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Research Article

Effect of CYP2C9*3 RS 1057910 Polymorphism on the Tolerability of Ibuprofen after Molar Tooth Extraction in Pakistani Population

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ABSTRACT

Post-extraction pain is frequently managed with ibuprofen, a widely used NSAID metabolized by CYP2C9. Genetic polymorphisms, particularly CYP2C9*3 rs1057910, may alter drug metabolism, influencing efficacy and tolerability. This study evaluated the impact of this variant on ibuprofen tolerability in a Pakistani population. A prospective observational study was conducted from August 2022 to August 2023 at Islamic International Medical College, Rawalpindi, and affiliated dental clinics. Two hundred adults undergoing unilateral molar extraction were enrolled. Standardized procedures were followed, and patients received ibuprofen 400 mg thrice daily for three days. Blood samples were collected for CYP2C9*3 genotyping. Adverse effects, especially gastropathy, were assessed on day four using the General Assessment of Side Effects (GASE) questionnaire. Associations between genotype and adverse effects were analyzed with Chi-square test. Allele frequencies were 82.8% (A) and 17.2% (C). The AA genotype was identified in 65.5% of participants, while 34.5% participants resulted in AC genotype. Adverse effects were significantly more common among AC carriers (43.5%) compared with AA carriers (16.8%) ($p<0.001$). The CYP2C9*3 rs1057910 polymorphism is associated with reduced ibuprofen tolerability in the Pakistani population, with heterozygous carriers showing a higher risk of gastropathy. These findings support the clinical relevance of pharmacogenetic screening to optimize NSAID prescribing and reduce adverse outcomes. Broader studies across diverse populations are recommended to validate these results and inform genotype-based dosing guidelines.

Keywords: Dental Extractions, Genetic Polymorphisms, Allele Frequency, Ibuprofen.

INTRODUCTION

Tooth extractions are one of the surgical procedures that are most performed in dental practices. These procedures are linked to a variety of post-operative problems such as pain, bleeding, and edema as well as infections and abscesses at the surgical site.[1] Acetaminophen and medications from the Non-Steroidal Anti-inflammatory Drugs (NSAIDS) group are suggested to be useful in the preliminary stage of pharmacological pain management with normal functioning of the liver. In pain situations with relevant inflammatory components, medications from the NSAIDS group show better clinical efficacy that efficiently alleviate the pain and inflammation. This criterion has been recommended by the American Pain Society and World Health Organization (WHO) pain ladder.[2]

Pharmacological therapy with NSAIDS offers efficacious pain relief with superior tolerability over opioids, [3] therefore, clinical practitioners most frequently use NSAIDs including ibuprofen for the relief of dental pain and inflammation after uncomplicated dental extraction. [4]

Ibuprofen, a propionic acid derivative, has potent analgesic, anti-inflammatory, and antipyretic actions. Ibuprofen is useful at low over-the-counter doses (400-1200 mg/day), in managing common inflammatory conditions that can cause acute and chronic pain. [5] Ibuprofen works by non-selectively inhibiting

cyclooxygenase (COX) isoenzymes, thereby minimizing the production of prostaglandins which induce pain and inflammation at a site of injury or disease. The analgesic, antipyretic, and anti-inflammatory effects of ibuprofen are achieved mainly by the blockade of the COX-2 pathway. COX-1 pathway causes the production of prostaglandins that play a protective role in the gastrointestinal tract. [6] As Ibuprofen blocks the production of mucosal protective prostaglandins, it can therefore result in Ibuprofen induced gastropathy.[7]

Ibuprofen undergoes extensive metabolism (99%) to its pharmacologically inactive metabolites by biotransformation reactions that include hydroxylation that is followed by glucuronide conjugation. [8] This metabolism depends on hepatic Cytochrome P450 (CYP) enzymes including CYP2C8 and CYP2C9. These enzymes are found in the endoplasmic reticulum of the hepatocytes. [9] The CYP2C9 gene is distinctly polymorphic, having almost 61 variants of alleles and numerous sub-alleles. [10] This genetic evaluation led to the identification of the two most prevalent variants of CYP2C9. These variants are considered as the influential and clinically significant alleles. These include rs1799853, and rs1057910, also known as CYP2C9*2 and CYP2C9*3 polymorphisms, respectively. These alterations usually occur due to single nucleotide polymorphisms (SNP). For instance, CYP2C9*3 modified variant is achieved when there is an amino acid substitution from Isoleucine (Ile) to Leucine (Leu) with a transition, A>T in the CYP2C9 gene at position 359. [11]

CYP2C9*3 rs1057910 genotype is observed to be more prevalent in the Pakistani population with the Minor Allele Frequency (MAF) of 9.9%. Haplotype analysis proposes that approximately 30% population who have modified CYP2C9 genotype with the above-mentioned SNPs, show somewhat compromised function of enzymes associated with the CYP2C9 gene. [12]

In previous some years, a few pharmacogenomics studies have been done to identify and evaluate the impact of CYP2C9 polymorphisms on the pharmacokinetics of ibuprofen in various parts of the world. These studies identified that the allele carriers with CYP2C9*2 and mainly CYP2C9*3 SNPs display high plasma concentrations of the drug. This happens due to the slow metabolism of the drug as the enzymes involved in the biotransformation have reduced functionality. [13] The drug remains in the activated form for longer durations resulting in increased chances of developing the adverse effects. So, these individuals with prevalent CYP2C9 polymorphisms are expected to have a disparity in tolerability and safety profile of the drug due to alterations in the enzymes that metabolize the drug.[14]

A previous meta-analysis study done in genetically different populations clearly reveals that an obvious association occurs between the altered CYP2C9 genotype and the compromised metabolism of ibuprofen. [15] This results in increased plasma levels of the drug which can further lead to increased chances of ibuprofen-induced gastropathy. By assessing this specific genetic variant in patients taking ibuprofen, the study aims to gain valuable insights into how genetic makeup influences drug metabolism resulting in modified tolerability of ibuprofen.

METHOD

Study Design and Duration: It was an observational prospective study with duration of one year, August 2022 to August 2023. The research synopsis was approved by the Ethical Review Committee Ref.no. Riphah/IIMC/IRC/22/2072 of Islamic International Medical College (IIMC), Rawalpindi conducted per the Declaration of Helsinki. The study was carried out in the Multidisciplinary laboratory of IIMC in collaboration with Fahad Dental Square Chaklala Scheme 3, Rawalpindi. The study was registered at clinicaltrial.gov with clinical trial identifier (NCT05983042). [16] Genetic Association Study (GAS) Power Calculator – Center for Statistical Genetics was used to calculate the sample size of the study. [17] In this power calculator, by setting minor allele frequency of 9.92%, power study of 90% and a significance level of 0.05%, the sample size obtained was 200.

Study Participants: The study was carried out on 200 Pakistani individuals in total, including both genders and above 18 years of age by Simple Convenient sampling technique. The participants were selected based on the following criteria: patients indicated for upper or lower unilateral molar tooth extraction. The extractions were advised based on panoramic radiographs, with or without intraoral and extra-oral swelling.

Inclusion Criteria:

The inclusion criteria included the following:

- Pakistani individuals of age between 18 to 55 years.
- Absence of systemic diseases that could interfere with the study.

- Extractions required based on orthodontic, periodontal, and endodontic indications were included.

Exclusion Criteria:

The exclusion criteria included the following:

- History of bleeding or gastrointestinal ulcers.
- Allergic sensitivity to ibuprofen or any other NSAIDs.
- Pregnancy
- Use of anticoagulants within 2 months before surgery
- Hepatic, intestinal, renal, cardiovascular, and pulmonary dysfunction.
- Individuals not of Pakistani origin, below 18 years and above 50 years.

Surgical Intervention: All the patients were selected according to inclusion criteria and informed verbal and written consents were taken. 2ml blood samples were withdrawn for genetic sequencing by venipuncture following proper antiseptic measures. The patients underwent unilateral molar extraction following a standardized protocol. [18] Patients were instructed to consume one tablet of ibuprofen 400mg thrice a day for 3 days. Patients were called for follow-up visits on the 4th day of tooth extraction. Ibuprofen induced gastropathy was recorded systematically during the follow-up visit using the General Assessment of Side Effects (GASE) questionnaires. [19] All the study participants were genotyped for CYP2C9*3 polymorphism.

Genotyping of the Samples:

DNA Extraction from the Blood Sample:

Deoxyribonucleic acid (DNA) was extracted from whole blood by the Chelex method. [20]

Primer Designing and Amplification Refractory Mutation System (ARMS)-Polymerase Chain Reaction (PCR) Amplification for CYP 2C9*3:

The concentration of extracted genomic DNA was checked by Nano Drop2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to take the correct volume of sample for PCR amplification.

Primer Mixture Formation:

The primers were designed for the amplification of CYP2C9*3 (rs1057910, c.1075 A>C) in the multidisciplinary laboratory of IIMC using the software Primer3plus supplied by M/s Macrogen ™ (Korea).

ARMS PCR:

ARMS PCR detected the above SNP. [21] Veriti 96 well thermal cycler was used to perform PCR reactions. PCR cycling conditions were optimized as follows: Stage 1 (initial denaturation); 1 cycle of 5 minutes' duration and the temperature was 95°C, followed by Stage 2 of 30 cycles. These 30 cycles include three steps: denaturation at 95°C for 30 seconds, annealing at 60°C for 60 seconds, and extension at 72°C for 30 seconds. The final extension was attained at 72°C for 60 seconds. The amplified PCR products were fixed on 2% agarose gel containing agarose and TBE buffer (Tris, borate, and Ethylenediamine tetra acetic acid) to see the DNA bands under ultraviolet light in the gel documentation system. Different alleles were identified according to their amplification sizes.

Statistical Analysis: The data was entered and analyzed using SPSS.27 (Statistical Package for Social Sciences). Chi Square test was performed to evaluate the association between polymorphism and presence of adverse effects. A p-value of < 0.05 was considered statistically significant.

RESULTS

For the given dataset, genotype was successfully acquired in all samples, adhering to Hardy-Weinberg Equilibrium (HWE). [22] Gel images of extracted DNA samples from the study subjects were obtained by placing gels on UV transilluminator (Gene Box ™ by Syngene G ™, USA). An image of DNA fragments is pasted below (figure 1) showing amplified bands of interest along with 50 bp DNA ladder in labelled lanes.

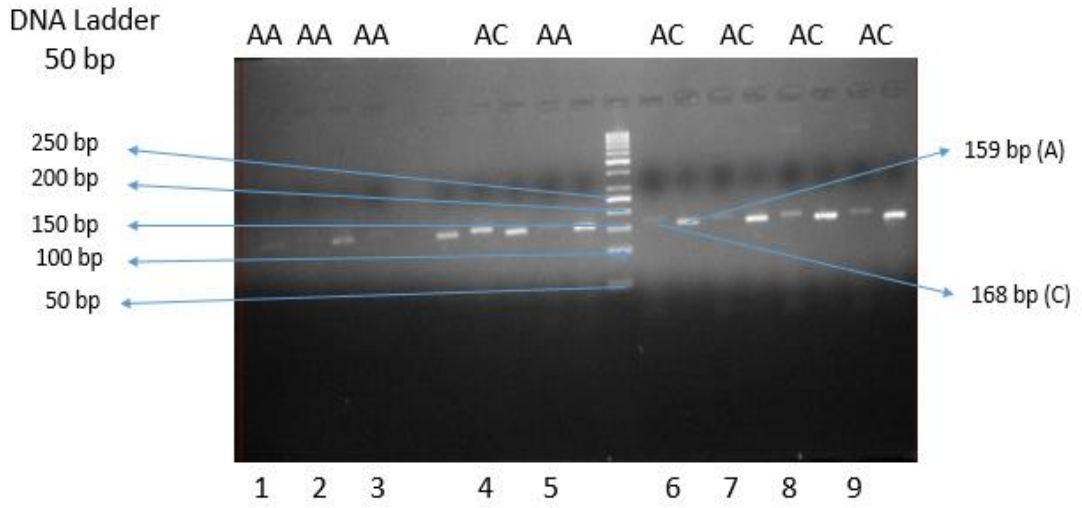

Figure 1: Amplified A and C Bands Separated on Gel Electrophoresis

The table 1 provides an overview of allele frequency and genotype frequency along with their respective 95% Confidence Intervals for CYP2C9*3 rs 1057910 polymorphism which were calculated according to HWE. These frequencies are further elaborated in graphical pattern in figure 2.

Table 1: Allele Frequency of CYP2C9*3 Rs 1057910 in Pakistani Population

Gene	Allele	Allele Frequency (%)	Genotype	Frequency (%)	95% Confidence Interval
CYP2C9*3 rs 1057910	A	131 (82.8%)	AA	131 (65.5%)	0.58 – 0.72
	C	69 (17.2%)	AC	69 (34.5%)	0.27 – 0.41

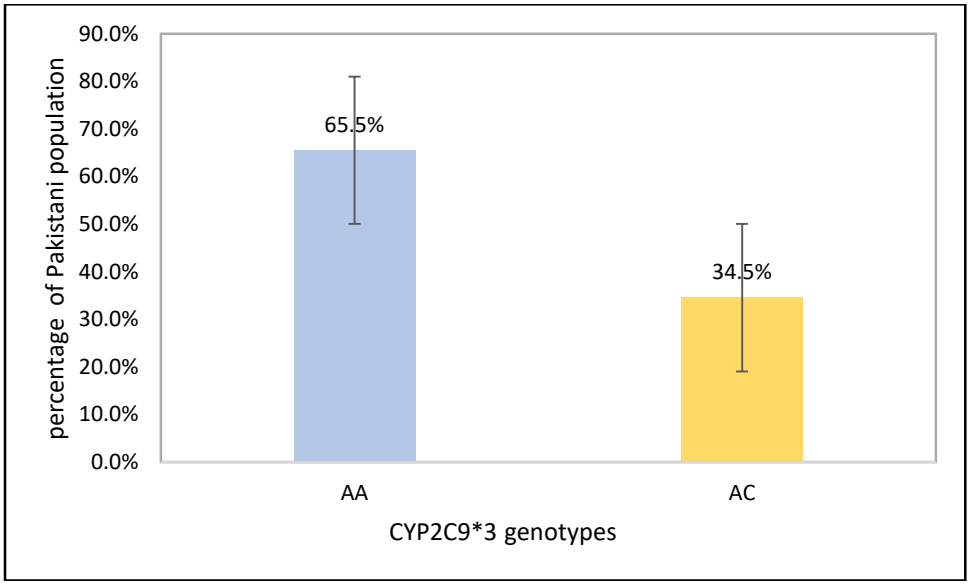

Figure 2: Genotype Distribution of CYP2C9*3 Rs 1057910 in Pakistani Population

The association between the different genotypes and adverse effects showed that patients identified with the genotype AC had more adverse effect (43.5%) as compared to genotype AA (16.8%) patients showing a statistically significant association. (p-value < 0.001).

Table 2: Association of Adverse Effects with CYP2C9*3 Rs 1057910 Alleles (Chi Square Test)

Adverse Effects	CYP2C9*3 rs 1057910	p- value

	AA (n=131)	AC (n=69)	
No	109 (83.2%)	39 (56.5%)	**< 0.001**
Yes	22 (16.8%)	30 (43.5%)	

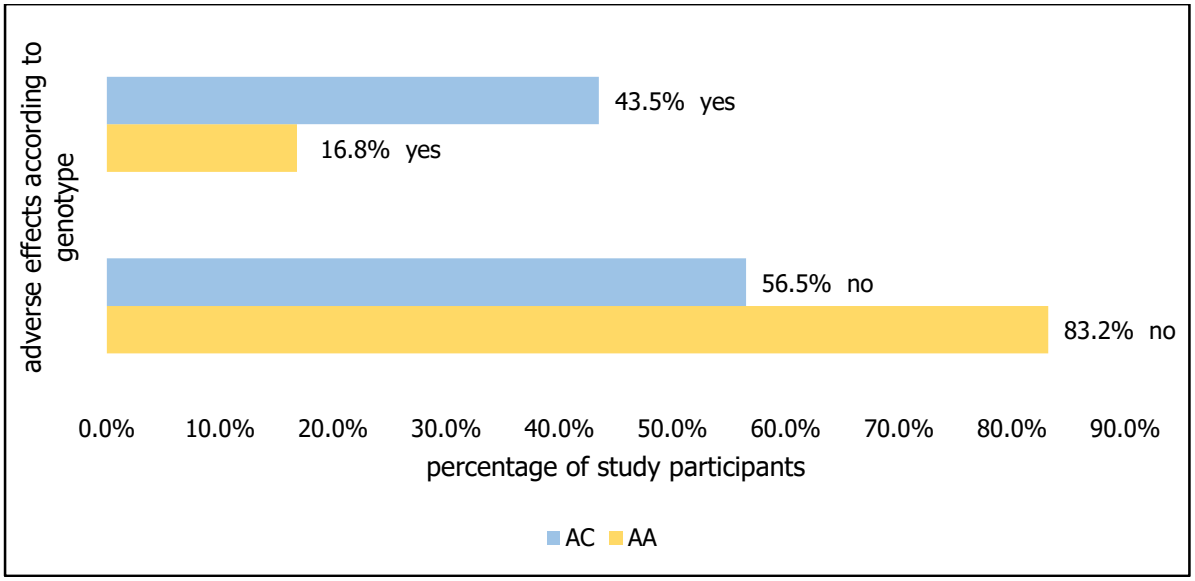

Figure 3: Response Percentage of Study Participants With Respect To Adverse Effects and Genotype

DISCUSSION

Pain is a complicated multidimensional mechanism that consists of an interaction between nociceptor, affective and cognitive processes. The diverse pathophysiological mechanisms of the pain can lead to substantial variability among different individuals in the effectiveness of all analgesics, including ibuprofen. [20] Ibuprofen is completely absorbed from oral rout. It undergoes hepatic metabolism which is chiefly done by the cytochrome P450 (CYP) enzymes that include CYP2C9. This enzyme metabolizes ibuprofen to form the inactive metabolites hydroxy ibuprofen and carboxy ibuprofen. Several factors can affect the rate of metabolism of ibuprofen including age, hepatic dysfunction and genetic variations in CYP enzymes. [21] In the present study, out of total 200 normal healthy individuals, 69 participants were found to have heterozygous CYP2C9*1/*3 polymorphism. There was a statistically significant association found between the altered efficacy and tolerability of Ibuprofen and interindividual variability when the genotyping of these patients was performed.

Aisha Qayyum et al (2017) conducted a study in Pakistani population. The study investigated the minor allele frequency for CYP2C9*3 allele to be 22.85% in, whereas our study revealed 17.2% (0.172) minor allele frequency for the same variant. The same study also showed 7.4% frequency of CYP2C9*3/*3 allele [22] whereas our study did not identify any CYP2C9*3/*3 (CC) variant in the Pakistani population. The study done by AR Khan et al in 2022 concluded the frequency of CYP2C9*3 to be 10% in Punjabi whereas 11% in Pathan population of Pakistan.[23] Similarly the minor allele frequency obtained from our study is also different from the frequency obtained by https://www.ncbi.nlm.nih.gov/snp/rs1057910. According to their data the minor allele frequency of CYP2C9*3 is only 0.043 (4.3%) in the people of South Asia.

Although, ibuprofen is considered as one of the safest NSAIDS, and the gastrointestinal complications are also rare. Nevertheless, Elena Garcia Martin et al (2004) reported that individuals with modified CYP2C9 variants are at a greater risk of acute gastrointestinal bleeding with ibuprofen.[24]

As the rate of metabolism is delayed, the drug plasma concentration of the drug increases. This leads to increased chances of occurrence of adverse effects compromising the tolerability of the drug. We found out the same results in our study regarding the occurrence of adverse effects in the CYP2C9*3 allele carriers analyzed by Chi square test. As the association between the CYP2C9*3 rs 1057910 genotype and the ibuprofen induced gastropathy reported by the patients on the follow-up visit was proved to be statistically significant with p-value of <0.001.

Only 16.8% patients reported ibuprofen induced gastropathy with wild type genotype whereas 43.5% patients experienced gastropathy with CYP2C9*3 polymorphism. A study conducted by N Carbonell et al on 188 patients, treated by different types of NSAID including ibuprofen concluded that the patients who had CYP2C9*3 genotype had greater risks of developing acute upper gastrointestinal bleeding.[25] Similarly, Akiko Shiotani et al investigated that CYP2C9*3 variant was involved in the increased chances of developing NSAID induced gastropathy. [26] But the study done by Gerardo Blanco et al reported that the presence of both CYP2C8*3 and CYP2C9*2 genotype, was a potent determinant in the risk to develop NSAID induced gastropathy.[27] Vonkeman et al found out that there was no significant association between CYP2C9*3 polymorphisms and higher risks of NSAID induced gastropathy.[28]

The guideline of CPIC has suggested the recommended doses of NSAID including ibuprofen according to the CYP2C9 genotypes. Since CY2C9*3 rs 1057910 is categorized as an intermediate metabolizer,[10] these individuals can have have more than normal risk of developing adverse events. This is more particular for the individuals with presence of other factors affecting clearance of drugs like hepatic dysfunction or advanced age.

It was a single center study with a sample size of only 200 patients with no control group that did not use ibuprofen for pain control. Because of interethnic differences in the frequencies of CYP2C9*3 variant, the findings of the study cannot be applied other than in Pakistani population. The findings of the study should stimulate further research in ethnically different populations. Furthermore, impact of CYP2C9*2 on the metabolism of ibuprofen can be evaluated.

CONCLUSION

This study concludes that the MAF of CYP2C9*3 rs 1057910 allele in Pakistani population is found to be 17.2%. The presence of CYP2C9*3 rs 1057910 SNP in the genetic makeup of individuals increases the occurrence of ibuprofen induced gastropathy by decreasing its metabolism. This can compromise the tolerability of ibuprofen in Pakistani population who have CYP2C9*3 rs 1057910 SNP in their genome.

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