



# Effectiveness of Different Local Anesthesia Application Methods in Postoperative Pain Control in Laparoscopic Appendectomies: A Randomized Controlled Trial

✉ Anıl Ergin<sup>1</sup>, ✉ Mehmet Mahir Fersahoğlu<sup>1</sup>, ✉ Hüseyin Çiyiltepe<sup>1</sup>, ✉ Aziz Bora Karip<sup>2</sup>, ✉ Ahmet Yalın İşcan<sup>3</sup>,  
✉ Nuriye Esen Bulut<sup>1</sup>, ✉ Yasin Güneş<sup>1</sup>, ✉ Yıldız Yiğit Kuplay<sup>4</sup>, ✉ Ahmet Çakmak<sup>1</sup>, ✉ Ali Cihan Bilgili<sup>1</sup>,  
✉ İksan Taşdelen<sup>1</sup>, ✉ Kemal Memişoğlu<sup>1</sup>

<sup>1</sup>Fatih Sultan Mehmet Training and Research Hospital, Clinic of General Surgery, İstanbul, Turkey

<sup>2</sup>İstanbul Oncology Hospital, Clinic of General Surgery, İstanbul, Turkey

<sup>3</sup>İstanbul University, İstanbul Faculty of Medicine, Department of General Surgery, İstanbul, Turkey

<sup>4</sup>Fatih Sultan Mehmet Training and Research Hospital, Clinic of Anesthesiology and Reanimation, İstanbul, Turkey

## ABSTRACT

**Aim:** Postoperative pain management has long been clinically challenging. Several methods have been attempted to prevent postoperative pain. In this study, we compared the effects of local anesthetic infusion to the incision site (LAIS), transversus abdominis plane block (TAPB), and intraperitoneal local anesthetic administration (IPLA) methods on postoperative pain and patient satisfaction in acute appendicitis cases who underwent laparoscopic appendectomy.

**Method:** Overall, 160 patients who underwent laparoscopic appendectomy for uncomplicated acute appendicitis between September 2018 and September 2019 were included. Patients were divided into four groups: Control group, LAIS group, TAPB group, and IPLA group. All patients were assessed in terms of visual analog scale (VAS) scores for pain, hemodynamic parameters, and patient satisfaction at postoperative 1, 2, 4, 6, 12, and 24 hours.

**Results:** VAS scores at postoperative 1, 2, 4, 6, 12, and 24 h were higher in the control group than in the LAIS, TAPB, and IPLA groups. The VAS scores of the IPLA group were significantly higher than the LAIS and TAPB groups. No significant difference was observed between the LAIS and TAPB groups. The rate of additional analgesic dose administration in the control group (97.5%) was significantly higher than in the LAIS (17.5%), TAPB (7.5%), and IPLA groups (72.5%) ( $p_1 < 0.001$ ;  $p_2 < 0.001$ ;  $p_3 = 0.005$ ;  $p < 0.05$ ). Further, the rate of additional analgesic dose administration in the IPLA group (72.5%) was significantly higher than in the LAIS (17.5%) and TAPB (7.5%) groups ( $p < 0.001$ ;  $p < 0.05$ ).

**Conclusion:** All preemptive analgesia methods were more effective in postoperative pain management compared to the control group. Furthermore, TAPB and LAIS methods were better at controlling patient-reported pain than IPLA.

**Keywords:** Laparoscopic appendectomy, preemptive analgesia, transversus abdominis plane block, intraperitoneal anesthesia, local anesthesia, postoperative pain management, pain relief, analgesia

## Introduction

Appendectomy is the most common emergency surgical intervention in general surgery. The risk of acute appendicitis is about 7% in the life of an individual in the US.<sup>1</sup> The causes of postoperative pain after acute appendicitis surgery include surgical incision, peritoneal inflammation, and visceral

peritoneal pain due to infection.<sup>2</sup> Although laparoscopic surgery is less painful than open surgery, laparoscopic interventions are not painless, especially during the early postoperative period.<sup>3-6</sup> Postoperative pain management has long been a clinical challenge for both surgeons and anesthesiologists.



Address for Correspondence: Anıl Ergin, MD,

Fatih Sultan Mehmet Training and Research Hospital, Clinic of General Surgery, İstanbul, Turkey

E-mail: dranilergin@gmail.com ORCID ID: orcid.org/0000-0001-6450-7124

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Preemptive analgesia is the use of drugs that regulate nociceptive activity before the emergence of stimulation that will cause pain, and it has become an important approach in reducing postoperative pain and postoperative use of opioids. The aim of preemptive analgesia is to trigger nociceptive activity with afferent stimulations that play a key role in reducing postoperative pain and preventing over-excitation of the central nervous system.<sup>7,8</sup> Damage to tissues and peripheral nerves cause proinflammatory cytokine release and initiates a local inflammatory process, thereby resulting in over-excitation of the peripheral and central nervous systems.<sup>9</sup> Some studies claim that preemptive analgesia inhibits this proinflammatory process and reduces the need for opioid use by reducing postoperative pain.<sup>10,11</sup> However, there are insufficient studies about the superiority of preemptive analgesic method in laparoscopic appendectomy to provide definitive evidence.

Preemptive analgesia methods include local anesthetic infusion to incision site (LAIS), transversus abdominis plane block (TAPB), and intraperitoneal local anesthetic administration (IPLA). In this study, we compared the effects of LAIS, TAPB, and IPLA methods on postoperative pain and patient satisfaction in cases of acute appendicitis that underwent laparoscopic appendectomy.

## Materials and Methods

In this double-blind, randomized, controlled study, we included 160 patients aged 16-74 years who underwent laparoscopic appendectomy for acute appendicitis between September 2018 and 2019. These patients were randomized into four groups: Control group that did not undergo any additional procedure, LAIS group, TAPB group, and IPLA group. Approval for this study was obtained from the Ethics Committee Fatih Sultan Mehmet Training and Research Hospital (approval number: 56, date: 13.12.2018).

The number of patients to be included in the study was determined using 80% power and a two-tailed alpha value of 0.05. The following patients were excluded from the study: those with an American Society of Anesthesiologists (ASA) score of >4, body mass index (BMI) of >55 kg/m<sup>2</sup>, who underwent open surgery instead of laparoscopy for any reason, those with complicated (perforated, gangrenous, or phlegmonous) appendicitis, those with >500 cc of bleeding during the operation, those with known local anesthetic allergies, those with chronic analgesic addiction, whose operative time was >120 min, and who did not agree to participate in the study. Informed consent was obtained from all patients.

The local anesthetic method to be used in each individual procedure was written in a sealed envelope and sent to

the operating room. Then the solution to be applied was prepared by the surgical nurse who opened the envelope in the operating room.

All operations were performed by experienced surgeons, each having performed >500 laparoscopic appendectomies. Laparoscopic appendectomy was performed using three trocars with an intra-abdominal pressure of 14 mmHg. The placement of the trocars is shown in Figure 1. Standard anesthesia procedure was performed in all patients; anesthesia induction was performed using 2-3 mg/kg propofol, 2 µg/kg fentanyl, and 0.6 mg/kg rocuronium with Bispectral Index (BIS) of <60. After the patients were intubated, 1.5-2% sevoflurane inhalations were performed to maintain the BIS value between 40 and 60 in 40% oxygen and 60% air. In case of 20% increase in blood pressure and increase in heart rate during the operation, 0.5 mcg/kg fentanyl was administered intravenously and the administered dose of fentanyl was recorded.

In the LAIS group, after endotracheal intubation, patients were administered a total of 20 cc of 0.5% bupivacaine (vial box of 1x20 mL vial of buvasin 0.5% injection solution) solution percutaneously and subcutaneously following suitable skin staining and sterile covering (8 cc of 0.5% bupivacaine for the trocar access points of 10 mm at infraumbilical and left lower quadrant trocar entries, 6 cc of 0.5% bupivacaine for the other trocar access point of 5 mm). In the TAPB group, after endotracheal intubation, patients had a camera trocar inserted to facilitate pneumoperitoneum after appropriate skin staining and sterile covering. The patients were then injected with 20 cc of 0.5% bupivacaine (vial box of 1x20 mL vial of buvasin 0.5% injection solution) including 10 cc to the right and 10 cc to the left side using a needle inserted at the location described in Figure 2 right

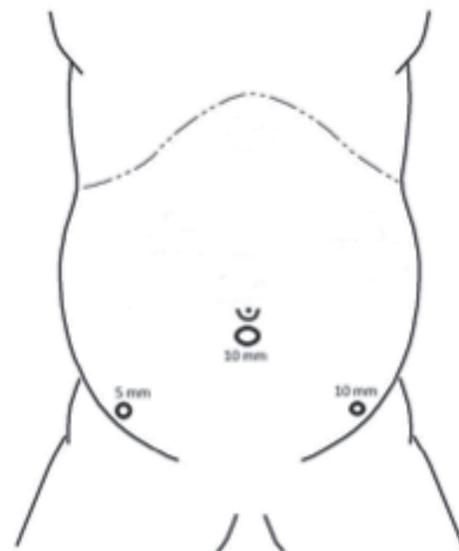


Figure 1. The placement of the trocars in laparoscopic appendectomy

between the transversus abdominis muscle and the internal oblique muscle under direct vision. In the IPLA group, after endotracheal intubation, patients were injected with a total of 20 cc of 0.5% bupivacaine solution into the appendiceal and periappendiceal areas after skin staining and sterile covering followed by pneumoperitoneum (Figure 3). The control group was not given any local anesthetic.

Approximately 30 min before the end of the operation (following removal of the appendix from the abdomen), 1 g of paracetamol and 4 mg of ondansetron were administered intravenously. At the end of the operation, the muscle relaxant effect was antagonized with 0.02 mg/kg atropine and 0.05 mg/kg neostigmine, and then the patients were extubated.

For postoperative pain follow-up, the visual analog scale (VAS) was used for pain assessment. All patients were evaluated in terms of VAS scores, hemodynamic parameters, and patient satisfaction level (5: not satisfied and 1: highly satisfied) at postoperative 1, 2, 4, 6, 12, and 24 hours,

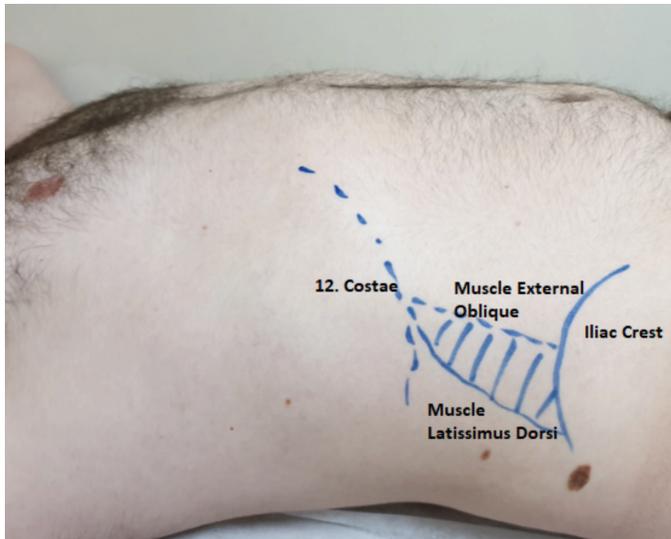


Figure 2. Transversus abdominis plane (TAP) Block application site



Figure 3. Intraperitoneal local anesthetic application around the appendix site

and the results were recorded. Patients with VAS score of  $>4$  were administered 50 mg intravenous tramadol, and those with high pain levels after 30 min were administered 100 mg tramadol. Non-steroidal anti-inflammatory drug (tenoxicam 20 mg) was administered intravenously at postoperative 8 hours routinely to all patients. Thereafter, 25 mg of meperidine was administered intravenously as salvage analgesic to the patients with pain, regardless of any other existing treatment. All analgesics taken, except for routine tenoxicam treatment, were calculated and recorded as additional analgesic dose. The doses of analgesics administered during the postoperative period as well as the hours of administration were recorded.

### Statistical Analysis

SPSS, version 21 was used for statistical analyses (IBM Inc., Armonk, NY, USA). To analyze the study data, the normality of distribution of the parameters was assessed using the Shapiro-Wilk test. Descriptive statistical methods (mean, standard deviation, and frequency) were used to analyze the study data, and One-Way ANOVA was used for intergroup comparisons of the normally-distributed parameters. Kruskal-Wallis test was used to make intergroup comparisons of non-normally-distributed parameters, and Mann-Whitney's U test was used to determine the group that caused the difference. Chi-square test and Fisher-Freeman-Halton test were used to compare the descriptive data. Pearson's correlation analysis was used to analyze the correlation between the normally-distributed parameters, whereas Spearman's rho correlation analysis was used to analyze the correlation between the non-normally-distributed parameters. Significance level was set at  $p < 0.05$ .

### Results

We enrolled 160 cases, including 104 (65%) males and 56 (35%) females. The age of the patients ranged from 17-69 years with a mean age of  $34.74 \pm 13.81$  years. The values of BMI ranged from 20-40  $\text{kg/m}^2$ , with a mean BMI of  $25.45 \pm 3.36 \text{ kg/m}^2$ . There were four groups as follows: IBLA group, 40 patients (25%); TAPB group, 40 patients (25%); IPLA group, 40 patients (25%); and control group, 40 patients (25%).

The operative times ranged from 20-90 min, with mean and median operative times of  $42.25 \pm 11.43$  and 40 minutes, respectively. The length of hospitalization varied between 1 and 7 days, with mean and median lengths of hospitalizations of  $1.60 \pm 0.83$  and 1 day, respectively.

No significant difference was observed between the study groups in terms of age, BMI, ASA score, gender distribution ratios, previous abdominal surgery rates, incidence of

comorbid disease, operative time, and incidences of peroperative and postoperative complications ( $p>0.05$ ).

The length of hospitalization in the LAIS group was significantly lower than in the IPLA and control groups [ $p_1=0.023$  (IPLA group);  $p_2<0.001$  (control group);  $p<0.05$ ] and that in the TAPB group was significantly lower than in the control group ( $p=0.008$ ) (Figure 4).

The rate of additional analgesic dose administration in the control group (97.5%) was significantly higher than in the LAIS (17.5%), TAPB (7.5%), and IPLA groups (72.5%) ( $p_1<0.001$ ;  $p_2<0.001$ ;  $p_3=0.005$ , respectively). Further, the rate of additional analgesic dose administration in the IPLA group (72.5%) was significantly higher than in the LAIS (17.5%) and TAPB (7.5%) groups (both  $p<0.001$ ). No significant difference was observed between the LAIS and TAPB groups in terms of the additional analgesic dose administered ( $p>0.05$ ; Table 1) (Figure 5).

It was found that all pre-emptive analgesic methods caused significantly less postoperative pain than the control group. VAS values of all measurement hours were significantly higher in the control group than in the LAIS, TAPB, and IPLA groups, whereas the patient's satisfaction level was lower in the control group than in the three study groups ( $p<0.001$ ). VAS values of all measurement hours were significantly higher in the IPLA group than in the LAIS and TAPB groups, whereas patient satisfaction level was lower in the IPLA group than in the LAIS and TAPB groups (both  $p<0.001$ ). No significant difference was observed between the LAIS and TAPB groups in terms of VAS values of all measurement hours and patient satisfaction levels ( $p>0.05$ ; Table 2) (Figure 6, 7).

Postoperative complications were observed in two patients in the LAIS group, three patients in the TAPB group, and one patient in the IPLA group. Abscess occurred in the abdomen in two patients in the LAIS group and regressed with antibiotic treatment without the need for drainage. In

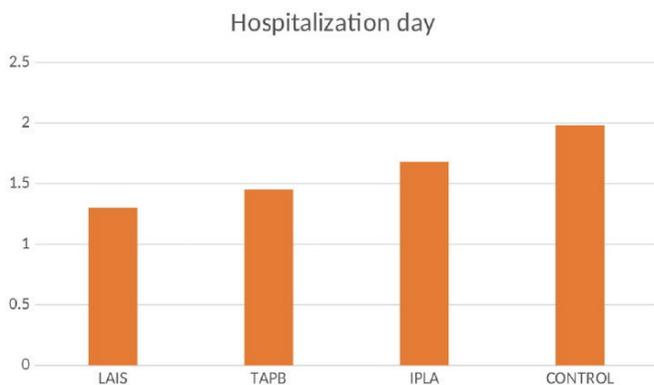
the TAPB group, two patients developed intra-abdominal abscess and one patient developed wound infection. These complications also regressed with antibiotic treatment without any drainage. Wound infection developed in one patient in the IPLA group and regressed with antibiotic treatment. No postoperative complications were detected in the control group.

## Discussion

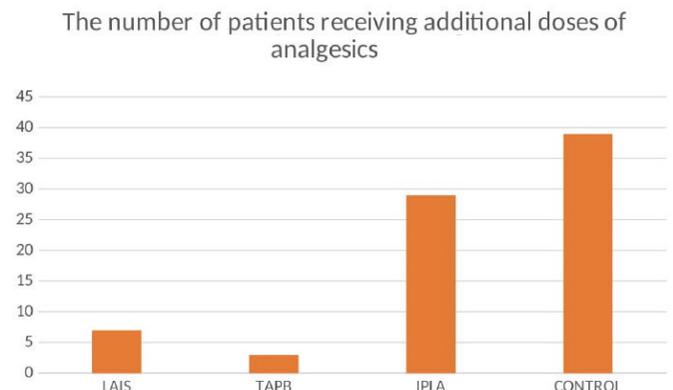
We compared the effects of different intraoperative local anesthetic application methods on postoperative pain and patient satisfaction over the short term in patients who underwent laparoscopic appendectomy for acute appendicitis. All the local anesthesia application methods reduced postoperative pain and increased patient satisfaction. Factors such as age, gender, BMI, ASA score, and operative time did not affect postoperative pain. Thus, we believe that peroperative local anesthetic application methods reduce postoperative pain and increase patient satisfaction.

Laparoscopic appendectomy is one of the most common urgent procedures. There are many studies on the advantages of peroperative local anesthetics in eliminating pain that occurs after this operation.<sup>4,11,12</sup> Ekstein et al.<sup>5</sup> reported that the causes of early pain after laparoscopic surgeries include creating wide peritoneal irritation due to pneumoperitoneum, postoperative intra-abdominal blood accumulation, or diaphragmatic irritation.

In appendectomy, local anesthetic injection at the preincision site reduces postoperative pain scores compared to placebo.<sup>12</sup> Blocking the somatic nerve fibers located between the transversus abdominis muscle and the internal oblique muscle, TAP block is reported to reduce postoperative pain in laparoscopic cholecystectomy and open appendectomy.<sup>13-16</sup> Intraperitoneal local anesthetics before laparoscopic procedures also prevent postoperative pain, reduce stress response and the need for analgesics, and



**Figure 4.** Number of hospitalization days of patients according to the groups



**Figure 5.** Number of patients who received additional analgesic doses according to the groups

extend the elapsed time until first postoperative analgesic administration.<sup>4,11,17</sup> However, there is no clear evidence about the superiority of these methods compared with each other. In this study, we investigated the advantages of these methods over each other. In our study, we found that these applications reduce postoperative pain and the need for additional analgesics after surgery, as well as increasing patient satisfaction. Similar to our results, Maestroni et al.<sup>4</sup> found that blocking pain receptors with preoperative local anesthesia reduces postoperative pain and the need for additional analgesics.

Some of the methods used to prevent pain after laparoscopic surgeries are postoperative opioid use, non-steroidal anti-inflammatory drugs, pre-incision and post-incision injections of local anesthetic drugs at the incision sites, local anesthetic spraying at the area that will cause intra-abdominal trauma or at the subdiaphragmatic region before and after the dissection, reduced pneumoperitoneum pressure, nongaseous laparoscopy, and traumatized intra-abdominal lavage with saline.<sup>18,19</sup> However, none of these have proven to be superior when compared to each other. The effect of local anesthetic infiltration in the incision

Table 1. Assessment of parameters among the study groups

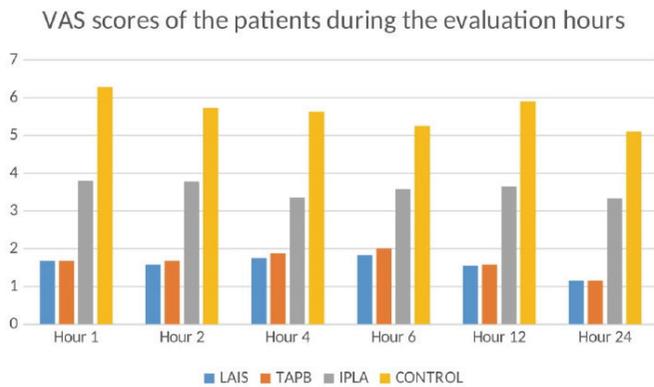
LAIS	Local anesthesia group				Total	p-value	
	TAPB	IPLA	Control				
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
Age	33.18±14.17	35.68±12.93	38.43±14.27	31.68±13.39	34.74±13.81	0.137 <sup>1</sup>	
BMI	25.48±3.57	25.1±3.13	26±3.34	25.23±3.45	25.45±3.36	0.644 <sup>1</sup>	
ASA (median)	1.4±0.67 (1)	1.48±0.64 (1)	1.5±0.68 (1)	1.43±0.59 (1)	1.45±0.64 (1)	0.861	
Operative time (minute) (median)	41.75±14.57 (40)	42.63±9.74 (42.5)	40.13±9.09 (40)	44.5±11.54 (40)	42.25±11.43 (40)	0.372 <sup>2</sup>	
Gender n (%)	Male	26 (65%)	27 (67.5%)	27 (67.5%)	24 (60%)	104 (65%)	0.883 <sup>3</sup>
	Female	14	13	13	16	56	-
Previous operations, n (%)	No	35 (87.5%)	28 (70%)	27 (67.5%)	31 (77.5%)	121 (75.6%)	0.154 <sup>3</sup>
	Yes	5	12	13	9	39	-
Presence of comorbid disease, n (%)	No	36 (90%)	36 (90%)	34 (85%)	38 (95%)	144 (90%)	0.567 <sup>4</sup>
	Yes	4	4	6	2	16	-
Comorbid diseases, n (%)	DM	2 (5%)	3 (7.5%)	3 (7.5%)	2 (5%)	10 (6.3%)	1.000 <sup>4</sup>
	HT	3 (7.5%)	4 (10%)	5 (12.5%)	2 (5%)	14 (8.8%)	0.787 <sup>4</sup>
	CVD	1 (2.5%)	1 (2.5%)	1 (2.5%)	1 (2.5%)	4 (2.5%)	1.000 <sup>4</sup>
	COPD	0 (0%)	0 (0%)	2 (5%)	0 (0%)	2 (1.3%)	0.245 <sup>4</sup>
Peroperative complications, n (%)	No	38 (95%)	40 (100%)	40 (100%)	37 (92.5%)	155 (96.9%)	0.694
	Yes	2	0	0	3	5	-
Postoperative complications, n (%)	No	38 (95%)	37 (92.5%)	39 (97.5%)	40 (100%)	154 (96.3%)	0.516 <sup>4</sup>
	Yes	2	3	1	0	6	-
Hospitalization (median)		1.3±0.46 (1)	1.45±0.64 (1)	1.68±0.83 (2)	1.98±1.12 (2)	1.6±0.83 (1)	0.0022*
Additional analgesic doses administered, n (%)	No	33 (82.5%)	37 (92.5%)	11 (27.5%)	1 (2.5%)	82 (51.2%)	<0.0013*
	Yes	7	3	29	39	78	-

<sup>1</sup>One-Way ANOVA, <sup>2</sup>Kruskal-Wallis test; <sup>3</sup>chi-square test; <sup>4</sup>Fisher-freeman-halton test; \*p<0.05, SD: Standard deviation, LAIS: Local anesthetic infusion to incision site, TAPB: Transversus abdominis plane block, IPLA: Intraperitoneal local anesthetic administration, Control: Control group, BMI: Body mass index, ASA: American Society of Anesthesiologists Score, DM: Diabetes mellitus, HT: Hypertension, CVD: Cardiovascular disease, COPD: Chronic obstructive pulmoner disease

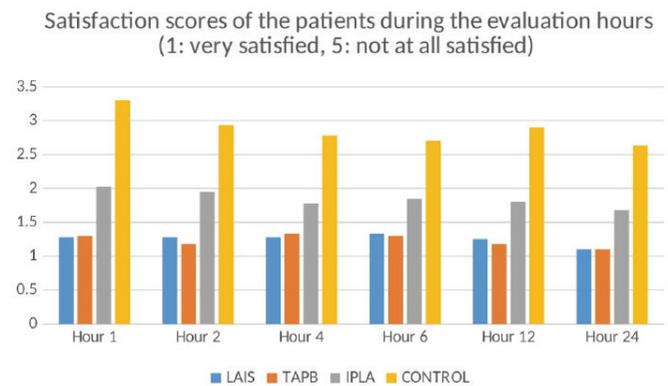
area on postoperative pain has been the subject of many studies.<sup>12,20-26</sup> When we examine the literature, we encounter studies that report that the LAIS method is effective in preventing postoperative pain<sup>12,22,24</sup> and in contrast, that LAIS has no effect.<sup>21,23,25-27</sup> It is not possible to reach a clear result since the application technique, application time and applied tissues of LAIS method differ between these studies. In the present study, we found that the LAIS method was effective in preventing postoperative pain.

The TAP block was first identified by McDonnell et al.<sup>13</sup> in 2004. There are three muscle groups in the abdominal wall: External and internal oblique muscles and transversus abdominis muscle. These muscles are innervated by somatic

nerve fibers located between the transversus abdominis muscle and the internal oblique muscle.<sup>28</sup> Blocking these nerve fibers in the anterior abdominal wall in laparoscopic cholecystectomy and open appendectomy reduces postoperative pain.<sup>13-16</sup> In our study, we found that the TAPB method reduces postoperative pain and increases patient satisfaction in laparoscopic appendectomy. In the present study, the TAP Block technique was applied under direct vision after the insertion of the camera trocar. In some studies in the literature, it has been found that the application of the TAPB technique before the incision reduces postoperative pain.<sup>12</sup> In the study conducted by Amr et al.<sup>29</sup>, it was found that performing TAPB application



**Figure 6.** VAS scores of the patients during the evaluation hours  
VAS: Visual analog scale



**Figure 7.** Satisfaction scores of the patients during the evaluation hours

**Table 2.** Evaluation of VAS and patient satisfaction levels among the study groups

LAIS		Local anesthesia group			p-value	
		TAPB	IPLA	Control		
		Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	
VAS	Hour 1	1.68±1.95 (1)	1.68±1.53 (2)	3.8±1.8 (3.5)	6.28±2.33 (6.5)	<0.001*
	Hour 2	1.58±1.57 (1)	1.68±1.47 (2)	3.78±1.56 (3.5)	5.73±2.35 (6)	<0.001*
	Hour 4	1.75±1.66 (1)	1.88±1.77 (2)	3.35±1.66 (3)	5.63±1.75 (6)	<0.001*
	Hour 6	1.83±1.82 (1)	2±1.97 (2)	3.58±1.92 (3.5)	5.25±2.44 (5)	<0.001*
	Hour 12	1.55±1.84 (1)	1.58±1.57 (2)	3.65±1.89 (3)	5.9±2.43 (6)	<0.001*
	Hour 24	1.15±1.49 (1)	1.15±1.41 (1)	3.33±1.86 (3)	5.1±2.33 (5)	<0.001*
Patient satisfaction	Hour 1	1.28±0.64 (1)	1.3±0.56 (1)	2.03±0.77 (2)	3.3±1.07 (3)	<0.001*
	Hour 2	1.28±0.51 (1)	1.18±0.38 (1)	1.95±0.68 (2)	2.93±1 (3)	<0.001*
	Hour 4	1.28±0.55 (1)	1.33±0.53 (1)	1.78±0.66 (2)	2.78±0.83 (3)	<0.001*
	Hour 6	1.33±0.57 (1)	1.3±0.76 (1)	1.85±0.83 (2)	2.7±0.94 (2.5)	<0.001*
	Hour 12	1.25±0.59 (1)	1.18±0.68 (1)	1.8±0.72 (2)	2.9±1.01 (3)	<0.001*
	Hour 24	1.1±0.38 (1)	1.1±0.3 (1)	1.68±0.76 (2)	2.63±1.03 (3)	<0.001*

Kruskal-Wallis test; \*p<0.05, SD: Standard deviation, LAIS: Local anesthetic infusion to incision site, TAPB: Transversus abdominis plane block, IPLA: Intraperitoneal local anesthetic administration, Control: Control group, VAS: Visual analog scale

before incision reduced postoperative pain more than after incision. However, it was found that the application of TAPB method after incision decreased postoperative pain compared to not being applied.<sup>29</sup>

In randomized controlled trials on the use of intraperitoneal local anesthetics in laparoscopic appendectomies in adults, IPLA was found to reduce the need for postoperative analgesics; low pain scores have been detected in three studies.<sup>30-32</sup> Our study also found that IPLA was advantageous for postoperative pain management compared to the control group.

Opioids are effective in reducing postoperative pain, but they cannot be used safely due to their possible side effects.<sup>33</sup> Some of these side effects include respiratory depression, sedation, postoperative nausea and vomiting, itching, urinary retention, ileus, and constipation and, therefore, delayed discharge.<sup>34</sup> Due to all these side effects, anesthesiologists and surgeons currently use nonopioid analgesia. In our study, we found that preemptive analgesia reduced the need for postoperative additional analgesics and opioid. Therefore, we believe that these methods might prevent the side effects caused by the overuse of opioids.

When considering early recovery programs, postoperative pain control added to the surgical protocol is important, which results in many advantages, such as early recovery, and short hospital stay.<sup>35,36</sup> Local anesthetic administrations reduce surgical stress response and the need for postoperative opioid use, as well as facilitate early recovery.<sup>36</sup>

#### Study Limitations

This study has some limitations. The TAPB technique applied in this study was performed under direct vision in anesthetized patients without using sonar probe in order to prevent prolongation of the operation time. Therefore, it is impossible to assume that all blocks were working perfectly. This point may have affected the validity of the results.

Another limitation of our study was that the age range of the evaluated patients was very wide. For the purpose of this study the possibility that the degree of pain that may occur after surgery may vary depending on age was ignored.

Finally, a limitation of our study was that postoperative follow-up was terminated within 24 hours due to the discharge of the patients. Single doses of local anaesthetics provide pain relief, but the short duration of effect can be a limiting factor.

#### Conclusion

Using peroperative preemptive analgesia methods to prevent postoperative pain after laparoscopic appendectomy facilitates early recovery, less need for additional analgesics,

and higher patient satisfaction during the postoperative period, thereby increasing the postoperative comfort of patients in the first 24 hours after surgery.

#### Ethics

**Ethics Committee Approval:** Approval for this study was obtained from the Ethics Committee Fatih Sultan Mehmet Training and Research Hospital (approval number: 56, date: 13.12.2018).

**Informed Consent:** Informed consent was obtained from all patients.

**Peer-review:** Externally peer-reviewed.

#### Authorship Contributions

Surgical and Medical Practices: A.E., Concept: A.E., A.B.K., A.Y.İ., N.E.B., Design: D.C.T., N.D., Data Collection or Processing: A.E., Analysis or Interpretation: A.E., A.C.B., İ.T., K.M., Literature Search: Y.G., Y.Y.K., A.Ç., Writing: A.E., M.M.F., H.Ç.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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