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ORIGINAL ARTICLE

Comparative Evaluation of Intra-articular Bupivacaine *vs* Intra-articular Bupivacaine and Dexmedetomidine for Postoperative Analgesia in Arthroscopic Knee Surgery

[1]Atish Pal, [2]Chitra Chatterji, [3]Chetan Mehra, [4]Varadarajan Muralidhar, [4]Harsha Madhok

ABSTRACT

Aim: To assess the postoperative analgesic effect of intra-articular dexmedetomidine, when administered as an adjuvant with bupivacaine in arthroscopic knee surgeries.

Materials and methods: A total of 60 patients undergoing elective unilateral knee arthroscopy under general anesthesia were randomly assigned to two groups (n = 30). Group Bupivacaine and Saline (BS) received intra-articularly 19 mL of 0.5% bupivacaine and 1 mL of isotonic saline (total volume 20 mL). Group Bupivacaine and Dexmedetomidine (BD) received intra-articular 100 µg dexmedetomidine (1 mL) added to 19 mL of 0.5% bupivacaine. Pain assessment using visual analog scale (VAS) was done at regular intervals for 24 hours and rescue analgesia given accordingly.

Results: Increased VAS scores (p-value 0.005, <0.001, 0.002) and increased use of supplementary analgesic (p-value 0.042, 0.026, 0.024) were seen in group BS (control group) compared with group BD (study group), at intervals of 30 minutes, 1 hour, and 2 hours. Mean duration of analgesia (time for first analgesic requirement) was longer in group BD (median 4 hours) compared with BS (median 1 hour) (p-value 0.012).

Conclusion: Intra-articular dexmedetomidine administered as an adjuvant to bupivacaine improves the quality and duration of postoperative analgesia after knee arthroscopy.

Clinical significance: A myriad of agents have been studied for their potential use in attenuating postoperative pain following knee arthroscopy, but despite multiple studies with various agents, no single agent has been found to be clearly superior to the rest. In such a scenario, dexmedetomidine provides an interesting option.

Keywords: Arthroscopy, Bupivacaine, Dexmedetomidine, Intra-articular, Postoperative pain.

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[1]Registrar, [2,4,5]Senior Consultant, [3]Consultant

[1-5]Department of Anesthesia and Critical Care, Indraprastha Apollo Hospitals, New Delhi, India

Corresponding Author: Atish Pal, Registrar, Department of Anesthesia and Critical Care, Indraprastha Apollo Hospitals New Delhi, India, e-mail: dr_atispal@yahoo.co.in

INTRODUCTION

Knee arthroscopy is a common procedure and very often is performed as a day-case surgery. It seems that ambulatory arthroscopic surgery of the knee is preferred by the majority of properly selected and well-informed patients. It has been reported that a significant number of patients have moderate-to-severe pain 24 hours after ambulatory surgery, in general, and knee arthroscopy, in particular, and pain affects the patient's activity level and satisfaction. Arthroscopic knee surgery is associated with significant postoperative pain that may require systemic analgesia and can delay hospital discharge. It is common to undertake the procedure as a day case. Efforts have been made to determine the best method of pain relief, to maximize analgesia and minimize side effects. Analgesic approaches include systemic opioids, systemic nonsteroidal anti-inflammatory drug , and various other pharmacological agents. The use of locally administered analgesia with various agents is appealing, and intra-articular local anesthetic agents and intra-articular opioids have been reported in literature to be effective.

Dexmedetomidine (Precedex; Abbott Laboratories, Abbott Park, Illinois) was approved in the United States by the Food and Drug Administration (FDA), at the end of 1999, for use in humans as a short-term medication (< 24 hours) for sedation/analgesia in the intensive care unit (ICU).[1] This approval was only for intubated patients on mechanical ventilator, but, in 2013, FDA approval was given to dexmedetomidine for sedation of nonintubated patients prior to and/or during surgical and other procedures.[2,3]

Dexmedetomidine is a potent and highly selective alpha (2)-adrenoreceptor agonist currently utilized for continuous infusion for sedation/analgesia in the ICU.[4,5] Dexmedetomidine offers remarkable pharmacological properties including sedation, anxiolysis, and analgesia along with its unique property to cause no respiratory depression. In addition, it also possesses sympatholytic and antinociceptive properties, which allow hemodynamic stability during surgical stimulation. Several studies have demonstrated its safety, although bradycardia and hypotension are the most predictable and frequent side effects. Dexmedetomidine has shown to consistently reduce opioids, propofol, and

benzodiazepines requirements, and, in the recent past, has emerged as an effective therapeutic drug in a wide range of anesthetic management, promising large benefits in the perioperative period.

Intra-articular dexmedetomidine may be useful to avoid the adverse hemodynamic effects of intravenous (IV) administration, while still providing the postoperative analgesia.

A myriad of agents have been studied for their potential use in attenuating postoperative pain following knee arthroscopy. Many of the reported studies have focused on amide local anesthetic agents, as these are the most widely used in clinical practice. Despite multiple studies, there is no agent that appears clearly superior to the rest.

This study was designed to assess and compare the postoperative analgesic effects of intra-articular dexmedetomidine administered as adjuvant with local anesthetic bupivacaine in patients undergoing arthroscopic knee surgery.

MATERIALS AND METHODS

After obtaining institutional ethical committee approval, the present study was conducted by the Department of Anesthesiology, Indraprastha Apollo Hospital, Sarita Vihar, New Delhi, India, with written informed consent taken from the 60 patients enrolled and scheduled for elective unilateral arthroscopic knee surgeries to be performed under general anesthesia.

Study Design

It was a randomized, double-blind, controlled, and prospective study.

Sample Size Estimation

Sample size estimation was based on previous studies using a dichotomous (success/failure, yes/no, etc.) primary variable. For example, 25% of the participants on the standard therapy had a successful outcome and it is of clinical relevance only if we observe a 40% (effect size) absolute improvement for those on the study therapy (i.e., 65% of the participants in the study will have a successful outcome). To determine the number of participants to observe a significant difference for a two-sided test of 5%, a simple formula to calculate the sample size is given by

$$n \text{ (size per group)} = C\,[pc\,(1 - pc) + pe\,(1 - pe)]/d2 + d/2 + 2$$

Where pc and pe are the proportion estimates, d = difference between pc and pe, expressed as positive quantity. Value of C is 7.85 for 80% power and significance level of 5%.

Thus, from the above example, pc = 0.25 and pe = 0.65. For 80% power, we have n (size per group) = 7.85 × [0.25 (1 – 0.25) + 0.65 (1 – 0.65)]/(0.25 – 0.65)2 +(0.065 – 0.25)/2 +2= 27.

Hence, 27 × 2 = 54 participants will be needed for the power of the study to be 80%. Considering 90% response rate, 60 patients were selected for the study, who were divided into two groups.

Sampling

Systematic sampling methodology was used for selecting the desired number of patients in each group. In systematic sampling, the first patient is randomly selected and the others are drawn from the clinical database according to a sample interval defined as N/n until achievement of the desired sample size.

Inclusion Criteria

After taking informed consent, 60 adult patients of either sex, and of American Society of Anesthesiologists (ASA) grade I and II, who underwent elective knee arthroscopic surgery at our institution, have been included in the study.

Exclusion Criteria

The various exclusion criteria taken for the study were as follows:

- Patient refusal
- Hypersensitivity or allergy to study drugs
- Age below 18 years and above 65 years
- History of cardiovascular or cerebrovascular disease
- Impaired renal or hepatic function
- Pregnancy
- On chronic pain medication
- Hypertension treated with methyldopa, clonidine, or beta-blockers

Allocation

The 60 patients included in the study were divided into two groups, each containing 30 patients.

Group BD: 30 patients receiving 0.5% bupivacaine and dexmedetomidine

Group BS: 30 patients receiving 0.5% bupivacaine and saline

Masking

The study has been double blinded with both the anesthesiologist and the orthopedic surgeon administering the drug, blinded to the study.

Preoperative Evaluation

In all patients, a complete preanesthetic evaluation was done. Age, body weight, and baseline vital parameters

were recorded, along with a complete physical examination and airway assessment. History regarding previous anesthesia, any previous surgery, any significant medical illness, medications, and allergy was also recorded. On preoperative rounds, the procedure was explained to patients and they were also taught to interpret the VAS (graded from 0 = no pain to 10 = maximum pain).

Anesthesia and Intraoperative Period

The ASA Task Force guidelines for preoperative fasting were carried out in all patients. On the operation table, routine monitoring [electrocardiogram, pulse oximetry, noninvasive blood pressure, end tidal carbon dioxide ($EtCO_2$)] along with baseline vital parameters like heart rate (HR), blood pressure (systolic, diastolic, and mean), and arterial oxygen saturation monitoring were done. After securing an IV line, induction of anesthesia was done by fentanyl 2 µg/kg and propofol 2 to 3 mg/kg. Airway was maintained with appropriate sized laryngeal mask airway (LMA) after muscle relaxation with atracurium besylate in a dose of 0.5 mg/kg. Anesthesia was maintained with 33% oxygen in nitrous oxide and isoflurane with a MAC level of 1. Muscle relaxation was maintained by intermittent bolus doses of atracurium. The patients were then put on mechanical ventilation to keep $EtCO_2$ between 35 and 40 mm Hg. For surgeries extending beyond 1 hour, all patients received fentanyl 1 µg/kg, 45 minutes after receiving the first dose of fentanyl. Following end of the arthroscopic procedure, patients then received one of the following intra-articular solutions (prepared by an individual not involved in the study), which was injected into the knee joint through the arthroscopic cannular sheath after withdrawal of camera, by the orthopedic surgeon (who was also unaware of the nature of the study drugs). In group BS, 19 mL of 0.5% bupivacaine and 1 mL isotonic saline (total volume 20 mL) was administered into the knee joint. Similarly, group BD patients received 100 µg dexmedetomidine (1 mL) added to 19 mL 0.5% bupivacaine (again making a volume of 20 mL). After this, the tourniquet was deflated, and then the closure of the wound completed. Subsequently, at the end of surgical procedure, LMA was taken out after adequate reversal of neuromuscular paralysis using IV glycopyrolate and neostigmine.

Postoperative Period

After completion of the surgery, patients were transferred from the operation theatre to the adjacent postanesthesia care unit, and intensity of pain and vital parameters were assessed after 30 minutes and then at 1, 2, 4, 10, 18, and 24 hours. The patients were also monitored for nausea and vomiting, sedation, hypotension (defined as systolic blood pressure > 20% decrease from baseline), and bradycardia (HR < 60 beats/minute) during this period of 24 hours. Pain intensity was monitored using the VAS and sedation scoring was done as per the Ramsay score. Tramadol 50 mg IV and diclofenac sodium 75 mg IV were then used as rescue analgesic drugs, in case of pain score greater than 3. The total amount of rescue analgesia received over 24 hours was noted and documented, along with the time of the first rescue analgesic requirement.

Statistical Analysis

Statistical analysis has been performed by the Statistical Package for the Social Sciences program for Windows, version 17.0. Continuous variables are presented as mean ± standard deviation, and categorical variables have been presented as absolute numbers and percentage. Data were checked for normality before performing statistical analysis. Normally distributed continuous variables were compared using the unpaired t-test, whereas the Mann–Whitney U test was used for those variables that were not normally distributed. Categorical variables were analyzed using either the chi-squared test or Fisher's exact test. For all statistical tests, p-value less than 0.05 was taken to indicate a significant difference.

RESULTS

The main salient finding in our study is that intra-articular dexmedetomidine in a dose of 100 µg, when used as an adjuvant to intra-articular 0.5% bupivacaine at the end of arthroscopic knee surgeries, enhanced postoperative pain relief. It also reduced the need for postoperative analgesia and prolonged the time to first analgesic request.

There were no significant differences between both the groups with regard to age, weight, gender, type, and side of surgery (Tables 1 to 3). There were also no significant differences in the baseline VAS, HR, and MAP. At the end of surgery, as per the protocol, the study group (group BD) received intra-articular 0.5% bupivacaine and 100 µg dexmedetomidine, while the control group

Table 1: Group distribution

Groups	*Frequency*	*Percent*
BD	30	50
BS	30	50
Total	60	100

Table 2: Sex distribution

	BS	*BS*	*BD*	*BD*	
Sex	*Frequency*	*Percent*	*Frequency*	*Percent*	*p-value*
1	23	76.7	18	60	
2	07	23.3	12	40	
Total	30	100	30	100	0.165

Table 3: Type of surgery

Surgery	BS Frequency	BS Percent	BD Frequency	BD Percent	p-value
Diagnostic	18	60	18	60	
ACL repair	09	30	07	23.3	
Menisectomy	03	10	05	16.7	
Total	30	100	30	100	0.687

Table 4: VAS score (mean)

Time	Control BS VAS Mean ± SD	Study BD VAS Mean ± SD	p-value
30 minutes	2.90 ± 0.89	2.23 ± 0.90	0.005
1 hour	2.79 ± 0.94	1.77 ± 0.86	<0.001
2 hours	2.36 ± 1.10	1.59 ± 0.63	0.002
4 hours	1.68 ± 1.04	1.89 ± 1.12	0.510
10 hours	2.50 ± 1.35	2.08 ± 1.38	0.471
18 hours	2.22 ± 1.09	2.20 ± 1.64	0.976
24 hours	1.50 ± 0.71	0 ± 0	–

Table 5: Use of tramadol

Time	BS Frequency	BS Percent	BD Frequency	BD Percent	p-value
30 minutes	9	30	2	6.7	0.042
1 hour	8	26.7	1	3.3	0.026
2 hours	6	20	0	0	0.024
4 hours	2	6.7	4	13.3	0.671
10 hours	4	13.3	4	13.3	1.000
18 hours	2	6.7	4	13.3	0.671
24 hours	0	0	0	0	–

Table 6: First analgesic requirement

	BS N	BS Median	BS IQR	BD N	BD Median	BD IQR	p-value
Time gap between 0 hour and the time of first tramadol use	26	1	0.5-2	11	4	1-10	0.012

(group BS) received 0.5% bupivacaine and normal saline intra-articularly (both groups receiving total volume of 20 mL). After completion of the surgery, patients were transferred to the postanesthesia care unit, and intensity of pain and vital parameters were assessed after 30 minutes and then at 1, 2, 4, 10, 18, and 24 hours. The patients were also monitored for nausea and vomiting, sedation, hypotension (defined as systolic blood pressure > 20% decrease from baseline) and bradycardia (HR < 60 beats/minute) during this period. Pain was monitored using the VAS, and sedation scoring was done as per the Ramsay sedation score.

The significant finding in our study was the increased VAS scores (Table 4) and the increased use of tramadol (Table 5) in the control group (group BS) as compared with the study group (group BD), at time intervals of 30 minutes, 1 hour, and 2 hours. These findings are consistent with the previous studies by Paul et al[6] and by Al-Metwalli et al,[2] and can be explained by the local analgesic effect provided by intra-articular dexmedetomidine. From 4 hours onward, there was no statistically significant difference of VAS scores and tramadol use, between the two groups. The 4th hour time interval showed slightly increased VAS score and tramadol usage in the study group as compared with the control group, although this difference was statistically insignificant. This could be due to the fact that the control group required more tramadol in the time period from 30 minutes to 4 hours; and at the 4th hour time interval, the patients in the control group were under the action of the rescue analgesic given. The patients in the study group, meanwhile, had significantly decreased VAS scores in the 30 minutes to 4 hour time period due to the effect of dexmedetomidine and thus, had received less analgesic as compared with the control group until 4 hours. From a pharmacokinetic perspective, dexmedetomidine has a half-life of 2 hours, and the duration of action of dexmedetomidine is approximately 4 hours after IV administration; however, the duration of action following intra-articular infiltration is not known. In our study, the effect of intra-articular dexmedetomidine probably begins to wear off at 4 hours, and hence, the patients in the study group start showing higher VAS scores at this point of time. Paul et al,[6] in their study, monitored the pain scores at 1, 2, 6, 10, 18 hours and found statistically significant difference in pain scores till 6 hours. The dose of dexmedetomidine was 100 µg, but they used 0.25% ropivacaine as the local anesthetic agent. There was no information available about the time of tourniquet release in relation to the time of intra-articular infiltration. In our study, the tourniquet was released after the infiltration. The other statistically significant finding in our study was that the mean duration of analgesia (delay between the intra-articular injection and the first postoperative analgesic demand) was longer in the study group BD (median 4 hours) compared with control group BS (median 1 hour). The interquartile range for group BS (control group) was 0.5 to 2 hours, while that for group BD was 1 to 10 hours (Table 6). The finding was statistically significant with a p-value of 0.012. We also saw found 50% of the patients in the study group did not require any supplementary analgesia over 24 hours in comparison with only 13.3% patients in the control group, and this is a statistically significant finding with a p-value of <0.001. About 30% of group BS patients required supplementary analgesia twice in 24 hours, while the percentage of such

Table 7: Use of diclofenac

	BS	*BS*	*BD*	*BD*	
Time	*Frequency*	*Percent*	*Frequency*	*Percent*	*p-value*
30 minutes	0	0	1	3.3	1.000
1 hour	4	13.3	2	6.7	0.671
2 hours	1	3.3	1	3.3	1.000
4 hours	1	3.3	2	6.7	1.000
10 hours	0	0	1	3.3	1.000
18 hours	0	0	0	0	–
24 hours	0	0	0	0	–

patients in group BD was only 6.7%, with a statistically significant p-value of 0.042. The difference in the requirement of the second analgesic (diclofenac) between the two groups was found to be statistically insignificant (Table 7). As regards the level of sedation also, the two groups were found to be comparable with no statistically significant difference.

We can conclude by saying that intra-articular dexmedetomidine, administered as an adjuvant to local anesthetic 0.5% bupivacaine, improves the quality and duration of postoperative analgesia after unilateral knee arthroscopic surgeries.

CONCLUSION

The main findings of the study after statistical analysis were as follows:

- The pain scores observed in the study group were significantly less during the first 2 hours after surgery, as compared with the control group.
- The requirement of analgesia was more in the control group during the first 2 hours.
- The time for the first analgesic requirement was significantly delayed in the study group, when compared with the control group.
- The pain score and the requirement of analgesia started to rise slightly in the study group at the 4-hour time interval, although it was statistically insignificant.
- Addition of intra-articular dexmedetomidine did not show increased levels of sedation in the study group, and it was comparable with the control group at all the time intervals.
- The difference between the two groups in the use of the second rescue analgesic (diclofenac) was found to be statistically insignificant.
- About 50% of the patients in the study group did not require any supplementary analgesia over 24 hours in comparison with only 13.3% patients in the control group, and this is a statistically significant finding with a p-value of <0.001 (Table 8).

Our study has shown that supplementation of intra-articular dexmedetomidine to intra-articular 0.5% bupivacaine improves the quality and duration of postoperative analgesia in patients undergoing arthroscopic knee surgeries under general anesthesia. It has also simultaneously shown a reduction in the total requirement of parenteral analgesics, and also perhaps their related side effects.

CLINICAL SIGNIFICANCE

Arthroscopy of the knee has spared patients large incisions and decreased morbidity compared with those of open procedures, but it has not eliminated pain.[7] Most of the intra-articular structures of the knee, including the synovial tissue, the anterior fat pad, and the joint capsule, have free nerve-endings that are capable of sensing painful stimuli and producing severe pain.[8] Arthroscopic procedures may cause enough pain and swelling to delay rehabilitation and return to work for up to 2 weeks after surgery.[3,9] Patients who cannot complete a rehabilitation program may be at an increased risk for postoperative complications (delay in strength recovery, prolonged knee stiffness, anterior knee pain).[3,9,10] Therefore, aggressive pain management in the early postoperative period is essential and can enhance convalescence after arthroscopy.[11]

In our study, the primary aim was to determine if intra-articular dexmedetomidine can be an ideal adjuvant to intra-articular bupivacaine in providing satisfactory postoperative analgesia to patients undergoing unilateral knee arthroscopies.

Dexmedetomidine is a α2-adrenergic receptor (α2-AR) agonist like clonidine. The α2-AR agonists produce clinical effects after binding to G-protein-coupled α2-AR, of which there are three subtypes (α2A, α2B, and α2C) with each having different physiological functions and pharmacological activities.[12] These receptor subtypes

Table 8: Number of times analgesia required

Number of times rescue analgesics required over 24 hours	*Group BS (n = 30)*	*Group BS (n = 30)*	*Group BD (n = 30)*	*Group BD (n = 30)*	*p-value*
	Frequency	*Percent*	*Frequency*	*Percent*	
0	4	13.3	15	50.0	<0.001
1	16	53.3	11	36.7	0.195
2	9	30.1	2	6.7	0.042
3	1	3.3	2	6.6	1.000

are found ubiquitously in the central, peripheral, and autonomic nervous systems, as well as in vital organs and blood vessels. Dexmedetomidine is about 8 times more selective toward α2-AR than clonidine.[12]

Alpha-2 adrenergic agonists produce their analgesic effects through supraspinal, spinal, and peripheral actions. The analgesic effect of intra-articular dexmedetomidine appears to be mainly due to direct local action. However, a central analgesic effect resulting from systemic absorption cannot be excluded. The mechanism of analgesic effect of intra-articular dexmedetomidine might be similar to that of intra-articular clonidine. Clonidine produces analgesia mainly through inhibition of the transmission of nociceptive stimulation in the dorsal horn of spinal cord. Dexmedetomidine, like clonidine, may provide local anesthetic effects, which inhibit the conduction of nerve signals through C and Aδ fibers, and may stimulate the release of encephalin-like substances at peripheral sites.

Administration of intra-articular dexmedetomidine also did not show any of the known adverse effects of IV dexmedetomidine, such as bradycardia, hypotension, and transient hypertension. Addition of intra-articular dexmedetomidine did not show increased levels of sedation in the study group, and it was comparable with the control group at all the time intervals.

Knee arthroscopies are nowadays predominantly moving toward being daycare procedures, and, in this scenario, the opioid-sparing effects of our regimen, along with the absence of adverse effects, are highlighted more.

REFERENCES

1. Afonso J, Reis F. Dexmedetomidine: current role in anesthesia and intensive care. Rev Bras Anestesiol 2012 Jan-Feb; 62(1):118-133.
2. Al-Metwalli RR, Mowafi HA, Ismail SA, Siddiqui AK, Al-Ghamdi AM, Shafi MA, El-Saleh AR. Effect of intra-articular dexmedetomidine on postoperative analgesia after arthroscopic knee surgery. Br J Anaesth 2008 Sep;101(3): 395-399.
3. Shukry M, Miller JA. Update on dexmedetomidine: use in nonintubated patients requiring sedation for surgical procedures. Ther Clin Risk Manag 2010 Apr 15;6:111-121.
4. Grewal A. Dexmedetomidine: new avenues. J Anaesth Clin Pharmacol 2011 Jul;27(3):297-302.
5. Gertler R, Brown HC, Mitchell DH, Silvius EN. Dexme- detomidine: a novel sedative-analgesic agent. Proc (Bayl Univ Med Cent) 2001 Jan;14(1):13-21.
6. Paul S, Bhattacharjee DP, Ghosh S, Dawn S, Chatterjee N. Ef cacy of intra articular dexmedetomidine for postoperative analgesia in arthroscopic knee surgery. Ceylon Med J 2010 Dec; 55(4):111-115.
7. Highgenboten CL, Jackson AW, Meske NB. Arthroscopy of the knee. Ten-day pain pro les and corticosteroids. Am J Sports Med 1993 Jul-Aug; 21(4):503-506.
8. Dye SF, Vaupel GL, Dye CC. Conscious neurosensory mapping of the internal structures of the human knee without intra-articular anesthesia. Am J Sports Med 1998 Nov-Dec; 26(6):773-777.
9. Durand A, Richards CL, Malouin F. Strength recovery and muscle activation of the knee extensor and exor muscles after arthroscopic meniscectomy. A pilot study. Clin Orthop 1991 Jan;(262):210-226.
10. Shelbourne KD, Nitz P. Accelerated rehabilitation after anterior cruciate ligament reconstruction. Am J Sports Med 1990 May-Jun;18(3): 292-299.
11. Rasmussen S, Larsen AS, Thomsen ST, Kehlet H. Intra- articular glucocorticoid, bupivacaine and morphine reduces pain, inflammatory response and convalescence after arthroscopic meniscectomy. Pain 1998 Nov;78(2):131-134.
12. Kaur M, Singh PM. Current role of dexmedetomidine in clinical anesthesia and intensive care. Anesth Essays Res 2011 Jul-Dec;5(2):128-133.