: Salivary cortisol levels were measured at three time points as biomarkers of stress. Before onset of surgical preparation of patients (on the day of the surgery, in time of 8h–9h in the morning, i.e. 15 minutes before anesthesia administration), approximately 1–2 mL of unstimulated whole saliva was collected from each patient using sterile pipettes and transferred into sterile labeled Eppendorf tubes.
Saliva sampling was repeated 15 minutes immediately after the surgery, as well as on the first postoperative day at the time of 8h–9h in the morning. Samples were stored at −80°C at the

Institute for Biomedical Research, Faculty of Medicine, University of Niš, until biochemical analysis. All tubes were identified only by patient study codes. Cortisol analysis was performed using the ELISA method (Salimetrics Salivary Cortisol Enzyme Immunoassay Kit, USA).
Preoperative subjective stress was assessed using the Revised Dental Anxiety Scale. Patients completed the questionnaire containing 4 questions, 30 minutes before the procedure in the waiting area outside the operating room. The scale consists of four items scored from 1 to 5, with total scores ranging from 4 to 20. Higher scores indicate greater dental anxiety. Anxiety levels were classified as: 4 = normal, 5–8 = mild, 9–12 = moderate, 13–14 = high, and 15–20 = severe anxiety or phobia. Scores were recorded in patient study forms and used as a measure of preoperative psychological stress for correlation with physiological and perioperative outcomes.

Outcome measures
The duration of conduction anesthesia was defined as the interval between the initial onset of the lower lip numbness and its complete resolution.
The duration of analgesia was defined as the time from completion of the surgical procedure to the onset of the first postoperative pain requiring analgesic administration.
Postoperative analgesic parameters were evaluated, including the time to first analgesic intake (min) and the total number of analgesics consumed within 24 h following surgery.

Duration of treatment and follow-up
The total duration of treatment and follow-up for all treatment arms included measurement of salivary cortisol levels at three time points as biomarkers of stress (on the operative day, at 8h–9h in the morning i.e. 15 minutes before local anesthetic administration, 15 minutes after completing the surgery, and on the first postoperative day at 8h–9h in the morning).
The Norman Corah Revised questionnaire was returned just after the patient completed it, on the day of the surgery. The completed questionnaire about duration of anesthesia, duration of analgesia and number of painkillers used, was returned during the first follow-up examination on the first postoperative day in the morning between 8h and 9h. The sutures were removed on the 7th day postoperatively.

Details of the randomisation process
Participant confidentiality was ensured through a coded identification system and restricted access to personal data. Patient records from the central registry were handled exclusively by a designated dental nurse involved in the technical support of the study. Upon receipt, each patient file was placed in an envelope marked only with a unique study code.
After written informed consent had been obtained, the responsible dental nurse independently prepared the anesthetic solution without the presence of other personnel. Each syringe was labeled only with a Roman numeral corresponding to the study group (I–III), the date of surgery, and the patient's study code. The coding sequence did not correspond directly to the order of anesthetic groups described in the study protocol.
The clinician administering the local anesthesia received a syringe containing 5 mL of prepared solution (4 mL local anesthetic with either 1 mL dexamethasone or 1 mL more of local anesthetic) without any indication of the anesthetic type. The type of anesthetic administered was recorded separately by the dental nurse in both written and electronic registries together with the patient's study code. Therefore, the anesthetic type remained blinded to the surgeon, operating room staff, investigators involved in intraoperative assessment, and the patient.
At discharge, patients received a questionnaire for recording the duration of analgesia and postoperative pain. The completed questionnaire was returned during the first postoperative follow-up visit. The collected data were then forwarded to the responsible dental nurse, who added the recorded outcome parameters to the database under the corresponding patient study code.
Biochemical results were collected and integrated with the study documentation by an

authorized investigator. Electronic records remained accessible only to the responsible dental nurse, while written study records were stored in sealed envelopes and opened only after completion of the study.

Intervention Type
Procedure/Surgery

Primary outcome(s)
1. Perioperative salivary cortisol levels measured using enzyme-linked immunosorbent assay (ELISA) at baseline on the day of the surgery, 15 min before anesthesia (08:00–09:00 am), 15 minutes after surgery and postoperative day 1 (08:00–09:00 am)

Key secondary outcome(s)
1. Preoperative anxiety measured using the Revised Norman Corah Dental Anxiety Scale at baseline (on the day of the surgery, 30 min preoperatively)

2. Duration of conduction anesthesia, defined as the time from onset of lower lip numbness to full measured using data collected from study records at one time point

3. Duration of postoperative analgesia, defined as the time from the end of surgery to first pain requiring analgesic intake measured using data collected from study records at one time point

4. Postoperative analgesic consumption, defined as the total number of ibuprofen doses taken within 24 hours post-surgery measured using data collected from study records at one time point

Completion date
07/07/2021

Eligibility

Key inclusion criteria
1. Patients scheduled for surgical extraction of impacted mandibular third molar for the first time
2. Diagnosed as horizontal impaction according to Winter’s classification
3. Patients classified as American Society of Anesthesiologists (ASA) physical status I (ASA I)
4. Absence of pain, swelling or trismus during the 2 weeks prior to surgery
5. Absence of systemic infection or pericoronitis
6. No known allergies to local anesthetics, analgesics, or dexamethasone
7. No antibiotic therapy within 14 days prior to the surgical removal of the impacted mandibular third molar teeth

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
20 Years

Upper age limit

50 Years

Sex
All

Total final enrolment
90

Key exclusion criteria
1. Patients classified as ASA II–VI according to the American Society of Anesthesiologists classification
2. Patients with systemic diseases
3. Individuals who had experienced major stressful life events within the previous 6 months (e.g., death of a close family member, divorce, or job loss)
4. Patients engaged in high-stress occupations and shift work
5. Smokers
6. Patients who had used or were currently using psychoactive medications, sedatives, anxiolytics or antidepressants
7. Pregnant or breastfeeding women
8. Women using oral contraceptive pills

Date of first enrolment
15/07/2019

Date of final enrolment
05/07/2021

Locations

Countries of recruitment
Serbia

Study participating centre
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Study participating centre
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Sponsor information

Organisation
University of Niš

Funder(s)

Funder type

Funder Name
Ministarstvo Prosvete, Nauke i Tehnološkog Razvoja

Alternative Name(s)
Ministry of Education, Science and Technological Development of the Republic of Serbia, Ministry of Education, Science and Technological Development, Министарство просвете, науке и технолошког развоја, Ministarstvo Prosvete, Nauke i Tehnološkog Razvoja Republike Srbije, MPNTR, MEST

Funding Body Type
Government organisation

Funding Body Subtype
National government

Location
Serbia

Funder Name
Faculty of Medicine, University of Niš

Alternative Name(s)
МЕДИЦИНСКОМ ФАКУЛТЕТУ У НИШУ, Медицински факултет Универзитета у Нишу, Faculty of Medicine, University of Nis, University of Niš Faculty of Medicine, Faculty of Medicine-University of Niš

Funding Body Type
Government organisation

Funding Body Subtype
Universities (academic only)

Location
Serbia

Results and Publications

Individual participant data (IPD) sharing plan

IPD sharing plan summary
Not expected to be made available