PAULO ZUPELARI GONÇALVES

Influência do genótipo do citocromo P450 (CYP2C9) na eficácia clínica do tenoxicam após cirurgias de terceiros molares inferiores

Araçatuba

2019

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Tese apresentada à Faculdade de Odontologia do Campus de Araçatuba – Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP, para obtenção do Título de DOUTOR EM ODONTOLOGIA (Área de concentração em Cirurgia e Traumatologia Bucomaxilofacial).

Orientadora: Professora Associada Roberta Okamoto
Coorientador: Professor Titular Carlos Ferreira dos Santos

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DEDICATÓRIA

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Dedico este trabalho à minha família.

O motivo de tudo valer a pena.

A razão que me faz buscar ser uma pessoa melhor.

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"Have the courage to follow your heart and intuition. They somehow already know what you truly want to become".

(Steve Jobs)

ZUPELARI-GONÇALVES, P. **Influência do genótipo do citocromo P450 (CYP2C9) na eficácia clínica do tenoxicam após cirurgias de terceiros molares inferiores.** 2019. 67 f. Tese (Doutorado) – Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2019.

RESUMO

Atualmente, com os avanços da Farmacogenética, estudos estão demonstrando que a resposta individual de medicamentos pode ser diretamente afetada pela alteração da farmacocinética induzida pela genética de cada paciente, e isto pode induzir à ausência, redução, alteração ou aumento da atividade enzimática associada. Esse fato pode modificar a eficácia clínica de determinados medicamentos e, nos casos de anti-inflamatórios não esteroidais (AINEs), alterar sua capacidade de lidar com a dor e até aumentar a frequência e a gravidade dos efeitos adversos. Este estudo teve como objetivo genotipar e fenotipar o gene CYP2C9 em 89 pacientes saudáveis submetidos à cirurgia de terceiro molar inferior, sob medicação de 20 mg de tenoxicam por dia durante 4 dias, comparando a influência do gene na dor pós-operatória, edema, trismo, quantidade de medicamentos de socorro consumidos pelos pacientes, avaliação global e satisfação do paciente em relação à ingestão do medicamento. Trata-se de um ensaio clínico randomizado, desenvolvido no Departamento de Cirurgia e Traumatologia Bucomaxilofacial da Faculdade de Odontologia de Araçatuba (FOA/UNESP) e na Disciplina de Farmacologia do Departamento Ciências Biológicas da Faculdade de Odontologia de Bauru (FOB/USP). Foi realizado o sequenciamento genético dos participantes do estudo, a fim de verificar polimorfismos do gene CYP2C9, e estes dados foram cruzados com as características pós-operatórias acima mencionadas. Oitenta e nove participantes foram selecionados: 64 (74%) foram incluídos no grupo “Metabolizadores Normais” (*CYP2C9 * 1 / * 1*) e 25 participantes no grupo “Metabolizadores Intermediários/Lentos” (*CYP2C9 * 1 / * 2, * 1 / * 3 e CYP2C9 * 2 / * 3, * 3 / * 3*). Não foram encontradas diferenças estatisticamente significantes em todos os parâmetros avaliados. Em relação à dor, apesar dos dois grupos referirem baixos níveis de dor durante o pós-operatório, o grupo “Metabolizadores Normais” apresentou mais dor ($p<0,05$) nos períodos de 4, 5, 6, 7, 8, 10, 48 e 72 horas de

pós-operatório, quando comparado ao ponto de tempo "zero", diferente do grupo “Metabolizadores Intermediários/Lentos” que relatou dor significativa apenas em 6 horas de pós-operatório, quando comparados com o tempo “zero”. Na prática clínica, isso significa que indivíduos com atividade anormal do CYP2C9 (metabolizadores intermediários e lentos) apresentam uma exposição aumentada ao tenoxicam e provavelmente mostram níveis mais baixos de dor, mas também mostram provavelmente um risco maior de efeitos colaterais, que incluem sangramento gastrointestinal, distúrbios hemorrágicos e cardiovasculares, sendo provavelmente necessário que a dose habitual do medicamento seja revisada.

Palavras-chave: Cirurgia. Terceiro Molar. Anti-Inflamatórios não Esteroides. Dor. Citocromo P-450 CYP2C9. Farmacogenética.

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NSAIDs - Non-steroidal anti-inflammatory drugs

PK - Pharmacokinetics

CYP - Cytochrome

VAS - Visual Analogue Scale

DNA - Deoxyribonucleic acid

NM - Normal Metabolizers

ISM - Intermediate/ Slow Metabolizers

INDEX

TITLE: Influence of cytochrome P450 (CYP2C9) genotype on clinical efficacy of tenoxicam after lower third molars surgeries[1,2]

SHORT TITLE: Influence of CYP2C9 on tenoxicam

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Keywords: Surgery. Third molar. Anti-Inflammatory Agents, Non-Steroidal. Pain. Cytochrome P450. CYP2C9. Pharmacogenetics.

[1] **Este trabalho foi formatado de acordo com as normas do periódico International Journal of Oral and Maxillofacial Surgery – IJOMS (Anexo 2)*

ABSTRACT

One of the most accepted pharmacological protocols on third molar extraction surgeries involves the use of the non-steroidal anti-inflammatory drugs, as tenoxicam. Many studies present that the individual drug response could be directly affected by genetics induced pharmacokinetics alteration. Our study aimed to genotype and phenotype CYP2C9 gene in 89 health patients that were submitted to wisdom teeth surgical removal under medication of tenoxicam, comparing the gene influence on postoperative pain, edema, trismus, amount of rescue medication consumed, global evaluation and patient satisfaction regarding the medication. CYP2C9 gene was screened to evaluate polymorphisms and the genetic characteristics were crossed to aforementioned postoperative findings on a randomized clinical trial. 89 volunteers were splitted in two groups: 64 (74%) Normal Metabolizers (NM) group and 25 (26%) Intermediate/Slow Metabolizers (ISM) group. There were not found statistically significant difference between groups. The NM group referred more pain ($p<0,05$) at 4, 5, 6, 7, 8, 10, 48 and 72 postoperative hours time points when compared to time zero. In clinical practice it means that individuals with CYP2C9 abnormal activity (ISM) presented an augmented exposition to tenoxicam and referred lower pain levels, but also, they were probably more susceptible to adverse effects.

Keywords: Surgery. Third molar. Anti-Inflammatory Agents, Non-Steroidal. Pain. Cytochrome P450. CYP2C9. Pharmacogenetics.

INTRODUCTION

Pain, edema and trismus are common characteristics after lower third molar removal surgery as the result of histological trauma and the normal response of the organism[1]. These classic symptoms could have an immediate negative impact at the social and professional life of patients[2].

There are uncountable different therapeutic proposals aiming to reduce these signs and symptoms on dentistry. One of the most accepted protocols for pain control after these surgeries involves the use of the non-steroidal anti-inflammatory drugs, the NSAIDs[3,4,5,6,7,8]. Usually well accepted by the patient and easy to be purchased, this pharmacological class rules an important paper in order to control pain, but also edema and trismus[6,7,8].

Through a well studied and explained pathway that involves the inhibition of cyclooxygenase 2, NSAIDs can prevent the formation of proinflammatory agents and, therefore, avoid postoperative undesired characteristics. Nowadays it could be find more than 50 different NSAIDs available on the global market and many of them have already been studied directly to wisdom surgeries[9].

tenoxicam has a place with the class of NSAIDs known as oxicams. It is also used to reduce inflammation, swelling, and pain related to rheumatoid joint inflammation, osteoarthritis, ankylosing spondylitis, tendinitis, bursitis, and periarthritis of the shoulders or hips[10]. Oral absorption of tenoxicam is rapid and complete. After oral administration, the peak plasma concentration is reached within 2 h. The recommended dosage regimen is 20 mg once a day for oral surgical procedures[11,12]. It inhibits cyclooxygenase, thus preventing the

formation of prostaglandins and leukotrienes that play an essential role in inflammation by diminishing active oxygen radicals and inhibiting migration and phagocytosis of leucocytes. In addition to its anti-inflammatory effects by these mechanisms, it also has analgesic and antipyretic effects[13]. Similar to many other NSAIDs, it also inhibits thrombocyte aggregation[14].

Lately, as a natural rise of the pharmacogenetics, studies are demonstrating that individual drugs response could be directly affected by genetics induced pharmacokinetics (PK) alteration[15]. In many cases, these variability is straight connected to genetics polymorphism that could induce to absence, reduction, alteration or increase of enzymatic activity[16,17]. This fact could also modify the clinical efficacy of the drug, change its capacity to handle with pain, and even rise the frequency and severity of adverse effects[18,19,20].

This modified enzymatic activity, while regarding about the NSAIDs, could be prevailing induced by the CYP2C9 gene, that concerns about the P450 cytochrome family[21,22,23,24]. The CYP2C9 gene, is highly polymorphic, and is responsible to metabolization and excretion of ibuprofen, indomethacin, celecoxib, valdecoxib, lornoxicam, piroxicam, meloxicam and tenoxicam[25]. *CYP2C9 * 1* is considered the wild-type allele when no variants are detected and is categorized by normal enzyme activity. Individuals who have 2 normal-function alleles (e.g., *CYP2C9 * 1 / * 1*) are classified as “Normal Metabolizers”. Two allelic variants associated with reduced enzyme activity are *CYP2C9 * 2* and * *3* (Figure 1).

Likely phenotype	Genotype	Examples of diplotypes
Normal metabolizer (normal activity)	An individual with 2 normal-function alleles	** 1 / * 1*
Intermediate metabolizer (heterozygote or intermediate activity)	An individual with one normal-function allele plus one decreased-function allele	** 1 / * 3, * 1 / * 2*
Poor metabolizer (homozygous variant, low or deficient activity)	An individual with 2 decreased function alleles	** 2 / * 2, * 3 / * 3, * 2 / * 3*

Figure 1: *CYP2C9* Phenotype based on Genotype[26].

Source: Dean L. Piroxicam Therapy and CYP2C9 Genotype. 2019 Feb 11. In: Pratt V, McLeod H, Rubinstein W, et al., editors. Medical Genetics Summaries [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2012. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537367/?report=classic

These individual genetic variations seem to assume an important role on pain and inflammation management since each individual could present a singular characteristic depending on his/her genetic polymorphism. And allied to other important factors as pain threshold, personal pain response, body weight and excretory system, one could be able to properly manage pain of each patient and also be able to cause minimal side effects. Worth mentioning that the NSAIDs are an over the counter medication, with poor or absolutely no control of use, and some individuals, with CYP2C9 mutations could present a lower clearance tax of the drug leading to possible gastrointestinal bleeding and hemorrhagic condition[19]. These are some reasons why the pharmacogenetics raised significantly last years and for sure will keep in expansion for the future.

The individual genetic variability is, already, an essential screening process that can avoid seriously adverse effects of some kind of drugs and can check the possibility of a person receive a determinate donate organ, for example. Also, the identification of the polymorphisms can improve the patient management,

indicating which patient should avoid a determinate drug or should receive a higher dose of other. The adequate approach on this strategy could potentially reduce the medical costs and improve the process as general, leading to a successfully drug therapy.

Our objective, in this study, was to genotype and phenotype CYP2C9 gene in healthy patients submitted to a wisdom tooth removal, under medication of 20 mg tenoxicam a day for 4 days, comparing the gene influence on postoperative pain, edema, trismus, amount of rescue medication consumed by patients, global evaluation and the satisfaction of the patient regarding the medication intake.

MATERIAL AND METHODS

This was a randomized clinical trial study developed in the Department of Oral and Maxillofacial Surgery at School of Dentistry of Araçatuba, São Paulo State University (FOA/UNESP) and Discipline of Pharmacology at Bauru School of Dentistry, University of São Paulo (FOB/USP). The surgeries were performed on the premises of Pharmacology and Clinical Physiology Laboratory (LAFFIC) (FOB/USP), coordinated by Prof. Dr. Carlos Ferreira dos Santos.

The study was approved by the Ethics Committee on Human Research of FOA/UNESP and also FOB/USP (Process 2.247.253 and 2.390.143) (APPENDIX 1) and was also registered on Clinical Trials as Number (NCT04182191).

Inclusion and exclusion criteria

For selection of the study participants, the following eligibility criteria was previously defined: be aged 18 years or more; have one third molar included and/or impacted; lack of inflammation or infection in the extraction sites; absence of systemic diseases that could interfere with the recorded data.

The exclusion criteria was, at least, one of the aspects described below: history of allergy to local anesthetics, or any inability to receive articaine anesthetic; history of bleeding or gastrointestinal ulcers, kidney disease, asthma, or allergic sensitivity to aspirin or any other non-steroidal anti-inflammatory agent; pregnancy or are breast-feeding; have used antidepressants at least one year before the research; have used anticoagulants, diuretics and/or antibiotics at last two months before the research; have used any illicit drug at any time of life, and patients under any treatment to quit addictions like alcohol or other drugs; hepatic, kidney, intestinal, cardiac, pulmonary, circulatory and/or brain dysfunction.

Patients

The screening of patients' sector analyzed patient documentations and referred 156 patients who fulfilled the inclusion criteria for this study. Of these, 65 patients were excluded from the study. Seven of them were not living at the city of the research by the time of the experiment; 29 had already performed the surgery in other services; one patient was pregnant and 28 leaved the study by personal reasons. In total, there were analyzed 91 extractions of third molars (91 patients).

Surgical procedure

The local anesthetic used in all study participants was articaine 4% with epinephrine 1: 200,000[6,7,42]. Anesthesia was performed with block of inferior alveolar, oral and lingual nerves, administered initially a cartridge (1.8 ml) of anesthetic. If after 5 min the patient does not feel anesthesia of the lower lip, would be given another cartridge (1.8 ml) and so on until the complete anesthesia-recounting patient without being exceeded the maximum amount of anesthetic to the patient. Achieved anesthesia of the lower lip, 0.9 mL of another cartridge still unused and of the same anesthetic solution, was administered under terminal infiltration technique to reduce bleeding and ensuring mucosal anesthesia.

The steps of the surgery were as follows: after complete anesthesia of the patient, an incision was made on the top of the alveolar bone above the impacted third molar and at the buccal–distal aspect of the second molar in a vertical descending direction, thus creating a triangular incision in this region. This triangle-shaped flap was folded down, allowing a direct view of the surgical field. Using a number 702 bur under constant irrigation with distilled water, an osteotomy was performed to remove the bone around the impacted tooth. Finally, using the same drill and irrigation, crown sectioning was performed, if necessary. The tooth was removed completely, and the surgical cavity was cleaned, with the removal of bone spicules and with alveolar curettage. Abundant irrigation with 0.9 % sterile saline was carried out. Suturing was performed using a 4–0 nylon suture, with three simple stitches over the flap.

Patients' postoperative care

Postoperative care, which consisted of resting for a period of 48 hours. Making ice packs (4-5 ice cubes) placed in a plastic bag and wrapped in a wet washcloth. These compresses should be applied for 10 minutes with a 10 - minute rest continuously for a period of 48 hours. Were also told to follow with routine cleaning, brushing all his/her teeth, mucosa, tongue, and also gently brushing the area of the surgery as if they were "combing" the stitches without leading, however, to trauma to the surgical area.

The requested feed was liquid/pasty and cold. Patients were encouraged to use ice cream, juice, vitamins, yoghurts and shakes ready for consumption. Patients also received information pertaining to normal expected course of recovery, as well as the possibility of edema formation, lockjaw and sensitivity in the operated area. Finally, any questions of the participants were clarified.

Anti-inflammatory protocol

The anti-inflammatory protocol use was tenoxicam 20 mg tablet every 24 hours, for 4 days[6,2,7,8]. As rescue medication, or if the patient judged that the level of analgesia produced by the anti-inflammatory supplied was not enough, it was prescribed acetaminophen at the dose of 500 mg that the patient could use every 8 hours concomitantly until the pain was circumvented without exceeding four days of medication.

Data collection

Patients also received a card to write down the amount of pain that they felt at certain times. This subjective evaluation of postoperative pain was noted by the volunteer himself a Visual Analogue Scale (VAS) (0-100 mm), which contained at their ends "no pain (0 mm)" and "worst possible pain (100 mm)"[28]. The volunteer took the medications at times 0, 24, 48 and 72 h (time zero was considered as the end of surgery). Subjective ratings of pain were made by the volunteers exactly at the end of surgery, called time 0 as well as the gaps 15, 30, 45, 60 and 90 minutes, 2, 3, 4, 5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 24, 48, 72 and 96 hours[27,6,7,29,30,8,31,32,3]. During the first hour, the volunteer remained on the premises of Pharmacology discipline to make his/her notes in VAS, and then discharged.

The second form received by the patient was the Rescue Medication Record. This form contains the same scales for marking the pain, but without predetermined times. The patient himself mark the day and time as well as the amount of pain experienced at the time necessary to make use of rescue medication. Thus, each time the patient has used the rescue medication the same filled a pain scale, with the date and time of medication use.

In addition to the forms filled in by the patient, the team members also evaluated the following parameters:

- Intraoperative findings:

• Hemodynamic parameters evaluation (systolic, diastolic and media blood pressure, oximetry, heart rate) of the volunteer on the following moments: before surgery (patient comfortable and relaxed at the dentistry chair), right after the first injection of the local anaesthetic, incision, flap detachment, osteotomy, extraction of the teeth, cleaning of the surgical site and after the closing sutures. All measures were performed using (Monitor Sistem, model DX2010, Dixtal Biomédica Ind. e Com. Ltda, Marília/SP, Brazilian Health System number 10293490012, serial number 00V81411) following the factory orientations. The sphygmomanometer was allocated on left arm, aligned to the patient chest and the oximetry sensor was placed on the third finger of the right hand[467].

• Bleeding score through surgery (operator opinion based in a 3-points scale = 1 Normal bleeding; 2 Capillary bleeding; 3 Arterial and Venous bleeding)[3367].

• Anaesthetic quality (operator evaluation based in a 3-points scale = 1 No discomfort related by the volunteer; 2 Some discomfort related but no need to complement the anaesthetic technique; 3 Need to complement the anaesthetic) [33,6,7].

- Pre and Postoperative findings:

• Opening mouth (the distance in mm between the edges of the upper and lower incisors during the maximum aperture achieved by the volunteer) prior to surgery, on the 2nd and 7th postoperative day. The ability to open the mouth in the postoperative period was expressed as a percentage of preoperative measurement[5,27,7,30,29,8].

• Measurement of facial edema in preoperative period, at the second and seventh day of postoperative period. The method used by Ustun et al, 2003; was applied, which takes into account the sum of the following measures (obtained with flexible tape): A- distance between the lateral corner of the eye and the goniac angle, B- away from the tragus to the labial and C- away from the tragus to the soft tissue of pogonion. Preoperative sum of the three measurements was considered as the baseline. The difference between the values obtained in the postoperative period and baseline indicated facial edema in the second and seventh days of the postoperative period[27,7,30,29,31].

- Postoperative findings:

• Wound healing quality (operator opinion on time of suture points removal based in a 3-points scale = 1 normal healing; 2 delayed healing with no infection; 3 poor healing with or without local infection)[33,6,7].

• Overall assessment of the postoperative period performed by the voluntary at the time of stitches removal on a scale of "excellent", "very good", "good", "fair" or "poorly"[34].

Molecular Analysis

89 DNA samples were previously collected and processed at Laboratory of Clinical Pharmacology and Physiology, LAFFIC, at Bauru School of Dentistry - University of São Paulo, FOB-USP, Brazil, with the QIAamp DNA Mini Kit (250), (Cat No./ID: 51306) (QIAGEN®). For the DNA extraction were utilized QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers and Collection Tubes (2 ml) (QIAGEN®).

After the extraction, DNA samples concentration (ng/µl) were checked in the Nanodrop - 1000 (ThermoFisher®) and all these DNA Samples were lyophilized with the Labconco FreeZone® 4.5 Liter Benchtop Freeze Dry System, at the Laboratory of Clinical Pharmacology and Physiology, FOB-USP, Brazil, for their future transport to Kailos Genetics®, Huntsville, Alabama, USA.

For the genetic sequencing of CYP2C9 MiSeq® System (Illumina®) instruments with a 2 x 78 bp read length was utilized, with the proprietary system, TargetRich, for capturing the specific regions of the genome that are relevant to pharmacogenetics. After captured and enriched, 98 % of the resulting sequences were aligned to the targeted regions. Initially, the regions flanking the regions of interest are targeted with a type II restriction enzyme tethered to a guide oligonucleotide sequence, enabling precision cutting of the genome. Once released, patch oligonucleotides, a two-part oligo that is complementary

to the cut ends of the targeted region and also to a universal PCR primer. Additionally, each universal PCR primer ligated to the targeted sequence possesses a chemical protecting group. This allows the digestion of background genomic DNA without harming the targeted-patched regions. This method enables multiplexing of patient samples (up to 48 per run) and includes Unique Molecular Identifiers (UMI). UMIs tag each starting template molecule of DNA, allowing for counting of starting molecules, error correction and increased sensitivity of variant detection.

This part of the molecular analysis was performed by the author GMW.

Statistical Method

In order to follow the study objectives patients were grouped into metabolizers characteristics as follows: a) Normal metabolizers (*CYP2C9 * 1 / * 1*) and b) Intermediate/Slow metabolizers (*CYP2C9 * 1 / * 2, * 1 / * 3* and *CYP2C9 * 2 / * 3, * 3 / * 3*, respectively).

The data were properly analyzed by means and median of graphs and tables (GraphPad Prism, 4.0 version). To check if there was normal distribution the data were submitted to Kolmogorov-Smirnov normality test. When data were normally distributed, comparisons among and between groups were made using the unpaired t-test. For all non normally distributed data, statistical comparisons between two independent groups were performed using the Mann–Whitney U test. Anova Two Way test (significance level of 5%) was used to compare pain level and hemodynamic criteria through the time and between each group.

RESULTS

Ninety-one healthy volunteers joined this study. Of these, two volunteers were excluded from the study. Both of them developed infection at the surgical site and were treated with cephalexin 500 mg antibiotics (1 tablet every eight hour for seven days). The condition of them was stabilized and these patients were removed from the sample (CONSORT flow chart, Figure 2).

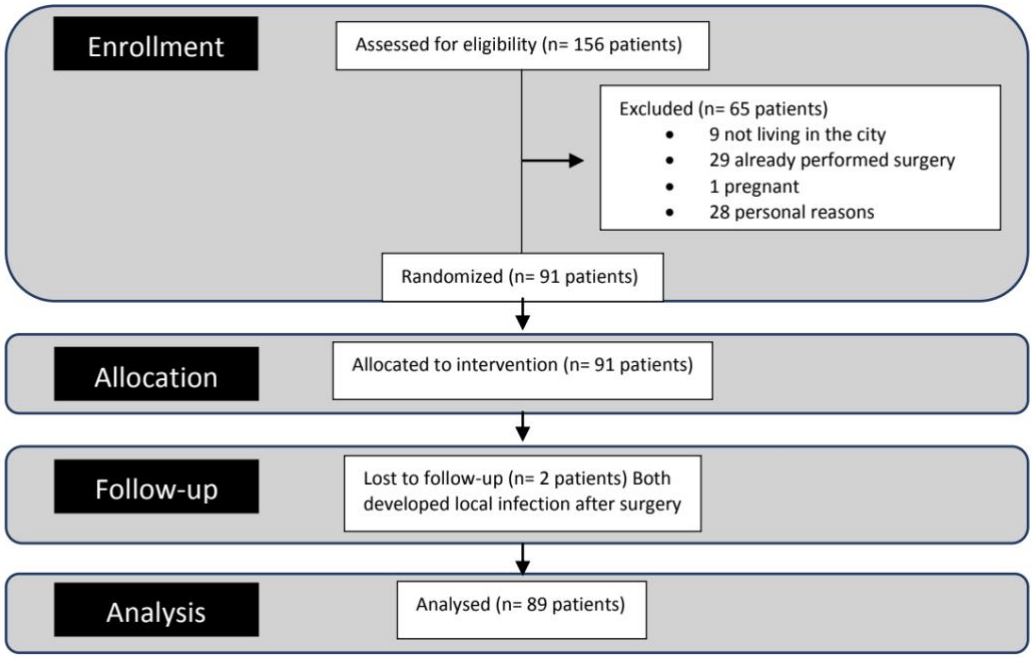

Figure 2: CONSORT flow diagram of the study design

The final sample was composed by 89 volunteers, 61 females (68,54%) and 28 males (31,46%). The mean age was 23,7 years (ranging from 18 to 50 years). The surgeries were surgeries were performed between August 2018, to July 2019.

The genotype distribution is presented in table 1 as follows: a) Normal metabolizers (*CYP2C9 * 1 / * 1*) and b) Intermediate/Slow metabolizers (*CYP2C9 * 1 / * 2, * 1 / * 3* and *CYP2C9 * 2 / * 3, * 3 / * 3*).

Table 1: Genotype frequencies of CYP2C9 (n = 89).

Groups	**Alelle**	**n**	**%**
Normal Metabolizers	*CYP2C9 * 1 / * 1*	64	74.41
Intermediate/ Slow Metabolizers	*CYP2C9 * 1 / * 2;* *CYP2C9 * 1 / * 3*	22	25.59
	*CYP2C9 * 2 / * 3;* *CYP2C9 * 3 / * 3*	3	
Total		89	100.00

Abbreviation: CYP, cytochrome P450.

Table 2 reports the demographic distribution and intraoperative parameters of the 89 volunteers submitted to lower third molar surgeries, splitted by metabolizers characteristics. The data analyzed shows the mean age, the total time of surgery (minutes) that's include the 5 initial minutes waited after the first injection of local anaesthesia, the quantity of anaesthetic utilized (cartridges), on set time of local anaesthesia, opening mouth and facial edema on preoperative period, 2 and 7 days postoperative, and the intraoperative evaluation about quality of anaesthesia, difficult of surgery, quality of wound healing and bleeding scores.

Table 2: Demographic data and the pre, intra and postoperative parameters of the patients submitted to surgery.

Parameters	Normal metabolizers	Intermediate/Slow metabolizers
Age (Mean; SD)	23.53 ± 5.74	24.44 ± 5.82
Sex		
Male	20.00 (31.25%)	8.00 (32.00%)
Female	44.00 (68.75%)	17.00 (68.00%)
Lower third molar position (Pell & Gregory Classification; n. %)		
IA	10.00 (15.63%)	3.00 (12.00%)
IB	1.00 (1.56%)	0.00 (0.00%)
IIA	9.00 (14.06%)	8.00 (32.00%)
IIB	19.00 (29.69%)	1.00 (4.00%)
IIC	4.00 (6.25%)	3.00 (12.00%)
IIIA	0.00 (0.00%)	1.00 (4.00%)
IIIB	1.00 (1.56%)	3.00 (12.00%)
IIIC	20.00 (31.25%)	6.00 (24.00%)
Local anesthetic. (median. IQR)		
Anaesthesia (cardrige)	1.50 ± 0.50	1.50 ± 0.50
On set of local anaesthesia (sec)	70.00 ± 33.50	74.00 ± 45.00
Quality of anaesthesia (1 to 3)	1.00 ± 2.00	1.00 ± 2.00

Table 2 (cont): Demographic data and the pre, intra and postoperative parameters of the patients submitted to surgery.

Surgery (median. IQR)		
Total time of surgery (min)	13.00 ± 5.0	14.00 ± 6.00
Surgical difficulty (1 to 3)	2.00 ± 1.0	2.00 ± 1.00
Intraoperative bleeding (1 to 3)	1.00 ± 0.0	1.00 ± 0.00
Quality of wound healing (1 to 3)	1.00 ± 0.0	1.00 ± 0.00
Mouth opening (mm) (Mean; SD)		
Preoperative period	47.83 ± 6.61	49.16 ± 5.68
Day 2	22.44 ± 7.52	24.08 ± 7.80
Day 7	31.63 ± 10.40	35.28 ± 11.49
Facial edema (difference from baseline) (Mean; SD)		
Day 2	1.52 mm (±1.43)	1.49 mm (±1.12)
Day 7	0.29 mm (±1.99)	± 0.10 mm (±1.65)
Body temperature (° C) (Mean; SD)		
Preoperative period	35.46 ± 2.53	36.08 ± 0.59
Day 2	35.62 ± 2.60	35.94 ± 0.53
Day 7	35.71 ± 2.51	35.90 ± 0.57
Rescue medication		
Time between first consumption & surgery (hr) (median. IQR)	4.00 ± 2.88	4.50 ± 2.25
VAS during first consumption (1-10) (median. IQR)	3.25 ± 3.00	3.00 ± 3.00
Total quantity consumed (mean; SD)	3.81 ± 3.30	4.00 ± 3.20

The mouth opening difference on the second and seventh postoperative day presented a slight but not significant different results for both groups where NM and ISM have shown, respectively, 22,44 mm and 24,08 mm on second postoperative day and 31,63 mm and 35,28 mm on seventh postoperative day. Besides that, the comparison among groups NM and ISM showed no statistically difference on the following parameters: swelling, surgery duration, intraoperative bleeding, onset of local anesthesia, total cartridges used of local anesthesia, total quantity of rescue medication, time to first rescue medication, VAS to first rescue medication and quality of wound healing.

Hemodynamic parameters obtained during surgery (heart rate, oximetry and arterial pressure) were similar between groups, fluctuated slightly during the different phases of extraction, yet no unusual peaks (data not shown). No statistically difference could be found between both groups in this parameter.

Regarding on adverse reactions related to tenoxicam, 13 (20,31%) NM volunteers referred any symptom on second or seventh postoperative day (vomit, sickness, headache, dizziness, stomachache, low blood pressure and loss of appetite). Seven (28,00%) ISM volunteers referred any of these symptoms. No statistically difference were found between groups ($p<0,441$).

Our study also analyzed the self-related pain by the volunteers in specific time points: 0 (right after the end of the surgery), followed by 15, 30, 45 60, and 90 minutes, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 24, 48, 72 and 96 hours (Figure 3).

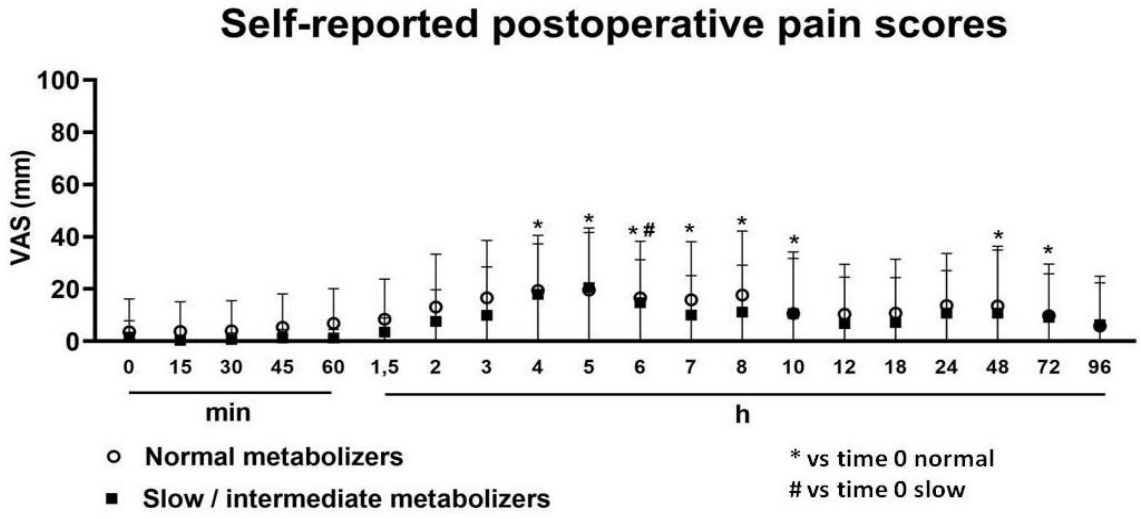

Figure 3: Postoperative pain scores reported by volunteers.

Notes: VAS of self-reported postoperative pain scores after lower third molar surgeries assessed at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18, 24, 48, 72 and 96 hours. Scores could range from 0 to 100 mm, with larger scores indicating increased pain.

It's possible to clarify that in both groups the NSAID tenoxicam was effective on pain control with no statistically difference between them. Meantime, when we analyze pain scores for each isolate group on different time points comparing them with time zero, interesting clinical results begin to appear. It is possible to confirm that among 4 and 10 hours after surgery, NM group showed higher pain scores (statistically significant data), when compared to time point 0. Furthermore, after 48 and 72 hours, the results were also significant. While on ISM group, the unique significant data (when compared to time zero) occurred only on 6 hours after surgery.

At the end of day 7 post-surgery, each patient reported their overall experience taking into account the surgery and pain relief, as well as factors such as side effects, the willingness to return to regular activities, and any other features that

the patient considered important (Figure 4). In general, most volunteers classified their experience with tenoxicam as "Excellent" and "Very Good". It was not found any statistically difference while comparing the results, despite NM group presented more volunteers that classified their experience as "Excellent" and "Very Good" than ISM group (55% versus 42%). By the same time, this same group (NM) presented more volunteers that classified their experience as "Fair" and "Poor" when compared to ISM group (20% versus 13%).

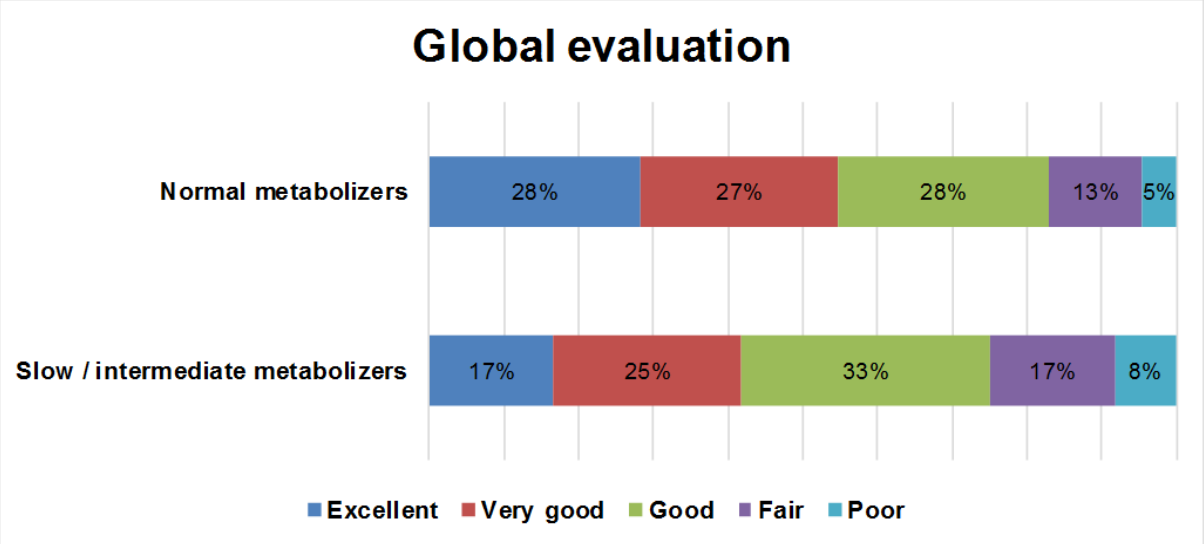

Figure 4. Self-reported global efficacy of tenoxicam (20 mg) comparing Normal metabolizers to Slow/intermediate metabolizers on seventh postoperative day, assessed using a 5-point Likert scale (n = 89). The Likert ratings were ‘excellent’, ‘very good’, ‘good’, ‘fair’, and ‘poor’.

DISCUSSION

Volunteers consumed 20 mg single dose per day (4 days) tenoxicam after wisdom tooth removal surgery in a randomized clinical trial. Eighty nine healthy patients were allocated in two groups: Normal metabolizers (*CYP2C9 * 1 / * 1*) and b) Intermediate/Slow metabolizers (*CYP2C9 * 1 / * 2*, ** 1 / * 3* and *CYP2C9 * 2 / * 3, * 3 / * 3*, respectively), and were evaluated regarding on pain, edema, trismus, amount of rescue medication consumed, global evaluation and the satisfaction of their treatment.

Data analysis shown that the compared factors were not significantly different between two groups but, concerning on pain, NM group referred more pain on 4, 5, 6, 7, 8, 10, 48 and 72 postoperative hours time points when compared to time point "zero", which was different from ISM group volunteers that reported significant pain only on 6 postoperative hours time point when compared to time point "zero" ($p<0,05$).

This reveals an interesting characteristic that was not yet well discussed in any other article. CYP2C9 normal metabolizers could experiment more pain levels while being medicated with NSAIDs when compared to intermediate/slow metabolizers. According to our data, normal metabolizers reported significant higher levels of pain in many postoperative time points. Interestingly, at the NM group, two specific time points comes to our spot. They were 48 ($p<0,0023$) and 72 ($p<0,038$) hours after surgery, which matches the medication consumption times (0, 24, 48 and 72 hours). It means that normal metabolizers volunteers were under significant pain at the time of medication consumption at 48 and 72

hours after surgery. Because of that, it is possible to assume that maybe, in these patients, the drug dosage could be higher or the interval among each dosage could be shorter. This supposition suggests that in future, patients may be medicated properly if we could know his/her genetic characteristic. It is also possible to assume how important and how embracing pharmacogenetics area are going to be in a real near future.

Of course we do know that these implications should be further studied and of course that we have to consider other implications that may interfere on drug absorption and distribution until we could really predict all characteristics of determined treatment, however it seems to be a very promising beginning of the clinical pharmacogenetics area .

As aforementioned, it was possible to remark that higher plasmatic levels of tenoxicam due to metabolic depuration (ISM group) may have influenced in lower pain levels presented by volunteers until 96 hours after surgeries. Now, when we compare NM and ISM individuals at the same time point, no difference is found on visual analogue scale. This may lead to a mistaken sensation that no difference between groups were found, but what it really shows is that the medication was effective enough to keep pain levels close to each other, despite the genotype difference between them. This finds are in agreement to a recent study[35], where authors, after third molars surgeries performed on 102 volunteers under use of piroxicam, concluded that independent of the polymorphism, pain levels were acceptable. Indeed, analyzing figure 3, it is clear that the NSAID could exert its effect, after all, the pain levels are low and did not reach 30 mm in any of the time points. What is new is that even in this

low pain pattern, it was possible to be seen a significant difference while comparing pain in many time points to time point zero.

Furthermore, other studies have stated that the pain control seems to be effective irrespective the presence or not of the polymorphism[36]. Once again it corroborates our findings and shows that the medication employed on this study was correct and able to manage correctly pain levels. But, as already stated, even inside a comfort range, our study shows a difference of pain perception between groups.

About the cytochrome P450 superfamily (CYP450) it is known that it refers to a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs[22]. The CYP2C9 gene presents high polymorphisms taxes and composes the main catabolic pathway for a wide range of clinically important drugs, as the NSAIDs and their products, and can regulate the enzymatic catalytic activities as reducing, inhibiting, or increasing[37], depending on its polymorphisms.

In the clinical practice, it means that individuals with abnormal CYP2C9 activity (Intermediate and slow metabolizers) present a augmented exposition to tenoxicam and probably shows lower pain levels, but also probably shows a higher risk of collateral effects, that includes gastrointestinal bleeding, hemorrhagic disturbances and may also impact cardiovascular morbidity by altering the metabolism of fatty acids, prostanoids, and steroid hormones, especially in poor metabolizers of CYP2C9 patients with pre installed conditions. And in such cases, the normal dose of the medicament should probably be revised[26].

In our research, data are consistent with other studies already stated that individuals with polymorphisms might have less pain or more side effects when compared to individuals with normal alleles for CYP2C9[35]. Anyway, it is imperative to clarify that our study was not specifically designed to address the risk of adverse events across genotype groups, and the study used a low dosage for a short duration that may not lead to appearance of any significant side effects.

Tenoxicam is a well studied NSAID commonly prescribed to manage postoperative inflammatory responses after third molar surgeries. Still, tenoxicam is primarily metabolized by CYP2C9[37], instead of other genes combinations that are possible to interfere on other NSAIDs depuration. In reason of that, our study selected tenoxicam in order to reduce the possible variables associated in the study design[38].

Brazilian population was studied regarding its genotype on CYP2C9 and it was revealed that its alleles *CYP2C9 * 1 / * 2* and *CYP2C9 * 1 / * 3* are associated to tenoxicam pharmacokinetics and pharmacodynamics parameters. There is a significant clearance reduction of oral tenoxicam in both genotypes' polymorphisms. It is reduced by 30 times while on action of *CYP2C9* * 3 when compared to *CYP2C9 * 1* [39,40]. Despite these differences, the maximum plasmatic concentration are similar to normal metabolizers once the absorption of the drug is not influenced by these genes[37].

It is worth noting that in addition to polymorphisms in metabolizing enzymes, there are additional genetic factors that may contribute to variable drug response among patients, as age, sex, past medical history and other

concomitant pharmacotherapy that have to be accounted for when dealing with patients[41].

This study is one of the first to present important clinical differences related to acute pain sensation on volunteers submitted to wisdom teeth removal surgeries and its genotype. Before effective improvements on health protocols, there is still a significative way on genotype and pain that needs to be further studied and revealed but for sure in not a distant future, the pharmacogenetics on NSAIDs will be as assure and useful as it is being for many other drugs[42,43].

After performing the genotype and phenotype of CYP2C9 in 89 healthy patients, it is possible to conclude that edema, trismus, amount of rescue medication, global evaluation and satisfaction of the patient presented no difference between groups.

Pain reported by Normal Metabolizers group presented significative difference when compared to time point zero on times of 4, 5, 6, 7, 8, 10, 48 and 72 postoperative hours.

Pain reported by Intermediate/Slow Metabolizers group presented significative difference when compared to time point zero on time of 6 postoperative hour.

CONCLUSION

Based on studied data it is possible to conclude that genetics polymorphisms on CYP2C9 could direct influence pain referred by volunteers after lower third molars extractions under consumption of 20 mg tenoxicam.

Genetics places a fundamental place on health and many complex treatments today are conducted based on its findings. Our team truly believe that Pharmacogenetics will also place an important role in future treatments, not as the major responsible for a prescription but yes as more one important information that includes a patient's history

STUDY LIMITATIONS

Despite our incessant work to produce a high-quality study, we could find some flaws that are listed below.

We observed a reduced number of participants in order to split them on normal, intermediate and slow metabolizers, because of so, intermediate and slow metabolizers groups were set together. Also, no difference between slow and intermediate metabolizers were found, and maybe in a larger sample it could be different.

In order to proper study adverse effects, we should consider, for further studies, to elect volunteers with related preexistent health conditions as gastrointestinal bleedings, hemorrhagic disturbances and controlled cardiovascular derangements.

REFERENCES

1. Osunde OD., Saheeb BD. Effect of Age, Sex and Level of Surgical Difficulty on Inflammatory Complications After Third Molar Surgery. *J Maxillofac Oral Surg* 2015;**14**(1):7–12. Doi: 10.1007/s12663-013-0586-4.
2. Bailey E., Worthington H., Coulthard P. Ibuprofen and/or paracetamol (acetaminophen) for pain relief after surgical removal of lower wisdom teeth, a Cochrane systematic review. *Br Dent J* 2014;**216**(8):451–5. Doi: 10.1038/sj.bdj.2014.330.
3. Christensen KS., Cawkwell GD. Valdecoxib versus rofecoxib in acute postsurgical pain: Results of a randomized controlled trial. *J Pain Symptom Manage* 2004;**27**(5):460–70. Doi: 10.1016/j.jpainsymman.2004.02.001.
4. Colombini BL., Modena KCS., Calvo AM., Sakai VT., Giglio FPM., Dionísio TJ., et al. Articaine and mepivacaine efficacy in postoperative analgesia for lower third molar removal: a double-blind, randomized, crossover study. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology* 2006;**102**(2):169–74. Doi: 10.1016/j.tripleo.2005.09.003.
5. Calvo AM., Sakai VT., Giglio FPM., Modena KCS., Colombini BL., Benetello V., et al. Analgesic and anti-inflammatory dose-response relationship of 7.5 and 15 mg meloxicam after lower third molar removal: a double-blind, randomized, crossover study. *Int J Oral Maxillofac Surg* 2007;**36**(1):26–31. Doi: 10.1016/j.ijom.2006.09.006.
6. Santos CF., Modena KCS., Giglio FPM., Sakai VT., Calvo AM., Colombini

BL., et al. Epinephrine Concentration (1:100,000 or 1:200,000) Does Not Affect the Clinical Efficacy of 4% Articaine for Lower Third Molar Removal: A Double-Blind, Randomized, Crossover Study. *J Oral Maxillofac Surg* 2007;**65**(12):2445–52. Doi: 10.1016/j.joms.2007.04.020.

7. Gregorio LVL., Giglio FPM., Sakai VT., Modena KCS., Colombini BL., Calvo AM., et al. A comparison of the clinical anesthetic efficacy of 4% articaine and 0.5% bupivacaine (both with 1:200,000 epinephrine) for lower third molar removal. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology* 2008;**106**(1):19–28. Doi: 10.1016/j.tripleo.2007.11.024.
8. Calvo AM., Brozoski DT., Giglio FPM., Gonçalves PZ., Sant'ana E., Dionísio TJ., et al. Are antibiotics necessary after lower third molar removal? *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;**114**(5):S199–208.
9. Costa FWG., Soares ECS., Esses DFS., Silva PGDB., Bezerra TP., Scarparo HC., et al. A split-mouth, randomized, triple-blind, placebo-controlled study to analyze the pre-emptive effect of etoricoxib 120 mg on inflammatory events following removal of unerupted mandibular third molars. *Int J Oral Maxillofac Surg* 2015;**44**(9):1166–74. Doi: 10.1016/j.ijom.2015.06.012.
10. Çebi AT., Kasapoğlu MB., Eren S., Kasapoğlu Ç. Comparison of the effects of diclofenac potassium and tenoxicam on postoperative pain, swelling, and trismus following third molar surgery. *Turkish J Med Sci* 2018;**48**(2):271–8. Doi: 10.3906/sag-1702-100.
11. Nilsen OG. Clinical Pharmacokinetics of Tenoxicam. *Clin Pharmacokinet*

1994;**26**(1):16–43. Doi: 10.2165/00003088-199426010-00003.

12. Merry AF., Webster CS., Holland RL., Middleton NG., Schug SA., James M., et al. Clinical tolerability of perioperative tenoxicam in 1001 patients - A prospective, controlled, double-blind, multi-centre study. *Pain* 2004;**111**(3):313–22. Doi: 10.1016/j.pain.2004.07.013.
13. Ilhan O., Agacayak KS., Gulsun B., Koparal M., Gunes N. A comparison of the effects of methylprednisolone and tenoxicam on pain, edema, and trismus after impacted lower third molar extraction. *Med Sci Monit* 2014;**20**:147–52. Doi: 10.12659/MSM.890239.
14. El-Gazayerly ON. Characterization and evaluation of tenoxicam coprecipitates. *Drug Dev Ind Pharm* 2000;**26**(9):925–30. Doi: 10.1081/DDC-100101319.
15. Medeiros-Albuquerque AF., Sampaio-Melo CM., Studart-Soares EC., Rodrigues-Ribeiro T., Roriz-Fonteles CS., Alves-Pereira KM., et al. Preemptive analgesia-related gene and protein expression in third molar surgeries under non steroidal anti-inflammatory drug protocols: A PROSPERO-registered systematic review of clinical studies. *Med Oral Patol Oral y Cir Bucal* 2018;**23**(6):e723–32. Doi: 10.4317/medoral.22576.
16. Meyer UA. Pharmacogenetics - Five decades of therapeutic lessons from genetic diversity. *Nat Rev Genet* 2004;**5**(9):669–76. Doi: 10.1038/nrg1428.
17. Martínez C., García-Martín E., Blanco G., Gamito FJG., Ladero JM., Agúndez JAG. The effect of the cytochrome P450 CYP2C8 polymorphism on the disposition of (R)-ibuprofen enantiomer in healthy subjects. *Br J*

Clin Pharmacol 2005;**59**(1):62–8. Doi: 10.1111/j.1365-2125.2004.02183.x.

18. Agúndez JAG., García-Martín E., Martínez C. Genetically based impairment in CYP2C8- and CYP2C9-dependent NSAID metabolism as a risk factor for gastrointestinal bleeding: Is a combination of pharmacogenomics and metabolomics required to improve personalized medicine? *Expert Opin Drug Metab Toxicol* 2009;**5**(6):607–20. Doi: 10.1517/17425250902970998.
19. Martínez C., Blanco G., Ladero JM., García-Martín E., Taxonera C., Gamito FG., et al. Genetic predisposition to acute gastrointestinal bleeding after NSAIDs use. *Br J Pharmacol* 2004;**141**(2):205–8. Doi: 10.1038/sj.bjp.0705623.
20. Sanderson S., Emery J., Higgins J. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: A HuGEnet™ systematic review and meta-analysis. *Genet Med* 2005;**7**(2):97–104. Doi: 10.1097/01.GIM.0000153664.65759.CF.
21. Lee CR., Goldstein JA., Pieper JA. Cytochrome P450 2C9 polymorphisms: A comprehensive review of the in-vitro and human data. *Pharmacogenetics* 2002;**12**(3):251–63. Doi: 10.1097/00008571-200204000-00010.
22. Kirchheiner J., Brockmöller J. Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin Pharmacol Ther* 2005;**77**(1):1–16. Doi: 10.1016/j.clpt.2004.08.009.
23. Miners JO., Coulter S., Tukey RH., Veronese ME., Birkett DJ.

Cytochromes P450, 1A2, and 2C9 are responsible for the human hepatic O'demethylation of R- and S-naproxen. *Biochem Pharmacol* 1996;**51**(8):1003–8. Doi: 10.1016/0006-2952(96)85085-4.

24. García-Martín E., Martínez C., Ladero JM., Agúndez JAG. Interethnic and intraethnic variability of CYP2C8 and CYP2C9 polymorphisms in healthy individuals. *Mol Diagnosis Ther* 2006;**10**(1):29–40. Doi: 10.1007/BF03256440.
25. Rodrigues a D. Cyclooxygenase Inhibitors the Same ? *Drug Metab Dispos* 2005;**33**(11):1567–75. Doi: 10.1124/dmd.105.006452.At.
26. Dean L. Piroxicam Therapy and CYP2C9 Genotype. In: In: Pratt V, McLeod H, Rubinstein W, et al. editors, editor. *Medical Genetics Summaries [Internet]*. Bethesda (MD): National Center for Biotechnology Information (US); 2019. p. 2–7.
27. Benetello V., Sakamoto FC., Giglio FPM., Sakai VT., Calvo AM., Modena KCS., et al. The selective and non-selective cyclooxygenase inhibitors valdecoxib and piroxicam induce the same postoperative analgesia and control of trismus and swelling after lower third molar removal. *Brazilian J Med Biol Res* 2007;**40**(8):1133–40. Doi: 10.1590/S0100-879X2006005000123.
28. Maxwell C. Sensitivity and accuracy of the visual analogue scale: a psycho- physical classroom experiment. *Br J Clin Pharmacol* 1978;**6**(1):15–24. Doi: 10.1111/j.1365-2125.1978.tb01676.x.
29. Trindade PAK., Giglio FPM., Colombini-Ishikiriama BL., Calvo AM., Modena KCS., Ribeiro DA., et al. Sublingual ketorolac and sublingual

piroxicam are equally effective for postoperative pain, trismus, and swelling management in lower third molar removal. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;**114**(1):27–34. Doi: 10.1016/j.tripleo.2011.05.027.

30. Trindade PAK., Giglio FPM., Colombini-Ishikiriama BL., Calvo AM., Modena KCS., Ribeiro DA., et al. Comparison of oral versus sublingual piroxicam during postoperative pain management after lower third molar extraction. *Int J Oral Maxillofac Surg* 2011;**40**(3):292–7. Doi: 10.1016/j.ijom.2010.10.026.
31. Senes AM., Calvo AM., Colombini-Ishikiriama BL., Gonçalves PZ., Dionísio TJ., Sant'Ana E., et al. Efficacy and safety of 2% and 4% articaine for lower third molar surgery. *J Dent Res* 2015;**94**(9_suppl):166S-173S.
32. Daniels SE., Desjardins PJ., Talwalker S., Recker DP., Verburg KM. The analgesic efficacy of valdecoxib vs. oxycodone/acetaminophen after oral surgery. *J Am Dent Assoc* 2002;**133**(5):611–21. Doi: 10.14219/jada.archive.2002.0237.
33. Sisk L. for Control of In tra- and Post-operative. *Blood* 1986:16–20.
34. Hillerup S., Bakke M., Larsen JO., Thomsen CE., Gerds TA. Concentration-dependent neurotoxicity of articaine: An electrophysiological and stereological study of the rat sciatic nerve. *Anesth Analg* 2011;**112**(6):1330–8. Doi: 10.1213/ANE.0b013e3182172a2e.
35. Calvo AM., Zupelari-Gonçalves P., Dionísio TJ., Brozoski DT., Faria FA.,

Santos CF. Efficacy of piroxicam for postoperative pain after lower third molar surgery associated with CYP2C8*3 and CYP2C9. *J Pain Res* 2017;**10**:1581–9. Doi: 10.2147/JPR.S138147.

36. Rollason V., Samer CF., Daali Y., Desmeules JA. Send Orders for Reprints to reprints@benthamscience.net Prediction by Pharmacogenetics of Safety and Efficacy of Non-Steroidal Anti-Inflammatory Drugs: A Review. *Curr Drug Metab* 2014;**15**:326–43.
37. Vianna-Jorge R., Perini JA., Rondinelli E., Suarez-Kurtz G. CYP2C9 genotypes and the pharmacokinetics of tenoxicam in Brazilians. *Clin Pharmacol Ther* 2004;**76**(1):18–26. Doi: 10.1016/j.clpt.2004.03.002.
38. Kaplan V., Eroğlu CN. Comparison of the Effects of Daily Single-Dose Use of Flurbiprofen, Diclofenac Sodium, and Tenoxicam on Postoperative Pain, Swelling, and Trismus: A Randomized Double-Blind Study. *J Oral Maxillofac Surg* 2016;**74**(10):1946.e1-1946.e6. Doi: 10.1016/j.joms.2016.05.015.
39. Takanashi K., Tainaka H., Kobayashi K., Yasumori T., Hosakawa M., Chiba K. CYP2C9 Ile359 and Leu359 variants: Enzyme kinetic study with seven substrates. *Pharmacogenetics* 2000;**10**(2):95–104. Doi: 10.1097/00008571-200003000-00001.
40. Tracy TS., Matthew Hutzler J., Haining RL., Rettie AE., Hummel MA., Dickmann LJ. Polymorphic variants (CYP2C9*3 and CYP2C9*5) and the F114L active site mutation of CYP2C9: Effect on atypical kinetic metabolism profiles. *Drug Metab Dispos* 2002;**30**(4):385–90. Doi: 10.1124/dmd.30.4.385.

41. Stamer UM., Zhang L., Stüber F. Personalized therapy in pain management: Where do we stand? *Pharmacogenomics* 2010;**11**(6):843–64. Doi: 10.2217/pgs.10.47.

42. Maruf A Al., Greenslade A., Arnold PD., Bousman C. Antidepressant pharmacogenetics in children and young adults: A systematic review. *J Affect Disord* 2019;**254**(May):98–108. Doi: 10.1016/j.jad.2019.05.025.

43. Han C., Wang SM., Bahk WM., Lee SJ., Patkar AA., Masand PS., et al. A pharmacogenomic-based antidepressant treatment for patients with major depressive disorder: Results from an 8-week, randomized, single-blinded clinical trial. *Clin Psychopharmacol Neurosci* 2018;**16**(4):469–80. Doi: 10.9758/cpn.2018.16.1.469.

ANEXOS

ANEXO 1: Aprovação Comitê de Ética

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Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Influência do genótipo do citocromo P450 (CYP2C9) na eficácia clínica do tenoxicam após cirurgias de terceiros molares inferiores

Pesquisador: Paulo Zupelari Gonçalves

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 2

CAAE: 66699717.3.1001.5420

Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.247.253

Apresentação do Projeto:

A farmacogenética é uma área da farmacologia que se encontra em ascensão e que estuda a contribuição de fatores genéticos para as respostas individuais aos fármacos. Esse ramo da ciência implica a variabilidade na farmacodinâmica e farmacocinética por meio do estudo dos polimorfismos, por exemplo em genes que codificam receptores, e no metabolismo dos fármacos, onde esta área da farmacologia vem crescendo e obtendo seus primeiros resultados com utilização clínica. Os AINES são metabolizados pela família do citocromo P450 (CYP), predominantemente CYP2C9. O objetivo do presente estudo é avaliar os diferentes haplótipos do gene para a eficácia clínica do tenoxicam após cirurgias de terceiros molares inferiores em relação à dor, edema e trismo, reações adversas, necessidade de utilização de medicação de socorro, satisfação do paciente em relação ao medicamento e a farmacocinética do medicamento entre os diferentes haplótipos do gene CYP2C9 que serão encontrados nessa população. Para tanto, 100 pacientes serão genotipados e fenotipados para esse gene e suas fichas pós-operatórias com todos esses dados confrontadas com os haplótipos encontrados na população brasileira. Para análise do gene proposto será coletada saliva, que servirá como fonte de DNA genômico. Para análise molecular será realizada a técnica de reação em cadeia da polimerase (PCR), sendo utilizados ensaios

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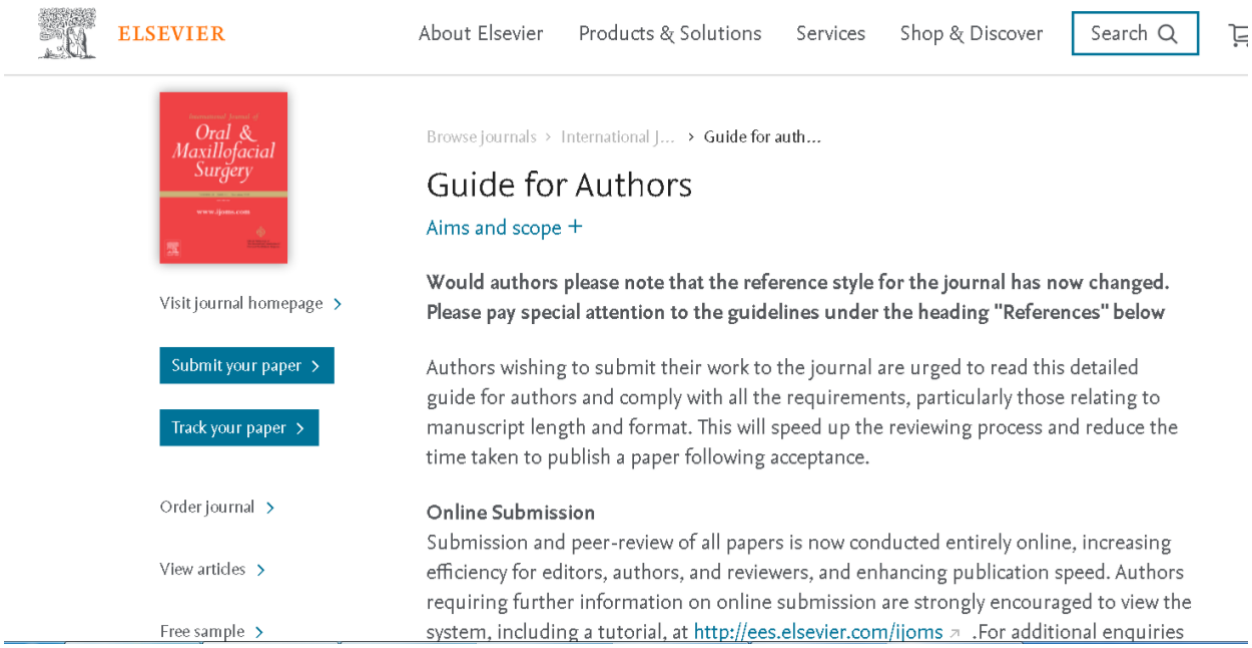

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The IJOMS welcomes Letters to the Editor. To facilitate submission of the highest quality of Letters to the Editor, the following guidelines should be followed:

1. Letters are meant to be focus pieces and, therefore, are limited to no more than 600 words, 6 references and a maximum of 2 figures. One reference should include a reference to the IJOMS article being addressed.
2. It is recommended that you limit your letter to one or two important and critical points to which you wish to provide a clear and precise discussion regarding the previously published article.
3. One should support all assertion by peer review literature which should be a primary research or large clinical studies rather than a case report.
4. Please include any financial disclosures at the end of the letter. This would include the potential conflicts of interest not just related to the specific content of your letter but also the content of the IJOMS article and other related areas.
5. Please recognize that letters that are essentially in agreement with the author's findings and offer no additional insights provide little new information for publication. Likewise, letters that highlight the writer's own research or are otherwise self promotional will receive a low publication priority.
6. There may be a need for additional editing. Should editing be required the letter will be sent back to the author for final approval of the edited version.
7. It is important to use civil and professional discourse. It is not advisable that one adopt a tone that may be misconstrued to be in anyway insulting.
8. Finally, it is not advisable to provide a letter that is anecdotal. While personal experiences can have great value in patient care, it is generally not strong evidence to be placed in a letter to the editor.