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Original Research Article

A PROSPECTIVE RANDOMISED, DOUBLE BLIND COMPARATIVE STUDY OF CLONIDINE AND BUPRENORPHINE AS EPIDURAL ADJUVANTS WITH PLAIN 0.2% ROPIVACAINE FOR POST OPERATIVE ANALGESIA IN INFRA-UMBILICAL SURGERIES

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Abstract

Background: Epidural analgesia is widely applied with adjuvants for the purpose of post-operative pain relief. But there is paucity of literature regarding the epidural administration of ropivacaine in combination with buprenorphine as adjuvant to produce differential blockade. The objective is to study and compare the efficacy of post-operative analgesia between epidurally administered Buprenorphine or Clonidine as adjuvant in combination with Ropivacaine in patients undergoing infra-umbilical surgeries. **Materials and Methods:** This Prospective randomized double-blind comparative study was conducted at Sri Sathya Sai Institute of Higher Medical Sciences, Whitefield, Bengaluru. **Result:** There was statistically significant difference (p value< 0.05) between RC and RB groups interms of heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure, starting from 30 minutes after epidural drug injection till 16hrs, with patients in Group RC showing lower values for heart rate. systolic blood pressure, diastolic blood pressure, and mean arterial pressure, than RB group. Statistically significant difference was noted in VAS scores between the groups and it was less in RB group (p value <0.01). **Conclusion:** Combination of Ropivacaine 0.2% with 150µ buprenorphine for post-operative epidural analgesia is superior when compared to Ropivacaine 0.2% with 75µg clonidine.

INTRODUCTION

Regional anaesthesia is the most preferred technique for lower abdomen and lower limb surgeries. Regional anaesthesia has various advantages compared to general anaesthesia in the patients undergoing infra-umbilical surgeries. The advantages are an awake patient, avoidance of poly-pharmacy, no airway manipulation, complete motor and sensory blockade, early food intake by the patient, less incidence of post-operative nausea, vomiting and good post-operative analgesia. Intrathecal (Spinal) anaesthesia and epidural anaesthesia are the most popular regional anaesthesia techniques used for lower abdominal surgeries.[1]

Epidural blockade is one of the most useful and versatile procedures in modern anaesthesiology.

It can be used to supplement general anesthesia, decreasing the need for deep levels of general anesthesia, therefore providing a more hemodynamically stable operative course and faster emergence from general anesthesia. It provides better postoperative pain control and more rapid recovery from surgery.

Leonard Corning administered the first epidural anaesthetic cocaine in dogin 1885. In 1930s, Archile Mario Dogliotti, building on Jansen's discovery of negative pressure in the epidural space, described a practical technique for administering lumbar segmental anesthesia. In 1949, Manuel Martinez Curbello modified a silk catheter for continuous

spinal anesthesia and inserted it into the epidural space, thus creating the first continuous epidural block. Epidural anaesthesia is unique in that it can be placed at virtually any level of the spinal spine, allowing more flexibility in its application to clinical practice.

Pain after infra umbilical surgery is a significant cause of post-operative morbidity. The intensity and frequency of postoperative pain may vary among patients. Post-operative pain results in various complications – most important being respiratory complications due to atelectasis, hypertension and tachycardia due to sympathetic stimulation. Numerous studies have demonstrated the benefits of epidural blockade. Epidural anesthesia or analgesia can reduce the adverse physiologic responses to surgery such as autonomic hyperactivity, cardiovascular stress, tissue breakdown, increased metabolic rate, pulmonary dysfunction, and immune system dysfunction.[2]

Well-conducted randomized trials have demonstrated the perioperative use of epidural anesthesia and analgesia may reduce overall mortality and morbidity by approximately 30% compared with general anesthesia using systemic opioids.

MATERIALS AND METHODS

This Prospective randomized double-blind comparative study was conducted at Sri Sathya Sai Institute of Higher Medical Sciences, Whitefield, Bengaluru. Study will be carried out from June 2017 to June 2018 for a period of 1year.

Study Population

Approval of the hospital Ethics and scientific Committee will be taken. 60 consecutive patients (30 plus 30 in each group) patients between the ages of 18-80 years undergoing elective infra-umbilical surgery and of ASA Grade 1 or 2 will be allocated to Group RB or RC, receiving either Ropivacaine plus buprenorphine (RB group) or Ropivacaine plus clonidine (RC group) respectively. Informed written consent from the patients will be taken from each of the patient.

Inclusion Criteria

All patients above the age of 18 years and <80years of ASA grade I and II scheduled for elective infraumblical surgeries under regional anesthesia.

Exclusion Criteria

Patients with ASA Grade > 2.

Patients with documented allergy to any of the three drugs used in the study - ropivacaine, buprenorphine and clonidine.

Patients with spinal deformities.

Patients with known major respiratory, cardiovascular, neurological, liver, renal disease, morbid obesity, hemodynamic instability, coagulation disorders or psychiatric disturbances.

Patients undergoing emergency surgery.

Patients not willing for regional anaesthesia.

Methodology

At the time of Pre Anaesthetic-Checkup, patient history will be noted; general physical and systemic examination will be carried out.

All patients will be trained about Visual Analogue Score day before the surgery.

The patient will be explained, in their native language, the nature of the study and will be given a Patient Information Sheet and their initials will be obtained on the Informed Consent Form.

Baseline Vital parameters like PR, BP, Spo2, RR, and will be recorded

All patients will receive a standard premedication of Tablet Alprazolam 0.5mg and Tablet Pantoprazole 40 mg on the night prior to surgery. The patients will be advised to remain NPO for 6 hours prior to the procedure. The sensitivity to the local anesthetics was tested in all the groups.

An intravenous line will be established with 18G IV cannula and preloading will be done with Ringer lactate -The patients will be premedicated with injection Ranitidine 50mg and injection Ondansetron 4 mg IV slowly preoperatively 5 minutes before surgery.

Spinal and Epidural Anaesthesia Technique

Under aseptic precautions L2-L3 / L3-L4 intervertebral space was identified in sitting position. Local infiltration of 2ml of 2% lignocaine given, then 18G Tuohy needle willbe introduced at L2-L3 space and epidural space will be identified by loss of resistance to normal saline. 18 G epidural catheter will be inserted so that 4-5cm of catheter is inside the epidural space,3cc test dose of 2% lignocaine with 1:2lakh concentration adrenaline solution injected after negative aspiration for blood and CSF. For 10 minutes we observed for signs of intravascular and subarachnoid placement of epidural catheter (tachycardia, motor block) when negative signs then catheter placement in the epidural space was assured.

Under strict aseptic precautions spinal anaesthesia was given in lower space with 25 gauze Quincke Babcock spinal needle at L3-L4 level, 15mg (3ml) of 0.5%bupivacaine(heavy) was injected. The surgery was done and standard anaesthesia monitoring was carried.

Postoperative Analgesia: At the end of the surgery, pain assessment was carried utilizing VAS score.

- Our study followed double blinding technique. Our patients in both study population groups were blinded of our interventions. The study drug combinations were prepared by a trained senior anesthesiologist in syringes labeled with the following codes,
  - CODE A- 0.2% Ropivacaine + Buprenorphine 3µ/kg body weight (bd wt.)
  - CODE B- 0.2 % Ropivacaine + Clonidine 1.5µ/kg body weight
- If VAS score is greater than 3 or patient complaining of mild pain, epidural study drugs loaded in coded syringes were administered by

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another anesthesiologist who was blinded of our study, study population groups and study drug combinations by coding them and all the study parameters were collated by the same anesthesiologist that is how in our study the double blinded technique was employed.

Data also obtained by same person.

- No surgical wound infiltration will be given at the end of surgery.
- In Group RB, 6ml 0.2% ropivacaine + buprenorphine 3µg/kg or 150 µg, and in Group RC 6 ml 0.2%ropivacaine + clonidine 1.5µg/kg or 75µg were given. The drug solutions will be injected through epidural catheter.

Statistical Analysis: Data were statistically described in terms of mean (±SD), frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using unpaired t-test for normally distributed data or by Mann Whitney test for non-normally distributed data. For comparing categorical data, Chi square test was performed. Exact test was used instead when the expected frequency is less than 5. A probability value (p value) less than 0.05 was considered statistically significant.

RESULTS

Group RC consisted of 18 (60.0%) female patients and group RB had 21 (70.0%) female patients, making a total of 39 female patients (65.0% of the total n=60). Group RC had 12(40%) male patients, and group RB had 9(30.0%) male patients, for a total number of 21 male patients (35.0% of the total n=60). There is no statistically significant difference in the gender distribution of the two groups (p value- 0.589).

the mean age of the study subjects was 45.63 ± 12.85 yrs. among subjects of RC group and 47.03±13.36yrs among RB group. The association between the study groups when comparing the age was statistically insignificant (p value 0.06).

the mean BMI of the study subjects was 24.06 ± 1.42kgs/m2 among RC group and 24.11 ± 1.48 kgs/m2 among RB group. The association between the study groups when comparing the BMI was statistically insignificant (p value- 0.901).

Among the RB group 53.3% were of ASA I and 46.7 % were ASA II and in the RC group 43.3% were of ASA I and 56.7 % were ASA II. In all 48.3 % were ASA I and 51.7 % were ASA II. Both groups with respect to ASA status were statistically insignificant (p=0.606) [Table1].

The mean of HR for the two groups was comparable at the baseline, mean of HR for RB group before starting epidural was 80.63±5.80 and for RC group, it was 80.23±5.59. The difference was statistically insignificant (p value = 0.79). A difference was observed in the mean of HR thereafter at different times of monitoring. It was observed that mean of HR was lower for RC group from 30 mins till 8 hrs which was statistically significant (in all readings the value was <0.05) [Table 2].

The mean of SBP for the two GROUPS was comparable at the baseline, mean of SBP in RC group was 124.33±8.03 mm hg and RB group, it was 126.87±7.49 mm hg. The difference was statistically insignificant (p value 0.21).

A difference was observed in the mean of SBP thereafter at different times of monitoring. It was observed that mean of SBP was lower for group RC from 30min till 8hr which was statistically significant (in all readings the p value was <0.05) like at 30min the mean SBP for RC group was 112.90±7.04 mm hg and for RB group was 118.77±7.15 mm hg which was statistically significant (p value <0.01). At 4hr mean SBP for RC group was 11.70±8.83mm hg and for RB group was 121.60 ± 4.86 mm hg which was statistically significant (p value <0.01). Overall it was seen the mean SBP was lower in the RC group. [Table 3]

The mean of DBP for the two groups was comparable at the baseline and before epidural, mean of DBP for RB group was 81.13±4.63 mm hg and for RC group, it was 80.37±8.76 mm hg. The difference was statistically insignificant (p value 0.53) .Even p value for DBP before giving epidural drug was less than 0.05.

A difference was observed in the mean of DBP thereafter at different times of monitoring. It was observed that mean of DBP was lower for the RC group from 45 min till 4hr which was statistically significant (in all readings the p value was <0.05) like at 45 mins the mean DBP for RC group was 73.17±4.50 mm hg and for RB group was 76.57±3.49mm hg which was statistically significant (p value <0.01). At 4hr mean DBP for RC group was 75.00 ±4.25mm hg and for RB group was 78.23±2.97 mm hg which was statistically significant (p value <0.01).Overall it was seen the mean DBP was lower in RC group. [Table 4]

The mean of MAP for the two groups was comparable before giving epidural drugs, mean of MAP for RC group was 93.66±4.24mm hg and for RB group, it was 95.56±5.5.13 mm hg. The difference was statistically insignificant (p value 0.12). A difference was observed in the mean of MAP thereafter at different times of monitoring. It was observed that mean of MAP was lower for RC group at 1hr,4hr 8hr which was statistically significant (in all readings the p value was <0.05) like at 1hr the mean MAP for RB was group 91.30±4.62 mm hg and for RC group was 85.67±6.46 mm hg which was statistically significant (p value <0.05).

At 4hr mean MAP for RB group was 92.77±3.13mm hg and for RC group was 87.20± 6.26 mm hg which was statistically significant (p value <0.01). Overall it was seen the mean MAP was lower in the RC group. [Table 5]

In case of RC group mean duration of onset of analgesia is 14.29±0.96 min, and in RB group it was

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14.42±0.82min. The difference obtained is statistically insignificant since p value >0.05.

Table also shows comparison of total mean duration of analgesia in two groups studied. In case of RC group, total mean duration of analgesia is 8.44±1.70 hrs and in RB group it is 17.90±3.54 hrs which was higher than the RC group, and the difference was found to be statistically significant with p value <0.01. [Table 6]

Since epidural was given postoperatively when patients started having mild pain (VAS>3), mean VAS for RC group was 3.26±0.76 and for RB group was 3.16±0.58 hence statistically insignificant (P =0.19).

The mean VAS at 1,2hr in both groups was '0' indicating no pain. So, none of the patients whether in the RC or RB group had pain till 2 hrs after getting epidural. The mean VAS score at 6hr in RB group 0.0 meaning patients not having pain at 6hr, where as in RC group some patients started having pain which showed Mean VAS score of 0.2 but the difference in both the groups was statistically insignificant p value 0.16.

A difference was observed in the mean of VAS thereafter at different times of monitoring, starting at 10hrs to 16hrs. At 10hr and 14hr, the mean VAS scores in group RB was lower than group RC with p value being significant (P<0.05). Mean VAS score of RB group at16,20,24hr were 0.28, 0.95,1.92 respectively. The VAS score monitoring was discontinued in patients when they received rescue analgesia, from both groups and exempted from statistical observation after that.

This gives impression that group RB had lower VAS scores than group RC.

The mean Bromage scale at 30min, in group RC was 0.17±0.38 and in RB group 0.07±0.25 and statistically insignificant since P value > 0.05. It means very few patients of both groups had bramage grade 1 of motor blockade i.e. patients had able to flex knees fully but mild affection of finger movements.

The mean bromage scores at 1hr showed 0.03±0.18 with P value >0.05, hence insignificant. Very few numbers of patients had bromage score grade I at 1hr which was statistically insignificant. Patients had no residual motor weakness at 4hr and 8hr interval. The initial motor blockade can be related to to recovery of motor blockade from the effect of the spinal anesthesia. The sensory blockade was more than the motor blockade.

Table 1: ASA grading distributionof the subjects

ASA grade	Group		Total
	RB	RC	
I	16	13	29
	53.3%	43.3%	48.3%
II	14	17	31
	46.7%	56.7%	51.7%
Total	30	30	60
	100.0%	100.0%	100.0%
p- value - 0.606			

Table 2: Mean heart rate variations with time

Pulse Rate	Group	N	Mean	SD	p- value
Baseline	RC	30	79.87	5.92	0.91
	RB	30	80.03	5.56	
Before EP	RC	30	80.23	5.59	0.79
	RB	30	80.63	5.80	
15 min.	RC	30	76.47	6.02	0.15
	RB	30	78.67	5.59	
30 min.	RC	30	72.43	5.59	<0.01
	RB	30	76.87	5.59	
45 min.	RC	30	67.73	6.01	<0.01
	RB	30	76.87	5.76	
1 hr.	RC	30	64.80	7.28	<0.01
	RB	30	77.00	5.85	
4 hr.	RC	30	61.57	9.48	<0.01
	RB	30	77.40	6.56	
8 hr	RC	30	66.50	8.14	<0.01
	RB	30	77.90	7.41	

Table 3: SBP variations with time with respect to the type of anaesthesiarecieved

SBP	Group	N	Mean	SD	p- value
Baseline	RC	30	124.33	8.03	0.21
	RB	30	126.87	7.49	
Before EP	RC	30	122.40	6.94	0.10
	RB	30	125.70	8.32	
15 min.	RC	30	117.90	7.20	0.06
	RB	30	121.63	7.96	
30 min.	RC	30	112.90	7.04	<0.01
	RB	30	118.77	7.15	

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45 min.	RC	30	108.87	8.16	<0.01
	RB	30	118.70	6.88	
1 hr.	RC	30	108.47	9.62	<0.01
	RB	30	119.63	6.93	
4 hr.	RC	30	111.70	8.83	<0.01
	RB	30	121.60	4.86	
8 hr	RC	30	116.07	6.13	<0.01
	RB	30	122.27	5.38	
DBP	Group	N	Mean	SD	p- value

Table 4: DBP Variations with Time in Both Groups

DBP	Group	N	Mean	SD	p- value
Baseline	RC	30	80.37	4.85	0.53
	RB	30	81.13	4.63	
Before EP	RC	30	76.90	14.96	0.19
	RB	30	80.67	4.29	
15 min.	RC	30	76.07	6.76	0.26
	RB	30	77.63	3.45	
30 min.	RC	30	76.20	9.96	0.85
	RB	30	76.57	2.97	
45 min.	RC	30	73.17	4.50	<0.01
	RB	30	76.57	3.49	
1 hr.	RC	30	73.17	4.55	<0.01
	RB	30	77.27	3.81	
4 hr.	RC	30	75.00	4.25	<0.01
	RB	30	78.23	2.97	
8 hr	RC	30	77.37	4.17	0.18
	RB	30	78.57	2.47	

Table 5: MAP Variations with Time in Study Groups

MAP	Group	N	Mean	SD	p- value
Before epidural	RC	30	93.66	4.24	0.12
	RB	30	95.56	5.13	
1 hr.	RC	30	85.67	6.46	<0.01
	RB	30	91.30	4.62	
4 hr.	RC	30	87.20	6.26	<0.01
	RB	30	92.77	3.13	
8 hr	RC	30	90.50	4.49	0.04
	RB	30	92.62	3.89	

Table 6: Mean Time of Onset and Duration of Analgesia

Variables	Group	N	Mean	SD	p- value
Analgesia Onset (min)	RC	30	14.29	0.96	0.55
	RB	30	14.42	0.82	
Duration of Analgesia (hrs)	RC	30	8.44	1.70	<0.01
	RB	30	17.90	3.54	

Table 7: Vas Score Variations with Time in Study Groups

VAS	Group	N	Mean	SD	p- value
Before EP	RC	30	3.26	0.76	P = 0.56
	RB	30	3.16	0.58	
1 hr.	RC	30	0.00	0.00	NA
	RB	30	0.00	0.00	
2 hr.	RC	30	0.00	0.00	NA
	RB	30	0.00	0.00	
6 hr	RC	30	0.20	0.76	0.16
	RB	30	0.00	0.00	
10 hr.	RC	30	2.13	1.74	<0.01
	RB	30	0.10	0.55	
14 hr.	RC	29	3.14	0.92	<0.01
	RB	29	0.28	0.84	
16 hr	RC	.	.	.	NA
	RB	26	0.95	1.46	
20 hr	RC	.	.	.	NA
	RB	26	1.92	1.62	
24 hr.	RC	.	.	.	NA
	RB	20	3.00	1.21	
32 hr.	RC	.	.	.	NA
	RB	12	3.33	1.44	
40 hr	RC	.	.	.	NA
	RB	.	.	.	NA

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DISCUSSION

In our study the demographic data in terms of age, height, weight and gender were comparable and showed no statistical difference between the two groups.

The onset of analgesia in both of our study groups was similar. In the group with Ropivacaine combined with clonidine the mean duration of onset of analgesia was 14.29±0.96 minutes and similarly in RB group it was 14.42±0.82 minutes. It was not statistically significant (p >0.05).

Similar findings were echoed in the study conducted by Christopher A and colleagues3, in which they studied 60 patients who were selected for post-operative analgesia following orthopaedic surgery. The patients were randomly divided into two groups of 30 each in which half of them were given 2.5 ml 0.5% bupivacaine in combination with buprenorphine 3µg/kg or 90 µg and the other half was given 2.5 ml of 0.5% bupivacaine combined with clonidine 1.5µg/kg or 75µg. They found out that the mean duration of onset of analgesia was 14.1±1.1 minutes in the buprenorphine group and 14.2±1.2 minutes in the clonidine group.

We observed that duration of analgesia in RB group was higher than RC group, which was statistically significant (P< 0.05). RB group's increased duration of analgesia can be explained by proposed mechanism of opiates, that they act locally and when deposited in epidural space diffuse through dura and bind to specific opiate receptors. The higher lipophilic substance, the greater will be its penetration through dura. The opiates with greater affinity for the receptor sites will produce longer duration of analgesia. Buprenorphine is a semi-synthetic opioid with partial agonist activity at the μ-receptor, partial or full agonist activity at the δ-receptor, and competitive antagonistic activity at the κ-receptor. It is a powerful analgesic, approximately 25–40 times as potent as morphine4.It also has a longer half-life with relatively lesser side effects. A preservative-free solution has high lipid solubility, strong affinity for opioid receptors and is, therefore, a logical choice to be used epidurally.[5]

In our study the mean VAS score before giving epidural drug for RC group was 3.26±0.76 and for RB group was 3.16±0.58 which was similar (P =0.19). The mean VAS at 1st and 2nd hour(hr) after the study drug was given through the epidural catheter in both groups had VAS score of '0' indicating no pain. Therefore, none of the patients in both groups experienced pain till 2 hrs after getting epidural. The mean VAS score at 6hr in RB group was 0, where as in the RC group 2 patients started having pain which showed Mean VAS score of 0.2 which was not statistically significant (p value=0.16).

A difference was observed in the mean VAS score thereafter at different times of monitoring, starting at 10hrs to 16hrs. At 10hr and 14hr, the mean VAS scores in group RB was lower than group RC with statistical significance (P<0.05). Mean VAS score of RB group at,[6] 20, 24hr were 0.28, 0.95 and 1.92 respectively. The VAS score monitoring was discontinued in patients when they received rescue analgesia and exempted from statistical observation after that time. It was implied that group RB had lower VAS scores than group RC.

The VAS scores in the study of Christopher A and colleagues,[3] at time intervals in minutes, T-480, T-540, T-600 and T-660 were significantly lower (P <0.05) in Buprenorphine group as compared to clonidine group. Similarly, our study group with Buprenorphine as adjuvant had lower VAS scores at 16^{th},$20^{th}$and 24^{th}hour compared to RC group of patients.

Profound analgesia of buprenorphine can be explained by its high affinity for spinal receptors. Additionally, higher lipid solubility of buprenorphine favours its diffusion into spinal cord and the diffusion from the spinal cord in to the blood stream is slower and does not approach the bulbar centres. Hence strong opiate receptor binding and high lipid solubility, were responsible for intense and prolonged duration of analgesic action.[6]

Mean Bromage scale in our study at 30min, in group RC was 0.17±0.38 and in RB group 0.07±0.25 and hence statistically insignificant (P > 0.05). 3 patients out of 30 in RB and 7 out of total 30 RC patients had bramage grade 1 motor blockade (patients were able to flex knees fully but mild affection of toes' movements). At 1st hour 2 patients had grade 1 motor blockade in RC group. At 4th ,8th hour, none of the patients in both groups had motor blockade. The residual motor blockade spinal anaesthesia given at the start of surgery must have contributed to the incidence of motor blockade to some extent. Patients had good sensory blockade than compared to motor blockade with use of 0.2% Ropivacaine as local anaesthetic. The maximum level of sensory block after epidural administration of study drugs in both the groups ranged between T6 to T10.

Similarly, in a study by Chhetty YK and colleagues,[7] who evaluated 80 parturients in active labour dividing them into two separate groups receiving lumbar epidurals with 0.125% and 0.2% ropivacaine in combination with fentanyl 2micrograms per ml in a volume of 15ml. Effective labour analgesia with no motor blockade was observed in both their study groups with no failure rate.

Similarly, SnigdhaPaddalwar and colleagues did a prospective,[8] randomized, double-blind study to compare the efficacy of Ropivacaine 0.125% and Bupivacaine 0.125% with Fentanyl 2 microgm/ml, in labour epidural analgesia and their effect on duration and course of labour. Authors quoted that Ropivacaine was introduced as S-enantiomer. In various human and animal studies, it was found to be having high sensory: motor differential blocking property and less of cardiotoxicity. Both these

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characteristics are beneficial for labour epidural analgesia. They noticed that no patient in group R(ropivacaine) developed motor block, whereas five patients in group B developed grade 2 (mild) motor block and concluded that Ropivacaine is equipotent, produces less motor block, has no adverse effect on the course and duration of labour, and can be used safely.

In our study we observed that high sensory: motor differential blockade with ropivacaine 0.2%. Thus, Ropivacaine with its efficacy, lower propensity for motor block appear to be an important option for regional analgesic pain management.[9]

CONCLUSION

Our study shows that the combination of Ropivacaine 0.2% with 150µ buprenorphine for post-operative epidural analgesia is superior when compared to Ropivacaine 0.2% with 75µg clonidine. The incidence of post-operative hypotension and bradycardia was also found to be lesser in the Ropivacaine- Buprenorphine group. The Ropivacaine - buprenorphine combination gives an extended duration of analgesia which is almost twice as Ropivacaine- Clonidine combination.

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