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CLINICAL RESEARCH

The Efficacy of Preemptive Pregabalin Administration on Pre- and Postoperative Anxiety and Postoperative Analgesia in Arthroscopic Shoulder Surgery: A Prospective, Randomized Double-Blind, Placebo-Controlled Clinical Study

Artroskopik Omuz Cerrahisinde Preemptif Pregabalin Yönetiminin Postoperatif ve Postoperatif Anksiyete ve Postoperatif Analjezi Üzerine Etkinliği: Prospektif, Randomize, Çift Kör, Plasebo Kontrollü Bir Klinik Çalışma

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ABSTRACT

Aim: Few studies have investigated pregabalin use as preemptive analgesia in the management of acute pain and anxiety following arthroscopic shoulder surgery. We hypothesized that the preemptive use of oral pregabalin might decrease pain and postoperative anxiety on arthroscopic shoulder surgery pain during the initial 48 hours.
Patients and methods: In this prospective, randomized, double-blind, placebo-controlled study, 65 eligible patients were randomly divided into two groups, the preemptive pregabalin 150 mg (group P) and the control group (group C). The primary outcomes were pain levels measured both rest and during active motion based on a visual analog scale (VAS). Secondary outcomes were the level of anxiety (STAI-S and STAI-T), patient satisfaction, and complications.
Results: Group P showed lower pain ($p < 0.001$), lower opioid consumption ($p < 0.001$), longer time to first requirement of analgesia ($p < 0.001$), and lower rescue analgesic dose ($p < 0.001$) than the control group at postoperative 48 h follow-up. Also, group P showed less preoperative and postoperative anxiety ($p < 0.001$) and greater patient satisfaction ($p < 0.001$) than group C. The rate of postoperative adverse effects was similar between the groups ($p > 0.05$).
Conclusion: The preemptive use of oral pregabalin received 150 mg daily for 2 days before surgery provided postoperative analgesia in both rest and active motion, and improved preoperative and postoperative anxiety levels and greater patient satisfaction in patients undergoing arthroscopic surgery.

Keywords: analgesia; anxiety; preemptive; pregabalin; orthopedic surgery

ÖZ

Arka plan: Artroskopik omuz cerrahisini takiben akut ağrı ve anksiyete tedavisinde pregabalinin preemptif analjezi olarak kullanımını araştıran az sayıda çalışma bulunmaktadır.
Amaç: Bu çalışmada, artroskopik omuz cerrahisinde oral pregabalinin preemptif kullanımının postoperatif ilk 48 saat boyunca pre-postoperatif anksiyeteyi ve postoperatif ağrıyı azaltabileceğini hipotezini kurduk.
Hastalar ve yöntemler: Bu prospektif, randomize, çift kör, plasebo kontrollü çalışmada, 65 uygun hasta rastgele iki gruba, preemptif pregabalin 150 mg (grup P) ve kontrol grubu (grup C) olarak ayrıldı. Birincil sonuçlar, görsel analog skalaya (VAS) dayalı olarak hem dinlenme hem de aktif hareket sırasında ölçülen ağrı seviyeleriydi. İkincil sonuçlar anksiyete düzeyi (STAI-S ve STAI-T), hasta memnuniyeti ve komplikasyonlardı.
Bulgular: Grup P, kontrol grubuna göre daha düşük ağrı ($p < 0.001$), daha az opioid tüketimi ($p < 0.001$), ilk analjezi ihtiyacına kadar daha uzun süre ($p < 0.001$) ve daha düşük kurtarma analjezik dozu ($p < 0.001$) gösterdi. Ayrıca grup P, grup C'ye göre daha az preoperatif ve postoperatif anksiyete ($p < 0.001$) ve daha fazla hasta memnuniyeti ($p < 0.001$) gösterdi. Postoperatif yan etkilerin oranı gruplar arasında benzerdi ($p > 0.05$).
Sonuç: Ameliyattan 2 gün önce günde 150 mg oral pregabalinin preemptif kullanımı, artroskopik cerrahi geçiren hastalarda hem istirahat hem de aktif harekette postoperatif analjezi sağladı ve preoperatif ve postoperatif anksiyete düzeylerini iyileştirdi ve hasta memnuniyetini artırdı.

Anahtar Kelimeler: analjezi; anksiyete; preemptif; pregabalin; ortopedik cerrahi

Introduction

Postoperative pain control after arthroscopic shoulder surgery optimizes postoperative rehabilitation, reduces anxiety, and can increase patient satisfaction and postoperative healing by providing amnesia and sedation (1). In these patients, intra-articular local anesthetic infiltration, regional nerve blocks, patient-controlled analgesia (PCA) with intravenous opioids, and oral nonsteroidal anti-inflammatory and gabapentinoid drugs are available for postoperative pain management (2,3).

Recently, gabapentinoids such as pregabalin have also been shown to have potential in the treatment of acute postoperative pain as part of multimodal analgesia due to their possible opioid consumption-reducing effects and prevention of post-surgical chronic pain (2). Pregabalin is an anticonvulsant drug that reduces calcium entry into the nerve terminals of the central nerve and also reduces levels of substance P, glutamate, and noradrenaline, all of which play a major role in creating a feeling of pain. It is well known that pregabalin reduces central sensitization and hyperalgesia after tissue injury by inhibiting calcium influx in voltage-gated calcium channels (3). These theoretical advantages have led to clinical trials confirming the analgesic effectiveness of oral pregabalin for postoperative pain management in various surgical procedures (4,5).

We hypothesized that the preemptive use of oral pregabalin received 75 mg of twice daily for 2 days before surgery might decrease total morphine consumption and extend the time to the first requirement for analgesia by reducing arthroscopic shoulder surgery pain during the initial 48 hours. The secondary objectives were to assess postoperative anxiety, patient satisfaction, and the adverse effects associated with pregabalin.

Materials and methods

Ethical statement

This prospective, randomized, double-blind, placebo-controlled, single-center study was conducted at Selcuk University, Medical school, Department of Anesthesiology and Reanimation between December 2019 and March 2021. This study was approved by the Clinical Research Ethics Committee of Selcuk University of School of Medicine (Ref No: 25.07.2019.2019/12) and registered on the Clinical Trials website (ClinicalTrials.gov Identifier: NCT: 04675671). Written informed consent was obtained from all participants.

Study design and population

Patients with American Society of Anesthesiologists physical status (ASA-PS) I-II, aged 18-65 years, who were scheduled for elective arthroscopic shoulder surgery (Bankart or rotator cuff repair) under general anesthesia were included in the study. Patients with major neurologic or psychiatric problems, cardiovascular, metabolic, respiratory, renal disease or coagulation abnormalities, body mass index (BMI) over 40 kg/m2, chronic alcohol and substance use, a history of upper gastrointestinal bleeding or perforation, using more than 5 mg/day of oral morphine or equivalent opioids (more than 1 month), and patients who were allergic to the drugs used in the study were excluded.

Study intervention and randomization

Two days before the scheduled surgery, all patients were randomly assigned to the pregabalin group (n=41) and the control group (n=41) by the first anesthesiologist according to a computerized randomization table (https://www.randomizer.org). All patients completed Spielberg's State-Trait Anxiety Inventory (STAI-T) and Spielberger's State Anxiety Scale (STAI-S) questionnaires on their own in the presence of an anesthesiologist before preoperative administration of pregabalin. The STAI-S test was completed again at the postoperative 24th hour. The hospital pharmacy prepared all medications in similar capsules, and all study drugs were administered orally with sips of water by a nurse who was not involved in other processes of this study. Patients in the pregabalin group received 75 mg of pregabalin twice daily for 2 days before surgery. The control group received a placebo capsule at the same point in time. The last doses were received one hour before induction of anesthesia. To ensure the double-blind design in this study, the randomization status of the participants were not be disclosed to the anesthesiologists collecting data, the orthopedic surgeon and the nurse giving the drugs

No other sedative premedication was given to the patients. Anesthesia was induced using propofol 2 mg.kg-1 and remifentanil 0.5-1 µg.kg-1, and tracheal intubation was facilitated with rocuronium 0.6 mg.kg-1. Anesthesia was maintained with a continuous infusion of remifentanil 0.05-0.2 µg.kg-1. min-1 and sevoflurane 2-2.5%. All surgeries were performed by an experienced orthopedic surgeon. At the end of the surgery, sevoflurane and remifentanil were stopped and residual neuromuscular paralysis was antagonized, and extubation was performed when the patient had sufficient respiration. Age, sex, BMI, the subtype of surgery, the time of surgery and anesthesia were recorded.

After the surgery was completed, all patients were transferred to the recovery room. Postoperative analgesia was standardized to morphine in patient-controlled analgesia (PCA; bolus 1 mg, lock-out 5 min, limit 25 mg/4 h). In patients with visual analog scale (VAS) values of less than 5, the PCA lock-out was extended to 10 min. Participants did not routinely receive non-steroidal anti-inflammatory drugs (NSAIDs). However, they were given dexketoprofen (Arveles, UFSA Ilaç, Istanbul Turkey) as a rescue analgesic during the first postoperative 48 hours in the event of VAS values of ≥3. Physical therapy was started 24 hours after surgery. Physical therapy sessions were conducted by the same physiotherapist. Twenty milligrams of IV metoclopramide was given only to patients with nausea and vomiting.

Data collection and outcome

The pre-postoperative pain was measured and evaluated using a VAS (0 = no pain to 10 = worst pain). This parameter was measured from the pre-postoperative pain on the first day until the second postoperative day (in the post-anesthesia care unit (PACU), 1, 3, 6, 12, 24, 36, and 48-hour intervals). VAS values were measured at 24 and 48 hours during both rest and active motion physical therapy because the shoulder is not moved within the first 24 hours. In addition, total morphine consumption, the time to the first requirement for analgesia, patient satisfaction

(Insufficient; 1, Satisfactory; 2, Good; 3, Excellent; 4) and the number of rescue analgesias required were recorded.

To measure anxiety levels, STAI-T and STAI-S questionnaires were used (6). Both STAI-T and STAI-S consist of 20 items with four-point Likert scales (Not at all, Somewhat, Moderately, Very much). The values obtained in each scale range between 20-80 points; 20-40 is defined as low-level anxiety, 41-60 as moderate anxiety, and 61-80 high-level anxiety. The Turkish form was validated by Le Compte and Öner (7) and a validity-reliability study was guided. The internal consistency and reliability of the Turkish form was between 0.94 and 0.96 for the STAI-S and between 0.83 and 0.87 for the STAI-T the Kuder-Richardson alpha reliability. In the present study, McDonald's w coefficient was 0.942 for the STAI-S and McDonald's w coefficient was 0.888 for STAI-T scale.

The adverse effects of pregabalin including nausea, sleepiness, headache, dizziness, and blurred vision were also evaluated during the postoperative 48-hour period.

Sample size

The primary outcome of the study was VAS scores at 48 postoperative hours. In Ahn et al.'s study (4), the mean VAS score at 48 hours was 4.5 ± 1.5 in the control group and 3 ± 1.7 in the pregabalin group. Accordingly, we determined that 37 patients would be required in each group to show this difference with a 5% significance level and power of 95% using the two-sided independent samples t-test. Allowing for a 10% drop-out rate during the study period, 41 patients were enrolled in each group. Power analysis was performed using the "pwr" package in R 3.6.0 (https://www.r-project.org).

Statistical analysis

All statistical analysis was performed using the R Version 3.6.0 software (www.r-project.org). The Shapiro-Wilk test and Q-Q plots were used to check the normality of the data, and Levene's test was used to evaluate the homogeneity of variances of the groups. The demographic and clinical characteristics of the participants are demostraded using descriptive statistics. Numerical variables are expressed as mean ± standard deviation or median (IQR, interquartile range: 25th percentiles – 75th percentiles), and categorical variables are described as counts (n) and percentages (%). The independent samples t-test, Chi-square test with Yates's continuity correction, and exact test with Monte Carlo simulation when expected counts in cells were greater than 20%, were used to compare the study groups according to the demographic and clinical characteristics. The Mann-Whitney U test was used to examine the difference of the groups according to VAS scores, both preoperative and postoperative. The exact test with Monte Carlo simulation, Fisher's exact test, and Chi-square test with Yates's continuity correction were used to determine the association between groups and adverse effects. The exact test with Monte Carlo simulation, the Mann-Whitney U test and the independent samples t-tests were used to examine the difference of the groups according to the delivery and demand of drugs on PCA, time of first analgesia, rescue analgesia, total morphine consumption, pre and post-operative STAI-S score, and preoperative STAI-T score. Moreover, the paired-samples t-test was used to compare preoperative STAI-S and postoperative STAI-S. A Wilcoxon test was conducted to determine whether there was a statistically significant difference between preoperative and postoperative STAI-S scores. All graphs are presented with mean ± standard deviation. A p-value of less than .05 was considered statistically significant.

Results

A total of 65 of 82 patients assigned for the study were included in this study. Seventeen patients from both groups (due to not meeting inclusion criteria, declining to participate, data loss, and admission to the intensive care unit) were excluded. The remaining eligible patients are presented in the Consolidated Reporting Standards (CONSORT) flowchart (Fig 1). The demographic and clinical characteristics of the patients are presented in Table 1. There were no significant differences between the groups including age, sex, BMI, ASA-PS, anesthesia, and surgical duration and surgery type ($p > 0.05$).

Preoperative and postoperative pain was evaluated using the VAS pain scale, shown in Figure 2. Although the postoperative VAS pain scores in the PACU at the 1st, 3rd, 6th, 12th, and 36th hours were statistically significantly lower in group P than in group C ($p < 0.001$, $p = 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.002$, and $p < 0.001$, respectively), there was no difference between the groups in terms of the preoperative measurement and postoperative measurement at 48 hours ($p = 0.118$ and $p = 0.306$, respectively). However, there was a difference between the groups' VAS pain scores with activity regarding the preoperative measurement and the postoperative 24th, 36th, and 48th hours ($p < 0.001$, $p < 0.001$, $p < 0.001$, and $p = 0.003$, respectively) (Fig 2).

The time to the first analgesia was significantly longer in group P than in group C (240 (180-308.75) vs. 97.5 (70-121.25) min; median (IQR); $p < 0.001$). Also, the rescue analgesic dose was significantly lower in group P than in group C (0 (0-1) vs. 2.5 (2-3); $p < 0.001$). The total morphine consumption was significantly lower in group P than in group C (12 (9.25-14) vs. 23 (14.25-30.75) mg; median (IQR); $p < 0.001$). Patient satisfaction was better in group P than in group C (3 (3-3.75) vs. 2 (2-3); $P < 0.001$) (Table 2).

The median preoperative STAI-T score in the pregabalin group was no difference between the groups (41 (37 – 47) vs. 44 (38 – 50); median (IQR); $p = 0.563$). There was no significant difference in the median preoperative STAI-S score assessments in between group P and C (46 (35.75 – 54) vs. 48 (42 – 54); median (IQR); $p = 0.176$). But, the median postoperative STAI-S score in group P was significantly lower than in group C (40 (34-45) vs. 46.5 (42.5-51.75); median (IQR); $p = 0.001$) (Table 3).

The group P showed significantly difference within the groups in terms of pre-postoperative STAI-S (p = 0.025) , but the group C showed no significantly difference within the groups in terms of pre postoperative STAI-S (p = 0.526) (Table 3).

In the postoperative period, the most common adverse effects were dizziness and headache, which were seen in both groups (p = 0.579 and p = 0.729, respectively). In this study, although other adverse effects such as pruritus, nausea, and vomiting were mostly reported among patients in group C, the difference was not statistically significant. Other adverse effects were sleepiness and blurred vision (Table 4)

Table 1. Demographic and clinical characteristics of the study groups

Parameters	Pregabalin (*n*=32)	Control (*n*=33)	
Age (years)	45.37 ± 13.54 (18 – 65)	45.93 ± 14.33 (18 – 65)	.875[a]
Gender (M/F)	24 (75.0) / 8 (25.0)	18 (54.5) / 15 (45.4)	.104[b]
BMI (kg/m^2)	26.87 ± 3.51	25.92 ± 3.17	.270[a]
ASA-PS (I/II)	19 (59.3) / 13 (40.6)	17 (51.5) / 16 (48.4)	.604[b]
Anesthesia time (min.)	95.67 ± 11.35 (80 – 120)	91.60 ± 9.34 (75 – 110)	.167[a]
Surgical time (min.)	73 ± 10.46 (54 – 100)	71.50 ± 9.02 (55 – 90)	.401[a]
Operation type (Bankard/RCR)	6 (18.7) / 26 (81.2)	8 (24.2) / 25 (75.7)	.794[b]

M/F; Male/Female, BMI; Body Mass Index, ASA-PS; American Society of Anesthesiologists physical status, RCR; Rotator Cuff Repair
Data were expressed as mean ± standard deviation (range: min – max), or counts (*n*) and percentages (%)
[a] Independent samples *t*-test
[b] χ^2 test with Yates continuity correction

Table 2. Postoperative Results

Parameters	Pregabalin (*n*=32)	Control (*n*=33)	
Time of analgesia for the first time (min.)	240 (180 – 308.75)	97.5 (70 – 121.25)	**< .001**[a]
Rescue analgesia dose	0 (0 –1)	2.5 (2 – 3)	**< .001**[a]
Total morphine consumption (ml)	12 (9.25 – 14)	23 (14.25 – 30.75)	**< .001**[a]
Patient Satisfaction	3 (3 – 3.75)	2 (2 – 3)	**< .001**[a]

Data were expressed as median (IQR: 25th percentile – 75th percentile)
Bold values indicated that statistically significant difference between groups.
a Mann-Whitney U test

Table 3. The pre-postoperative STAI-S and preoperative STAI-T score assessments in the groups.

Parameters	Pregabalin (*n*=32)	Control (*n*=33)	*p*-value[1]
Preoperative STAI-T	41 (37 – 47)	44 (38 – 50)	.563
Preoperative STAI-S	46 (35.75 – 54)	48 (42 – 54)	.176
Postoperative STAI-S	41 (34 – 45.25)	48 (45 – 52)	**.001**
***p*-value[2]**	**.025**	.526	

Spielberg's Trait Anxiety Inventory (STAI-T), Spielberger's State Anxiety Scale (STAI-S)
Data were expressed as median (IQR: 25th percentile – 75th percentile)
Bold values indicated that statistically significant difference between groups.
p-value[1] was calculated using Mann Whitney U test
p-value[2] was calculated using Wilcoxon test (Preoperative STAI-S vs. postoperative STAI-S)

Table 4. Adverse events

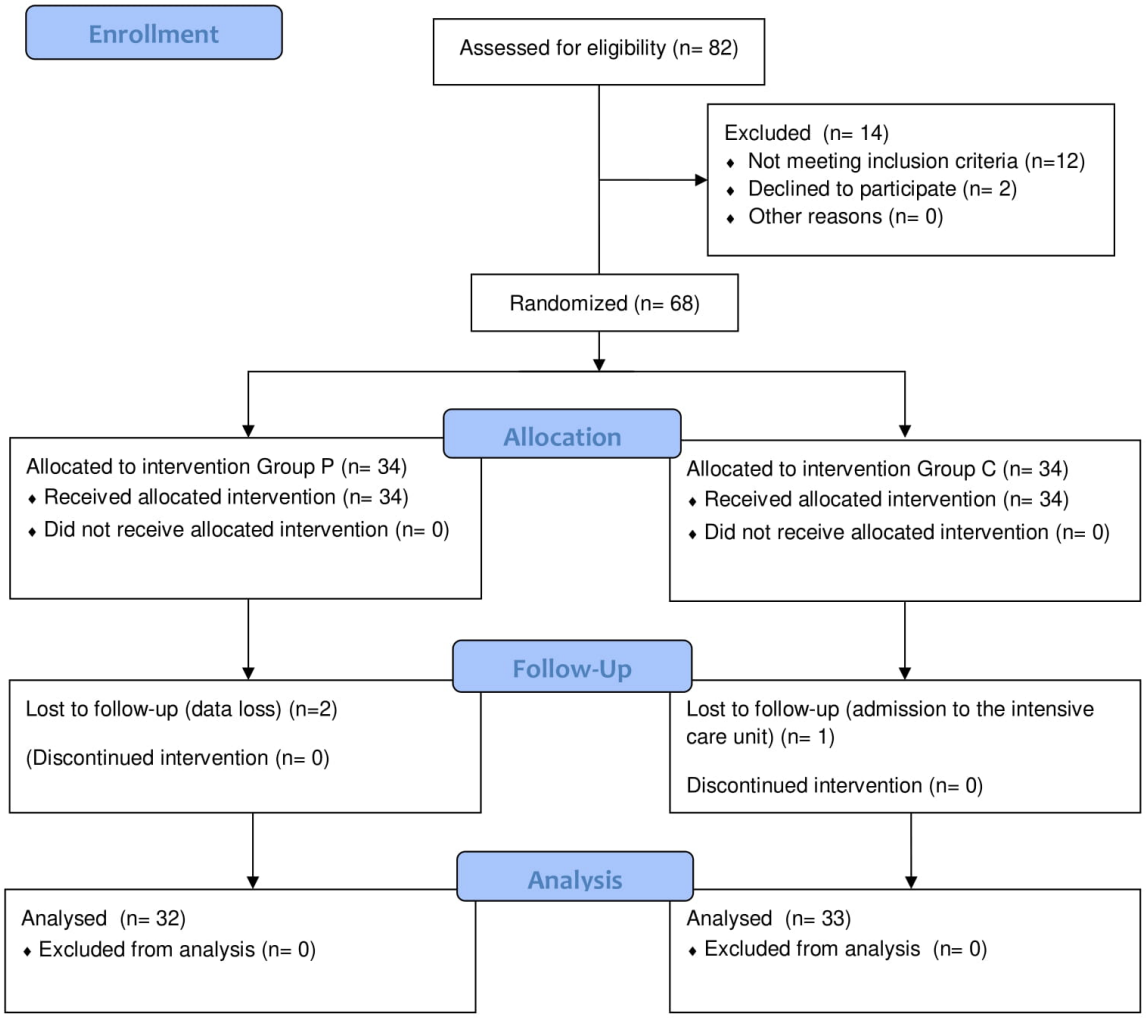
Events	Pregabalin (*n*=32)	Control (*n*=33)	*p*-value
Nausea	0 (0)	1(3.3)	.628[a]
Sleepiness	3 (9.3)	1 (3.3)	.612[b]
Headache	6 (18.7)	4 (13.3)	.729[c]
Dizziness	11 (34.3)	8 (26.7)	.579[c]
Blurred vision	3 (9.3)	1 (3.3)	.612[b]

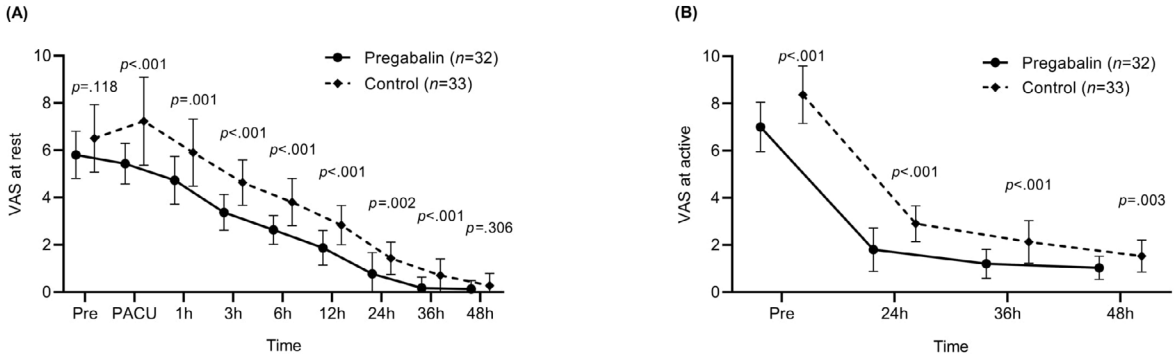
Data were described as numbers (*n*) and percentages (%)
[a] Exact test with Monte-carlo simulation
[b] Fisher exact test
[c] χ^2 test with Yates continuity correction

Discussion

This study was designed to evaluate the efficacy of preemptive pregabalin administration in adult patients undergoing arthroscopic shoulder surgery. Significant beneficial effects of using oral pregabalin received 150mg of daily for 2 days before surgery on n both rest and active motion pain intensity and anxiety were observed in the postoperative periods. In addition, receiving pregabalin preoperatively the time to first analgesia, the total morphine consumption, rescue analgesia and patient satisfaction also showed better results in the postoperative periods. Besides the beneficial effects mentioned above, pregabalin was not accompanied by an increase in adverse effects.

In addition to patient perceptions of discomfort, arthroscopic shoulder surgery is associated with severe post-operative pain that hinders healing and rehabilitation of the shoulder (8). The use of oral pregabalin for postoperative pain in orthopedics surgeries has led to clinical studies confirming its analgesic efficacy. In a study by Ahn et al., a preoperative dose of 150 mg pregabalin was benefecial in controlling pain intensity using a lower amount of opioids over 48 hours after arthroscopic shoulder surgery (4). Mishriky et al. shown that pregabalin was provided better postoperative analgesia compared with placebo at rest (5). Eskandar et al. demostrated that the VAS scores of the pregabalin group were significantly lower until postoperative 8 hours than in a control group when 300 mg was administered at 12 hours and 1 hour before surgery (9). The authors noted lower opioid consumption and higher satisfaction scores in the pregabalin group. But, the pregabalin group did not have a statistically significant increase in dizziness. In a similar study by Bang et al., the VAS scores up to the 12th hour postoperatively were significantly lower in the group that received 300 mg of pregabalin 2 hours before the preoperative compared with the placebo group. However, fentanyl consumption in postoperative 24th hour was similar. Although there





was no reduction in opioid use, the pregabalin group reported reduced respiratory distress, nausea, vomiting and urinary retention compared with the control group (10). There are also studies showing the effectiveness of preemptive pregabalin on postoperative pain in various laparoscopic abdominal operations (11-14). In our study, a dose of 150 mg of pregabalin daily for 2 days was used, similar to the literature, and pain scores at both rest and during activity were found to be lower in the pregabalin group compared with the control group for a long period, up to the postoperative 48th hour.

Although modern surgical techniques have developed and become safer, it is known that most patients with medium and large surgery experience preoperative anxiety (15). The effectiveness of pregabalin against general anxiety and its use as an adjuvant in the multimodal treatment of postoperative analgesia has led to recent research. Perioperative use of pregabalin suggests that it may be a attenuates preoperative anxiety, and reduces postoperative pain scores (16). There are many studies in the literature regarding STAI measurements of preoperative anxiety. Kindler et al. found that the mean preoperative STAI value was 39 (17). In the study of Kim et al., the mean STAI-S and STAI-T scores were 43.8 and 42.7, respectively (18). According to Domar et al., the average preoperative anxiety score was 45 on the STAI scale (19). In a recent study of urology patients by Demirkol et al., the authors demonstrated a preoperative mean STAI score of 39.16 ± 0.42 (20). In other studies in Turkey, mean preoperative STAI values ranged from 36 to 42.4 (21,22). In the present study, the mean preoperative STAI-S value was 46 and the postoperative STAI-S value was found as 40 in the group using preemptive pregabalin. The literature provides insufficient studies using pregabalin preoperatively for trait and state anxiety in patients undergoing a surgical procedure that produces pre- and postoperative pain and anxiety, such as arthroscopic shoulder surgery. In our study, the postoperative STAI-S score was significantly lower than in the control group. A total of 300 mg of pregabalin administration for 2 days preoperatively resulted in a significant decrease in anxiety levels.

STAI-S is the anxiety state that can change depending on the stress of a particular moment, whereas STAI-T reflects the personality tendency that affects the total anxiety of the person (23). For this reason, both STAI scales were used in our study, considering that the personality disposition of the patients may also affect their current anxiety.

Yucel et al. reported that the improved analgesia and anxiolysis attributable to pregabalin contributed to increased patient satisfaction (24).

In another study conducted by Myles et al. with 10811 patients, it was reported that moderate or severe pain was associated with low patient satisfaction (25). Dexter et al. reported that pain was associated with low patient satisfaction (26). Patient satisfaction levels in the pregabalin group in our study were found to be superior to the control group.

Pregabalin adverse effects are dose-dependent and usually temporary (27). We stated in our study that the main complications were nausea and dizziness. In addition, adverse effects such as headache, sleepiness, and blurred vision were seen. Previous study has been stated that pregabalin reduce nausea-vomiting (5). However, the incidence of these complications was similar between our groups and was consistent with another previous study (28).

This study has some advantages. This is a prospective randomized double-blind, plasebo-controlled clinical study, both pain levels and anxiety assessments were performed and evaluated regularly within the first 48 hours postoperatively. However, this study has some limitations. First, the study was part of a single-center self-answer questionnaire. Secondly, the sample size was small. Therefore, study results cannot be generalized to the general population. Thirdly, preoperative side effects were not followed up in patients receiving pregabalin. However, there were no preoperative adverse effects that were severe enough to cause exclusion from the study.Finally, the pregabalin dose was used preoperatively, it was not continued postoperatively.

Conclusion

In patients undergoing arthroscopic shoulder surgery, use of oral pregabalin received 150 mg of daily for 2 days before surgery reduced postoperative pain both at rest and with active movement. As a secondary result, it has been shown to improve anxiety and provide better patient satisfaction. Pregabalin may be a useful addition to a multimodal postoperative pain management strategy in selected patients.

Compliance with Ethics Guidelines

This study was approved by the Clinical Research Ethics Committee of Selcuk University of School of Medicine (Ref No: 25.07.2019.2019/12) and registered on the Clinical Trials website (ClinicalTrials.gov Identifier: NCT 04675671). All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Information Availability

The information sets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Disclosures

None

Informed Consent

Informed consent was obtained from all individual participants included in the article.

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Conflict of interest

No author has a conflict of interest that relates to the content discussed in this article.

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