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THE ROLE OF OPRM1 (rs1799971) GENETIC VARIANT IN MODULATING THE ANALGESIC EFFECT OF TRAMADOL IN POSTOPERATIVE PAIN

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Abstract:
Objective: This pioneering study aims to understand the influence of genetic polymorphism identified as rs1799971 in the exon 1 of mu-opioid receptor (OPRM1) gene on the safety and efficacy of tramadol in context to postoperative pain within Pakistani population. Insights from this research may contribute to more tailored approaches to pain management in this demographic.
Study Design: Uncontrolled Cohort Pharmacogenetics Studies.
Place & duration of study: Nawaz Sharif Medical College, Gujrat from March 2022- December 2022.
Methods: This study was conducted within Gujrat city population in Punjab, Pakistan. Participants were genotyped for the OPRM1 (rs1799971) SNP (AA, AG, GG) using Sanger sequencing technique. Postoperative pain scores, rescue analgesic requirements, and side effects such as nausea, vomiting, and sedation were assessed at multiple time points within 24 hours post-surgery among the three OPRM1 genotypes. The data were analyzed using IBM SPSS Statistics 26, comparing pain scores and categorical variables like sedation and nausea amongst different genotypes, considering a p-value of 0.05 or less as statistically significant.
Results: The variant allele "G" was observed in 31.5% of study population with GG genotype in 11% of our study population, adhering to Hardy-Weinberg equilibrium. Patients with GG genotype showed tramadol analgesic inadequacy as they reported statistically higher pain scores both at rest and on movement (<0.001 vs AA) and required more rescue analgesia in the immediate postoperative

phase. A discernible allele effect was noted with the G allele linked to reduced side effects ($p<0.05$) including nausea and vomiting sedation and other side effects of tramadol in the study participants. However, dizziness was more prevalent in AA genotype patients, while the AG and GG genotypes showed reduced occurrences.

Conclusion: This study underscores the significant influence of OPRM1(rs1799971) genotypes on efficacy and safety of tramadol in acute pain settings within the Pakistani population paving way for personalized analgesic strategies to optimize patient results

Keywords: OPRM1, rs1799971, Postoperative Pain, Tramadol, Efficacy, Safety, Genotypes, Pakistani Population, Personalized Medicine.

Introduction:

Postoperative pain management constitutes a critical cornerstone of the surgical patient's care pathway, significantly influencing morbidity, recovery trajectory, and overall quality of life [1]. Among the myriad of analgesics employed in postoperative care, tramadol has emerged as a key player.2 Its dual mechanism of action, invoking both opioid receptor activation and reuptake inhibition of norepinephrine and serotonin, renders it an effective and versatile agent [2].

Notwithstanding the broad utility of tramadol, there has been a consistent observation of inter-individual variability in its response, encompassing both its analgesic efficacy and side-effect profile. While an array of factors contributes to this variability, one of the most intriguing is the patient's genetic constitution. Central to this discussion is the μ-opioid receptor gene (OPRM1) [3]. It encodes for the μ-opioid receptors, the principal target of tramadol's analgesic effects, that exhibits a notable degree of polymorphism and are densely located in pain associated areas [4]. Upon interaction with tramadol, μ-opioid receptors undergoes a structural change that facilitates the exchange of G-protein-bound molecules leading to the inhibition of the adenylate cyclase enzyme, disruption of downstream Protein Kinase A (PKA)-dependent signaling pathways and decreased neuronal action potential firing due opening of potassium channels and inhibiting neurotransmitter release as N-type $Ca2+$ channels in the presynaptic regions of central and peripheral neurons are inhibited [4-5].

The rs1799971 single nucleotide polymorphism (SNP) SNP A118G, located in the coding section of the OPRM1 gene's exon 1, results in the substitution of an adenine (A) with a guanine (G) nucleotide as shown in figure 1. This change leads to the replacement of asparagine with aspartic acid at the receptor protein's 40th position (N40D) altering the glycosylation site on extracellular terminus that causes unstable receptor protein formation that translates into altered response to opioids[4]. The OPRM1RS1799971 polymorphism's impact on postoperative pain medication needs remains debated. Variations in opioid usage and pain levels have been noted in patients undergoing procedures like abdominal hysterectomy, Cesarean section, and total knee arthroplasty in relation to the A118G SNP genotype [6-8]. Furthermore, the G allele's presence may not only influence pain relief efficacy but also the frequency and intensity of tramadol-related side effects, potentially due to altered receptor binding with tramadol's metabolite [6-8]. However, others have failed to prove any association between rs1799971 polymorphism and efficacy and safety of opioids [8].

It is well established that the frequency of genetic variants, including SNPs like OPRM1 (rs1799971), can significantly vary among different populations across the globe and is documented to be as high as 36% in East Asians [9].This variation can profoundly influence drug responses and side effect profiles, necessitating population-specific genetic studies to inform individualized treatment strategies. In the context of the Pakistani population, there is a significant gap in the current body of research, with limited data available on the prevalence of critical pharmacogenomics variants and their impact on the response of tramadol in the post-operative pain.

In Pakistan, where diverse genetic backgrounds exists, understanding the prevalence and impact of pharmacogenomic variants like OPRM1 (rs1799971) on tramadol response during acute pain is crucial. Yet, this population has not been adequately represented in global studies. Given this backdrop and recognizing the potential to significantly enhance post-operative pain management in

Pakistan, this study aims to explore the frequency of the OPRM1 (rs1799971) SNP and its correlation with tramadol efficacy and safety for post-operative pain management. The insights from our study could provide an important stepping stone towards establishing personalized analgesic strategies within the Pakistani healthcare system.

Methods:

This was an uncontrolled Cohort pharmacogenetics study that allowed for an in-depth analysis of the safety and efficacy of Tramadol for postoperative pain management across different rs1799971 genotypes. The experimental protocols underwent thorough examination and received approval from the Ethical Review Committee (ERC) of Riphah International University (Riphah/IRC/20/103), and Institutional Review Board (IRB) of Nawaz Sharif Medical College (dated 11/07/2021), ensuring ethical standards. With a global Minor Allele Frequency (MAF) of rs1799971 of 0.30, and using a significance level of 0.05 and study power of 80%, the sample size was determined to be 100 [10]. A double-blind approach was used. Patients were unaware of their genotyping results as it was performed after clinical assessment. Efforts were taken to minimize others biases in this study. Specific inclusion and exclusion criteria were set to create a more homogeneous sample, thereby reducing the influence of confounding variables. We recognize that while these criteria, while essential also limits the generalizability of our findings. Patients aged 18 and above, undergoing major abdominal surgery lasting up to 1-4 hours and willing to use a numeric pain scale were recruited through random convenient sampling for this study after obtaining informed consent. Patients with prior liver or kidney disease, history of substance abuse, seizures, psychiatric disorders like bipolar disorder or schizophrenia, and respiratory depression or sleep apnea, history of substance use, chronic opioid intake and use of medications interacting with tramadol were excluded from the study [11]. Additionally, those experiencing preoperative pain were excluded so as to eliminate potential confounders in our study outcomes. Each study participant provided comprehensive data, including demographic information, medical history, and drug history, which were recorded using a customized form designed specifically for this research.

Anesthesia protocol was standardized across all study population to avoid any bias. All patients received oral midazolam (0.1 mg/kg, max 15 mg) as pre-medication. Anesthesia was induced with propofol (1-3 mg/kg) and atracurium (0.5 mg/kg) for endotracheal intubation, then maintained with sevoflurane or isoflurane. Intraoperative analgesia included IV ketamine (25 mg) and paracetamol (1 gram). Post-surgery, glycopyrrolate (0.5 mg) and neostigmine (2.5 mg) were used to reverse anesthesia. At the conclusion of surgery, Tramadol 50mg in 50ml 0.9% sodium chloride (NaCl) was administered intravenously every 6 hourly as a postoperative analgesia. Evaluations were conducted at six time-points within 24 hours after surgery, focusing on pain severity and incidence of adverse drug reactions [12]. Pain severity, both at rest and on movement (head raising), was gauged using a numeric rating scale (NRS), along with the requirement for rescue analgesia (paracetamol) [13]. A modified version of the Pasero Opioid-induced Sedation Scale (POSS) consisting of four specific stages: 'awake', 'can be awaken by verbal instruction', 'struggling to remain awake', and 'unresponsive' was employed to measure the sedation level produced by tramadol [14]. Any occurrence of tramadol induced nausea and vomiting was evaluated using a simple three-point ordinal scale, which allowed for an efficient and straightforward assessment. Participants were also evaluated for other potential adverse effects, including pruritus, dizziness, headaches, sweating, and dry mouth.

Blood samples were stored at -20°C until analysis, and genomic DNA was extracted using a phenol-chloroform method. The quality, integrity, and quantity of the extracted DNA was assessed using a gel electrophoresis followed by spectrophotometric evaluation employing Thermo Scientific Multi Skan Go Instrument with target ratio near 1.8–2.0.

The target genetic variant rs1799971 was amplified with primers designed through bioinformatics tools having a balanced GC content (40 60%) and an optimal melting temperature (Tm) within 51 58°C. The forward primer of the target site SNP rs1799971 had sequence of “CCCAGTGAAGAGACCTACT” whereas reverse primer had sequence

"ACACGATGGAGTAGAGGG". The reaction mix for PCR amplification included components like the DNA template, dNTPs PCR buffer, Taq polymerase and MgCl2 and was conducted in a thermocycler after fine-tuning magnesium ion concentration and annealing temperature. Post successful PCR amplification, the DNA samples were subjected to Sanger sequencing for detection of the rs1799971 SNP [15]. The sequencing involved incorporating fluorescently labelled ddNTPs into PCR products, generating DNA fragments of varying lengths that were then separated by electrophoresis based on their size. The DNA sequence was latter analyzed and identify any genetic variations, including the rs1799971 SNP.

Data analysis was performed using IBM SPSS Statistics 26 to determine the relationship between the OPRM1 (rs1799971) genotype and Tramadol's effectiveness and safety profile for post-operative pain. Allele and genotype frequencies for the SNP rs1799971 were calculated from the collected sample. To determine whether the observed genotype frequencies were consistent with the Hardy-Weinberg equilibrium (HWE), expected genotype frequencies were manually calculated then tested against the observed frequencies using the chi-square test. A p-value above 0.05 indicated alignment with HWE, while a value below showed a significant deviation. None of the expected frequencies in the chi-square analysis were less than 5, ensuring the reliability of the chi-square test result. Pain scores, presented as mean and standard deviation (SD), were analyzed using ANOVA. Incidences of postoperative outcomes (e.g., nausea, vomiting, sedation, and other side effects), which is categorical data, were presented as frequencies and percentages. These were analyzed using either the Chi-square Test or Fisher's Exact Test when expected cell counts were less than 5. The level of statistical significance for all tests was set at $p \leq 0.05$.

Table 1: Demograhic data of study participants

Demographic data		
Parameters	**Characteristics**	**Value**
Gender	Male	37%
	Female	63%
Material Status	Married	75%
	Unmarried	25%
Mean Age (Years)		34.95±13.47
Mean Weight (Kg)		70.77±11.13
Mean Height (feet)		5.45±0.28
Mean Body Mass Index		25.12±4.15
Smoking Status	Yes	5%
	No	95%
Mean Serum Creatinine		0.78±0.20
Mean Urea		28.32±7.81
Previous Medication	Yes	2%
	No	98%
Mean Serum ALT		38.01±21.32
Mean Serum AST		39.03±22.00
Alcohol consumption		0%
ASA Status	ASA Status	
	I. Healthy	69%
	II. Mild Systemic Disease	29%
	III. Sever Systemic Disease	2%
Duration of Surgery (Minutes)		30.82±4.70

Table 2: Genotypic frequency of OPRM1 rs1799971 SNP

Genotypic frequency							
Gene	**SNP**	**Allele Frequency (%)**	**Genotype/ Haplotype**	**Observed Frequency**	**Expected Frequency**	**95% Confidence Interval**	**p^{hwe}**
OPRM1 (A/G) rs1799971	A	137 (68.5%)	A/A	48	47	0.379 – 0.582	0.898
	G	63 (31.5%)	A/G	41	43	0.312 – 0.513	
			G/G	11	10	0.056 – 0.188	

Table 3: Postoperative Pain scores according to OPRM1 (rs1799971) Genotypes After Tramadol Administration.* Mean ±S.D, **p value ANOVA, ***P value Post Hoc tukey test .95% CI in brackets.

Time points	OPRM1(rs1799971) AA* (n=48)	AG* (n=41)	GG* (n=11)	p-value**	AA vs AG*** (95%CI)	AA vs GG*** (95%CI)	AG vs GG*** (95%CI)
Mean Pain Score at Rest							
1 Hour	5.22±1.18	6.00±0.18	7.09±1.22	0.048	< 0.001 (-2.59 to-0.98)	< 0.001 (-41.5 to-1.62)	0.001 (-.2.31to 0.18)
3 Hours	4.56±1.14	5.87±1.09	6.00±1.26	0.039	< 0.001 (-2.3532 -0.600)	0.005 (-4.4952 to 1.739)	0.735 (-3.04 to 0.241)
6 Hours	3.99±1.35	4.78±1.23	5.63±1.63	0.042	0.005 (-2.025 to -.581)	0.009 (-2.86 to-.5851)	0.805 (-1.57 to 0.746)
12 Hours	3.13±0.89	4.07±0.78	5.18±1.16	0.047	0.002 (-3.07 to 1.417)	0.015 (-3.356 to 0.75)	0.710 (-1.13 to 1.516)
24 Hours	3.00±0.83	3.29±0.71	4.45±0.69	0.014	0.08 (-1.102 to 0.569)	0.019 (-3.21 to 0.578)	0.507 (-2.96 to 0.29)
Mean Pain Score at head Raising							
1 Hour	5.27±1.19	6.78±1.10	7.01±0.48	0.033	0.001 (-.866 to 0.638)	0.001 (-2.79 to 0.43)	0.504 (-2.69 to 0.29)
3 Hours	4.93±1.15	5.73±1.09	6.78±1.55	0.046	0.012 (-1.36 to 0.16)	< 0.001 (-2.16to .275)	0.012 (-1.42 to 0.49)
6 Hours	4.79±0.18	5.46±1.12	6.77±1.73	0.005	< 0.001 (-1.6902 -.3840)	< 0.001 (-3.1990 -1.1457)	0.037 (-2.1781 -.0924)
12 Hours	4.64±0.10	4.73±0.86	6.09±1.13	0.026	0.042 (-0.4405 to0.721)	0.028 (-1.2537 to 0.5719)	0.054 (-1.408 to 0.446)
24 Hours	3.02±0.91	3.75±0.88	4.82±0.75	0.006	0.002 (-1.99 to 0.51)	0.001 (-2.12 to 0.2009)	0.060 (-.8913 to 1.468)

Table 4: Incidence of Tramadol-Induced Nausea and Vomiting across OPRM1 (rs1799971) Genotypes. *Data expressed as frequency (%). **Statistical analyses employed Chi-square Test or Fisher's Exact Test where appropriate. Statistical significance set at $p \leq 0.05$

Nausea Vomiting 30 Minutes after Tramadol							
	OPRM1 (rs1799971)			**p value****	**AA vs AG****	**AA vs GG****	**AG vs GG****
	AA(48)*	**AG(41)***	**GG(11)***				
At 1 hour post operatively							
No Nausea (n=48)	24 (50.0%)	18 (43.9%)	6 (54.5%)	0.04	0.368	<0.01	0.009
Feel Nausea (n=42)	20 (41.7%)	18 (43.9%)	4 (36.4%)				
Vomit (n=10)	4 (8.3%)	5 (12.2%)	1 (9.1%)				
At 3 hour post operatively							
No Nausea (60)	31 (64.6%)	20 (48.8%)	9 (81.8%)	<0.01	0.014	< 0.01	0.018
Feel Nausea(22)	4 (8.3%)	16 (39.0%)	2 (18.2%)				
Vomit(18)	13 (27.1%)	5 (12.2%)	0 (0%)				
At 6 hour post operatively							
No Nausea(68)	30 (62.5%)	29 (70.7%)	9 (81.8%)	0.006	0.121	<0.01	0.013
Feel Nausea (21)	11 (22.9%)	8 (19.5%)	2 (18.2%)				
Vomit(11)	7 (14.6%)	4 (9.8%)	0 (0%)				
At 12 hour post operatively							
No Nausea (92)	43(89.5%)	39 (95.1%)	10 (90.9%)	0.755	0.957	0.935	0.764
Feel Nausea (7)	4 (8.4%)	2 (4.9%)	1 (9.1%)				
Vomit(1)	1 (2.1%)	0 (0%)	0 (0%)				
At 24 hours post operatively							
No Nausea (93)	43(89.6%)	39 (95.1%)	11 (100.0%)	0.526	0.467	0.247	0.594
Feel Nausea (7)	5 (10.4%)	2 (4.9%)	0 (0%)				
Vomit(0)	0(0%)	0(0%)	0(0%)				

Table 5: Postoperative Sedation Levels across OPRM1 (rs1799971) Genotypes Using the Modified Pasero Opioid-induced Sedation Scale (POSS). *Data expressed as frequency (%). **Statistical analyses employed Chi-square Test or Fisher's Exact Test where appropriate. Statistical significance set at $p \leq 0.05$.

Tramadol induced Sedation							
	OPRM1(rs1799971)			**P value****	**AA vs AG****	**AA vs GG****	**AG vs GG****
Modified Pasero Opioid-induced Sedation Scale	**AA***	**AG***	**GG***				
At 1 hour post operatively							
Awake(n=16)	7 (14.6%)	1 (2.4%)	8 (72.7%)	0.005	0.658	< 0.001	0.022
Easily Awakened by Verbal Command (n=57)	32 (66.7%)	23 (56.1%)	2 (18.3%)				
Difficulty in staying awake (n=27)	9 (18.7%)	17 (41.5%)	1 (9.0%)				
No awakening(n=0)	0	0	0				
At 3 hours post operatively							
Awake (n=67)	31 (64.6%)	29 (70.7%)	7 (63.6%)	0.072	0.996	0.031	0.053
Easily Awakened by Verbal Command(n=33)	17 (35.4%)	12 (29.3%)	4 (36.4%)				
At 6 hours post operatively							
Awake(n=87)	42 (87.5%)	35 (85.4%)	10 (90.9%)	< 0.001	0.791	< 0.001	< 0.001
Easily Awakened by Verbal Command (n=13)	6 (12.5%)	6(14.6%)	1 (9.1%)				
At 12 hours post operatively							
Awake(n=88)	43 (89.6%)	38 (92.7%)	7 (63.6%)	0.561	0.610	0.030	0.012
Easily Awakened by Verbal Command(n=12)	5 (10.4%)	3 (7.3%)	4 (36.4%)				
At 24 hours post operatively							
Awake(n=99)	47 (97.9%)	41 (100.0%)	11 (100.0%)	0.579	0.247	0.592	0.923
Easily Awakened by Verbal Command(n=1)	1 (2.1%)	0 (0%)	0 (0%)				

Table 6: Incidence of Other Tramadol Side Effects across OPRM1 (rs1799971) Genotypes. *Data expressed as frequency (%). **Statistical analyses employed Chi-square Test or Fisher's Exact Test where appropriate. Statistical significance set at $p \leq 0.05$.

Other Side Effect of tramadol							
	OPRM1(rs179971)			**P Value****	**AA vs AG****	**AA vs GG****	**AG vs GG****
Other Side Effects	**AA***	**AG***	**GG***				
At 1 hour post operatively							
No side effects (n=70)	27 (56.2%)	33 (80.5%)	10 (90.9%)	0.237	0.036	0.360	0.429
Dizziness (n=16)	12 (25.0%)	4 (9.8%)	0 (0%)				
Headache (n=11)	8 (16.7%)	2 (4.9%)	1 (9.1%)				
Sweating (n=2)	0 (0%)	2 (4.9%)	0 (0%)				
Dry Mouth (n=1)	1 (2.1%)	0 (0%)	0 (0%)				
At 3 hours post-operatively							
No side effects (n=78)	38(79.2%)	32(78.0%)	8 (72.7%)	0.774	0.825	0.951	0.625
Dizziness (n=2)	1 (2.1%)	1 (2.4%)	0 (0%)				
Headache (n=14)	7 (14.6%)	5 (12.2%)	2 (18.2%)				
Sweating (n=2)	0 (0%)	2 (4.9%)	0 (0%)				
Dry Mouth (n=4)	2 (4.9%)	1 (2.1%)	1 (2.1%)				
At 6 hours post-operatively							
No side effects (n=81)	40 (83.3%)	32 (78.0%)	9 (81.8%)	0.533	0.085	0.956	0.897
Dizziness (n=3)	2 (4.9%)	1 (2.4%)	0 (0%)				
Headache (n=11)	5 (10.4%)	4 (9.8%)	2 (18.2%)				
Sweating (n=2)	0 (0%)	2 (4.9%)	0 (0%)				
Dry Mouth (n=3)	1 (2.1%)	2 (4.9%)	0 (0%)				
At 12 hours post-operatively							
No side effects (n=77)	37 (77.1%)	31 (75.6%)	9 (81.8%)	0.762	0.896	0.962	0.689
Dizziness (n=5)	3 (6.3%)	2 (4.9%)	0 (0%)				
Headache (n=11)	6 (12.5%)	4 (9.8%)	1 (9.1%)				
Sweating (n=2)	0 (0%)	2 (4.9%)	0 (0%)				
Dry Mouth (n=5)	2 (4.9%)	2 (4.9%)	1 (9.1%)				
At 24 hours post-operatively							
No side effects (n=77)	39 (81.3%)	30 (73.2%)	8 (72.7%)	0.834	0.765	0.623	0.098
Dizziness (n=5)	3 (6.3%)	2 (4.9%)	0 (0%)				
Headache (n=12)	5 (10.4%)	5 (12.2%)	2 (18.2%)				
Sweating (n=3)	0 (0%)	2 (4.9%)	1 (9.1%)				
Dry Mouth (n=3)	1 (2.1%)	2 (4.9%)	0 (0%)				

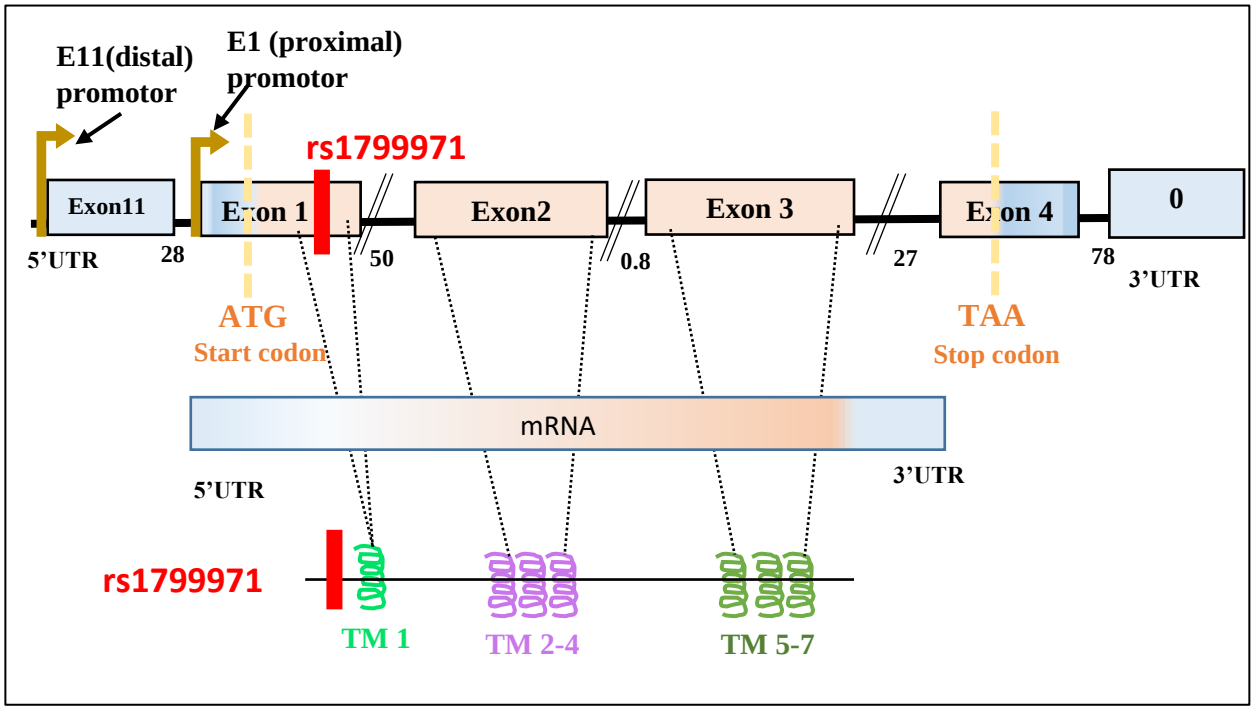

Figure 1: schematic presentation of OPRM1 gene with rs 1799971 SNP.

Results:

For detailed demographic and clinical characteristics of the study participants, please refer to Table 1. The analysis of the OPRM1 gene single nucleotide polymorphism (SNP) rs1799971 revealed the presence of both the A and G alleles in the population, with the A allele being more common at a frequency of 68.5% and G allele had minor allele frequency of 31.5% (Table 2). In terms of genotype distribution, the AA genotype was the most frequent at 48%. The population was within Hardy-Weinberg equilibrium.

This study demonstrated statistically significant differences in postoperative pain perception among individuals with differing genotypes of the OPRM1 gene (rs1799971) as depicted in Table 3. At rest, patients with the GG genotype reported the highest pain scores at each time point, from 1 hour to 24 hours, with p-values ranging from <0.001 to 0.014 when compared to the AA genotype, and from 0.001 to 0.507 when compared to the AG genotype (Table 3). When asked to raise their head, an indicator of pain during movement, patients with the GG genotype again reported the highest pain scores ($p<0.05$ vs AA and AG). Meanwhile, patients with the AG genotype generally reported intermediate pain scores, both at rest and during movement, which were significantly different from the AA and GG groups at various time points. Consequently examining post-operative rescue analgesia among patients with different OPRM1 (rs1 799971) genotypes, we observed initial high needs in GG and AG groups, decreasing over 24 hours. These observations point to a noteworthy trend of increased pain perception and rescue analgesia requirement with the presence of G allele in the OPRM1 gene (rs1799971), revealing a possible allele effect

The examination of tramadol-induced nausea and vomiting in patients with varying OPRM1 (rs1799971) genotypes similarly revealed distinct trends (Table 4). Initially, the AG and GG groups reported less nausea than the AA group, a pattern that persisted at the 3- and 6-hour marks for GG, with statistical significance ($p<0.05$). At 12 and 24 hours post-operatively, the incidence of nausea was relatively low across all genotypes, with no statistically significant differences noted. As for vomiting, the highest incidence was observed among AA genotype patients (27.1%). Correspondingly, anti-emetic administration was significantly more in the AA and AG genotypes when compared to GG genotype during the initial hours post-operatively ($p<0.05$ AA vs AG & GG). The evaluation of tramadol-induced sedation using the Modified Pasero Opioid-induced Sedation Scale (MPOSS), in relation to OPRM1(rs1799971) genotypes revealed significant differences in

sedation states at various post-operative times as shown in Table 5. At the 1-hour mark, the GG genotype patients were mostly awake (72.7%), a significant difference compared to the other genotypes ($p < 0.001$). This pattern continued at 6 hours and 12 hours with GG genotype patients reported statistically significantly ($p < 0.05$) less sedation when compared to other genotypes. By 24 hours post-operation, all patients in the GG group were awake, and no significant differences were noted ($p = 0.923$). Upon examining the tramadol side effects in relation to the OPRM1 (rs179971) gene variants, distinct trends become evident as demonstrated in table 6. In the immediate 1-hour postoperative period, patients with the AG and GG genotypes exhibited a higher proportion of no side effects (80.5% and 90.9%, respectively) compared to the AA genotype (56.2%). Dizziness was more frequently observed in patients with the AA genotype (25.0%), compared to the AG (9.8%) and GG (0%) genotypes. In the subsequent 3-hour, 6-hour, and 12-hour postoperative intervals, the majority of patients across all genotypes reported no side effects, with no significant difference among the genotypes. Sweating was reported only in the AG genotype, while dry mouth was minimal across all genotypes (Table 6).

Discussion:

Postoperative pain is a universal dilemma in healthcare that impairs patients' recovery, and provoke chronic complications. This calls for proficient postoperative pain control by tramadol with comparable efficacy to morphine and a more tolerable side effect profile[16]. However, genetics like SNP rs1799971in OPRM1 gene can significantly impact responses to tramadol, calling for it exploration in the Pakistani subset of population for personalized pain management to maximize efficacy and minimize side effects [17].

The minor allele frequency of rs1799971 was 31.5% in the studied Pakistani cohort, noticeably higher than the global average but closet to the frequencies reported for the East Asian (G=0.3929) and South Asian (G=0.418) populations, as provided by the 1000 Genomes project [9]. Moving onto genotypes, the population exhibited three combinations of the A and G alleles, namely AA, AG, and GG, with the GG genotype in a mere 11% of the population. The differences in MAF could be due to varying degrees of genetic admixture and selection pressures in these populations, underlining the intricacies of genetic variation.

The study results illustrated that the individual with the GG genotype experienced higher pain levels at rest and during movement, indicating a lessened effect of tramadol. These results were supported by previous researches as an analysis carried out Zhang Xueying and his fellows on more than 500 published studies till 2018 concluded that rs1799971 polymorphism required higher opioid dose for pain relief (MD: 0.17; 95% confidence interval [CI]: [0.12, 0.22]; $P < 0.001$) and displayed less nausea risk difference (RD): −0.04; 95% CI: [−0.06, −0.01]) and hence were associated with more adverse effects [18]. Similar conclusion were drawn by Heba Khalil and colleagues as they proved rs1799971 SNP to higher pain scores and more opioid consumption in post-operative period (β=0.46, 95%CI -0.008to - 0.085) [19]. The diminished effects of tramadol might result from the influence of the rs1799971 SNP on N-glycosylation sites of MOR that are GPCRs. These sites play a pivotal role in protein folding, stability, and function, impacting receptor trafficking, ligand-binding affinity, and receptor dimerization [20-21].

However in contrast to this study results, Miriam Saiz-Rodríguez and colleagues demonstrated that 'G' variant resulted in statistically significant ($p>0.05$) superior analgesic response to tramadol in their study population [22]. Likewise, similar observation was made by Eleonora Pettini and colleagues [23]. In their study, Eleonora Pettini and colleagues found no significant differences in the administration of analgesic "rescue" doses or in the incidence of moderate/severe post-operative pain (VAS > 3) between two groups across various timeframes post-operation. Specifically, the effect size, denoted as Proportion Difference (PD), and its accompanying Confidence Interval (CI) for VAS > 3 in the first 24 hours was -0.08 with a CI of -0.34 to 0.2 [23]. This discrepancy in results may be due to a combination of methodological, genetic, environmental, and statistical factors.

Tramadol-induced opioid nausea and vomiting results from its activation of mu and delta opioid receptors in the CTZ, serotonin reuptake inhibition affecting 5-HT3 receptors, enhanced vestibular sensitivity, and impairment of gastrointestinal motility [8]. Given these mechanisms, rs1799971 SNP emerges as a significant research interest. This polymorphism, linked to decrease tramadol affinity and signaling, might lead to reduced pro-emetic pathway activation in the CTZ. Moreover, reduced MOR expression in the gut may offer an anti-emetic effect in the 'G' variant [8]. This study data confirms the G allele's protective role against tramadol-induced nausea and vomiting, with the GG genotype showing the least OINV.

The reduced affinity of the MOR receptor for tramadol in rs1799971 carriers also dampens its inhibitory impact on NMDA receptors, leading to decrease neural excitability, a primary contributor to opioid sedation.8 Consequently, G allele carriers may exhibit resistance to tramadol's sedative effects as evident from the present study data. In terms of other adverse effects of tramadol, such as dizziness, headache, sweating, and dry mouth were also observed, though to a lesser degree in GG genotype. Dizziness was mostly confined to the AA genotype. Other studies have validated our results as they demonstrate that individuals with the wild-type genotype of the OPRM1 rs1799971 allele (A) frequently experience adverse effects, particularly gastrointestinal disturbances and nausea, more frequently than those who carry the polymorphic version (G) of this allele [24-25]. Mureil and fellow concluded that total number of opoiod induced adverse eventes were higher in AA genotyped carriers who reported almost two more AEs than AG/GG patients (7[5-11] vs 5 [3-9],P= 0.046). Additionally, nausea was notably more prevalent in the AA group (P= 0.015) [24]. Sia and colleagues demonstrated that the AA group exhibited the greatest occurrence of nausea, with 26 out of 272 individuals (9.6%; P = 0.02), in comparison to the AG group with 13 out of 234 (5.6%) and the GG group with 1 out of 82 (1.2%) [25]. Consistent with our study results, Kolesnikov and fellow scientists proved that during all postoperative observation intervals, patients with heterozygous genotypes for OPRM1 rs1799971 exhibited significantly ($p<0.05$) reduced scores for both nausea and sedation. Furthermore, only a minority (18% or 2 patients) from this group necessitated anti-nausea intervention [26]. Incidence of purities had also been documented to be lower in GG genotype [23].

In summary, this study thoroughly evaluated the impact of rs1799971 polymorphism on tramadol efficacy and safety profile that paves way for personalized postoperative pain management.

Although this study is first to elucidate the response of the OPRM1 receptor to tramadol within Pakistani population but it has its limitations. Our study limitation includes results generalizability due to relative homogeneity of our sample and focus on tramadol's efficacy, not necessarily translating to other opioids. Additionally, we focused on one SNP of the OPRM1 gene leaving room for future research to delve into a broader genetic analysis. It would also be of interest to determine whether these findings hold true in chronic pain settings or different surgical procedures.

Conclusion:

This study findings underscore a significant association between the OPRM1 gene variation rs1799971 and tramadol efficacy, with individuals possessing the GG genotype experiencing poor tramadol analgesia as evident by higher pain scores both at rest and during movement. Furthermore, the presence of the G allele in the OPRM1 gene (rs1799971) seems to confer protection against tramadol-induced nausea, vomiting, and sedation, pointing to its potential role in predicting opioid response and side effects. These findings represent a significant step towards developing more individualized, effective, and safer pain management protocols. This is a pivotal stride towards the practice of personalized medicine, particularly in regions like Pakistan, where this field is still in its early stages.

Ethical Consideration: The research protocols were reviewed and approved by the Ethical Review Committee (ERC) of Riphah International University (Riphah/IRC/20/103), and Institutional Review Board (IRB) of Nawaz Sharif Medical College (dated 11/07/2021),

Patient Consent: All participating individuals provided their written consent, acknowledging their understanding and agreement for their participation in study and data to be used for publication

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Authors' contribution:
Ammara Khan: data collection, data analysis, interpretation of the results, manuscript drafting
Akbar Waheed: study design formulation, study administration and supervision
Ayesha Afzal &Ajmal Afzal: manuscript drafting
Zahid Azam Chaudhry: statistical analysis
Syed Ihtisham Haider: manuscript revision
Shafia Arshad: critical review and manuscript revision

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