



A Comparative Study of Nerve Stimulator Versus Ultrasound-Guided Supraclavicular Brachial Plexus Block

Dr. Deepti N. Anandani¹, Dr. Shivangi Bhatt², Dr. Manisha s Kapdi³, Dr. Ami D. Patel⁴, Dr. Raju Baldaniya⁵, Dr. Vikas Singh Rajput⁶, Dr. Anushka Patel⁷

1) Assistant Professor, Department of Anaesthesiology, Smt.NHL MMC, Ahmedabad. 2) Consultant Anaesthesiologist, Civil hospital, Mansa, Gandhinagar. 3) Associate Professor, Department of Anaesthesiology, Smt.NHL MMC, Ahmedabad. 4) First year Resident, Smt.NHL MMC, Ahmedabad. 5) First year Resident, Smt.NHL MMC, Ahmedabad. 6) First year Resident, Smt.NHL MMC, Ahmedabad. 7) First year Resident, Smt.NHL MMC, Ahmedabad

Corresponding Author: Dr. Shivangi Bhatt,

E-603, Sarthak Era, Opp Hari Aalayam, Nr. Suryaretreat, Sargasan cross road, Gandhinagar-382421

Date of Submission: 17-07-2020

Date of Acceptance: 01-08-2020

ABSTRACT

BACKGROUND

Upper limb surgeries are mostly performed under peripheral nerve blocks such as the brachial plexus block. Peripheral nerve blocks not only provide intraoperative anesthesia but also extend analgesia in post-operative period without any systemic side effect. This study was carried out with the objective of comparing the supraclavicular brachial plexus block by using nerve stimulation and ultrasound guided techniques for upper limb surgeries.

AIMS AND OBJECTIVES

This study was carried out with the objective of comparing the supraclavicular brachial plexus block by using nerve stimulation and ultrasound guided techniques for upper limb surgeries in terms of block execution time, time of onset of sensory and motor block, time to achieve complete block, success rate of block procedure, duration of sensory and motor block, incidence of complications, time to first analgesic request.

MATERIAL AND METHODS

Hundred patients, aged 18-60 years, ASA grade I and II of either sex posted for elective upper limb surgeries were randomly divided into two groups, group US (n=50) and group NS (n=50).

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS Statistics software. The parametric data were analyzed with unpaired "t" test and the nonparametric data were analyzed with Chi-square test.

P value	Inference
>0.05	Non Significant
<0.05	Significant
<0.001	Highly Significant

RESULTS AND CONCLUSION

- Time taken for the block performed by ultrasound was shorter than the nerve stimulator guided technique.
- Onset of sensory and motor blockade were earlier in group US than group NS.
- Time to achieve complete block was shorter in group US than group NS.
- Success rate of the block was more in group US than group NS.
- Perioperative hemodynamic parameters were stable in both groups.
- Duration of sensory and motor blockade were prolonged in group US than group NS.
- Time to first analgesic request was also prolonged in group US as compared to group NS.
- Incidence of complications like vessel puncture, nerve injury was seen only in group NS.

KEYWORDS: Supraclavicular brachial plexus block, nerve stimulator, ultrasound guidance, upper limb surgeries.

I. INTRODUCTION

Kulenkampff^[2] first described the classical supraclavicular approach to the brachial plexus in 1912. Supraclavicular brachial plexus block provides consistently effective regional anaesthesia to the upper extremity. The brachial plexus block can be performed by conventional, nerve stimulator (NS)-guided or ultrasound (US)-guided technique.

The classical approach - paraesthesia technique is a blind technique associated with higher failure rate and injury to nerves and vascular structures^[3]. Peripheral nerve stimulator was introduced in 1962^[4] allowing better localization of



the nerves/plexus^[5,6], However, also had persistent risk of injury to surrounding structures especially vascular structures nerves and pleura leading to pneumothorax^[7,8].

Ultrasound (US) in regional anaesthesia offers a new standard in nerve-location and identification. The advantages of US guided regional Anaesthesia include: (i) ability to visualize and identify the target nerve(s) and their relationship to surrounding structures (ii) allow for patient variability (e.g. size, shape, anatomical variations); (iii) determine depth, angle, and path of the needle to the target nerve; (iv) real-time Visualization of the technique and guidance of the needle to the target; (v) visualization of the spread of local anaesthetic and placement of a catheter; (vi) allow the procedure to be carried out safely (e.g. children) and even to be repeated if ineffective; (vii) portability and safety (no ionizing radiation).

So, this study was planned to compare nerve stimulator guided technique and ultrasound guided technique of supraclavicular brachial plexus block for upper limb surgeries.

AIMS OF THE STUDY

This study was carried out with to compare supraclavicular brachial plexus block by using nerve stimulation and ultrasound guided techniques for upper limb surgeries in terms of

1. Block execution time
2. Time of onset of sensory and motor block
3. Time to achieve complete block
4. Success rate of block procedure.
5. Duration of sensory and motor block.
6. Incidence of complications.
7. Time to first analgesic request.

Equipments prepared: A portable sterile tray containing:

- | |
|--|
| • Disposable syringes of 10 ml. |
| • Disposable 23G 60mm needle (group US) or insulated needle (group NS). |
| • Bowls containing povidone iodine, spirit and normal saline solutions. |
| • Sponge holding forceps. |
| • Sterile towel and towel clip. |
| • Drugs injection 1.5% Lignocaine with 1:200000 adrenaline 20ml and injection 0.5% Bupivacaine 10ml. |

No sedation was given till evaluation of the block was completed.

Position: The patients were positioned supine and head turned to the opposite to side of intended block. A small pillow or folded sheet was placed below the shoulder to make the field more prominent. The proposed site of block was

II. MATERIALS AND METHODS

After approval by the IRB committee all,100 patients who satisfied the inclusion were selected

Inclusion criteria:

- | |
|---|
| • ASA grade 1 and 2 patients of either sex. |
| • Age between 18 and 60 years. |

Exclusion criteria:

- | |
|--|
| • Patients who refuses to participate in the study, |
| • Patient suffering from coagulopathy |
| • Known allergy to the local anesthetics |
| • Skin infection at the proposed site of block |
| • Pulmonary pathology |
| • Pre-existing neurological deficit in the upper limbs |
| • Patients less than 18 years. |
| • Pregnant females. |

Pre-anaesthetic assessment: Included

- History taking
- General and physical examinations
- Laboratory and radiological investigations
- Systemic examination
- Procedure to be carried out was explained.
- Informed written consent was taken from the patient and VAS score explained
- All patients advised nil by mouth

Preparation: An intravenous line was secured. Pulse oximeter, blood pressure and ECG monitoring was started. All patients were pre medicated with Inj. Glycopyrrolate 0.004 mg/kg IV and Inj. Ondansetron 0.08 mg/kg IV before surgery. Ultrasound machine and probe cleaned or Electrical nerve stimulator were kept ready for the procedure.

aseptically prepared with iodine, spirit and normal saline solution and draped properly.

Landmarks: A point 1cm above the midpoint of clavicle and pulsations of subclavian artery. The patients were randomly divided into two groups:

- **Group US:** Ultrasound guided
- **Group NS:** Nerve stimulator guided



III. PROCEDURE

Group US: - A Sonosite Micromax linear probe (6-13 MHz) was used for conducting the block. The probe was inserted into a sterile plastic sheath to maintain sterility. It was then placed in the coronal oblique plane in the supraclavicular fossa. The subclavian artery, vein, and the brachial plexus were visualized. The brachial plexus and its relationship to the surrounding structures were scanned. The plexus was identified superolateral to the subclavian artery consistently in all the cases. Next, a 23 G 60 mm needle was connected to a 10 cm extension line and primed with the drug. It was inserted using in-plane approach and the needle movement was observed in real time. Once the needle reached the plexus, after negative aspiration, drug was injected and the spread of the drug was observed. When necessary, the needle was repositioned to achieve an ideal perineural distribution of the drug.

Group NS: - In this group, the positive electrode of the NS was attached to an ECG lead and stuck on the ipsilateral arm. The subclavian artery was then palpated and immediately lateral to it, a 23G insulated needle attached to the negative electrode of the NS was inserted in a backward, inward, and downward direction. NS was set to deliver a current of 2 mA in the internal mode.

After finger flexion was elicited with stimulation, the current was reduced in steps of 0.2 mA till the

presence of a muscle twitch with 0.5 mA was observed and no twitch with a current of 0.2 mA was observed. This confirms the proximity of the needle tip to the nerve and the drug was injected after negative aspiration for air or blood.

Hemodynamic monitoring was carried out at every 5 minutes interval up to 15 minutes and from 30 up to 300 minutes at every 30 minutes.

Following parameters were noted:

Block execution time: 1. In the group US, it is calculated from the time of initial scanning to the removal of the needle.

2. In the group NS, it is from the time of insertion of the needle to its removal.

Onset of sensory blockade

It was assessed by pin prick every 2 min till the onset of sensory block. The time from the removal of block needle to the time when the patient first says he/she has reduced sensation when compared to the opposite limb.

Onset of motor blockade

The onset of motor blockade was assessed every 2 mins. It is the time of removal of the block needle to the time when the patient had weakness of any of the three joints – Shoulder, elbow, or wrist, upon trying to perform active movements.

Evaluation of sensory block: Hollmen scale [10]

1.	Normal sensation of pin prick
2.	Pin prick felt as sharp pointed but weaker compared with same area in the upper limb.
3.	Pin prick perceived as touch with blunt object
4.	Complete loss of pin prick sensation

Evaluation of motor block: Hollmen Scale [10]

1.	Normal muscle function
2.	Slight weakness in function
3.	Very weak muscular function
4.	Complete loss of function

Time to achieve complete block: Interval between block execution time to complete loss of pin prick sensation and motor function.

Success: We considered our block to be successful when the patient had a full block of all the sensory dermatomes and no power to move above-mentioned joints.

Failure of block: Inadequate analgesia, sensory blockade or motor blockade even after 30 minutes of injection of local anaesthetic solution.

Duration of sensory blockade: Interval between onsets of sensory block to the first time pin prick sensations are felt again.

Duration of motor block: Interval between onset of motor block to the time when the patient is able to move the blocked limb.

Time to first analgesic request: interval between onset of sensory block and the first time when the patient complains of pain.

Complications:

1.	Vessel Puncture (Hematoma)
2.	Horner's syndrome
3.	Pneumothorax
4.	Neurological sequelae



This table II shows that block execution time as well as time of sensory and motor block were shorter in group US than group NS.

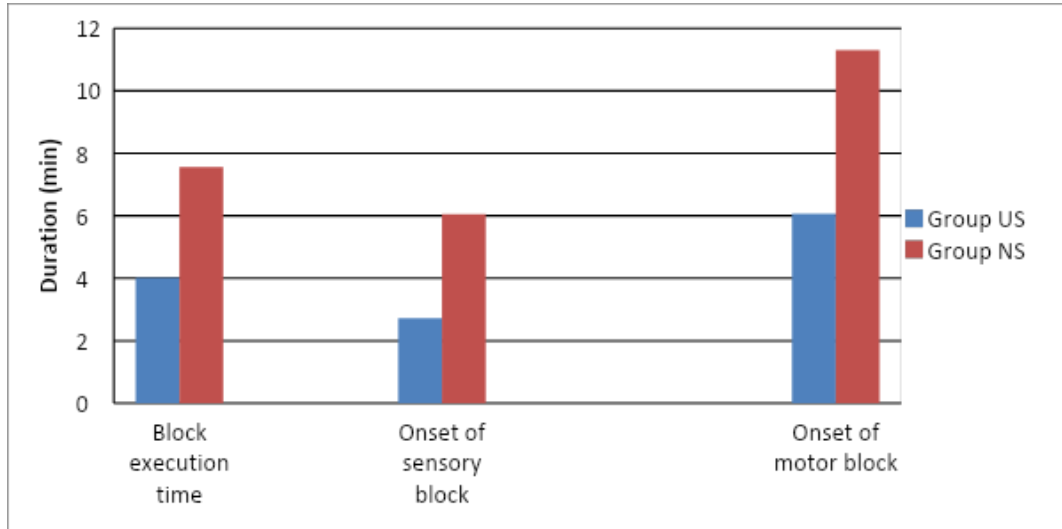


Chart-I: Characteristics of block
Table III-Success Rate of the block

Assessment of block	Group US	Group NS	P value	Inference
Successful	49 (98%)	46 (92%)	0.169 (Chi square test)	Non Significant
Failed	1	4		

This table III shows that failure of block resulted in 1 patient in group US and 4 patients in group NS and were supplemented with general anesthesia.

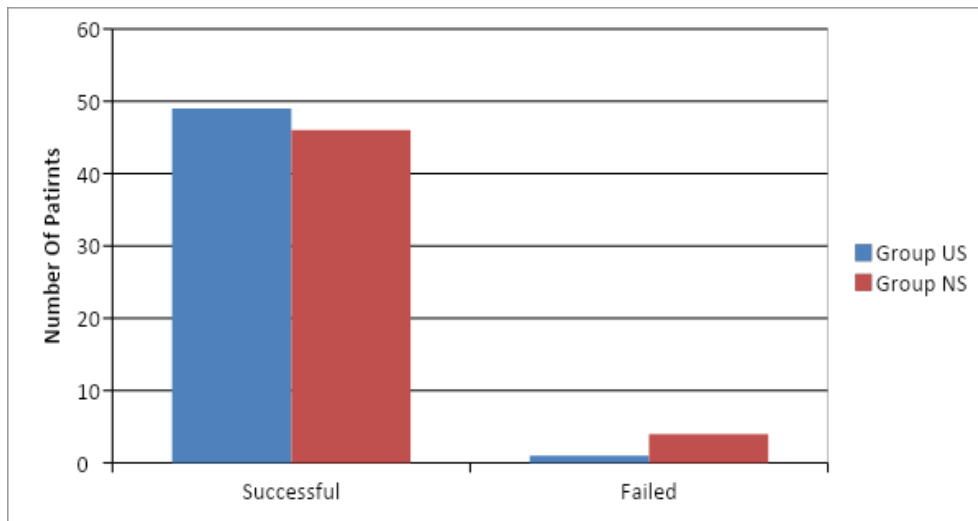


Chart-II: Success rate of block

Table IV- Time to achieve complete block

Duration (min)	Group US n=49	Group NS n=46	P value	Inference
	12.92 ± 1.11	17.07 ± 1.08	<0.0001	Highly Significant

This table IV shows that time to achieve complete block was shorter in group US than group NS and was statistically significant.

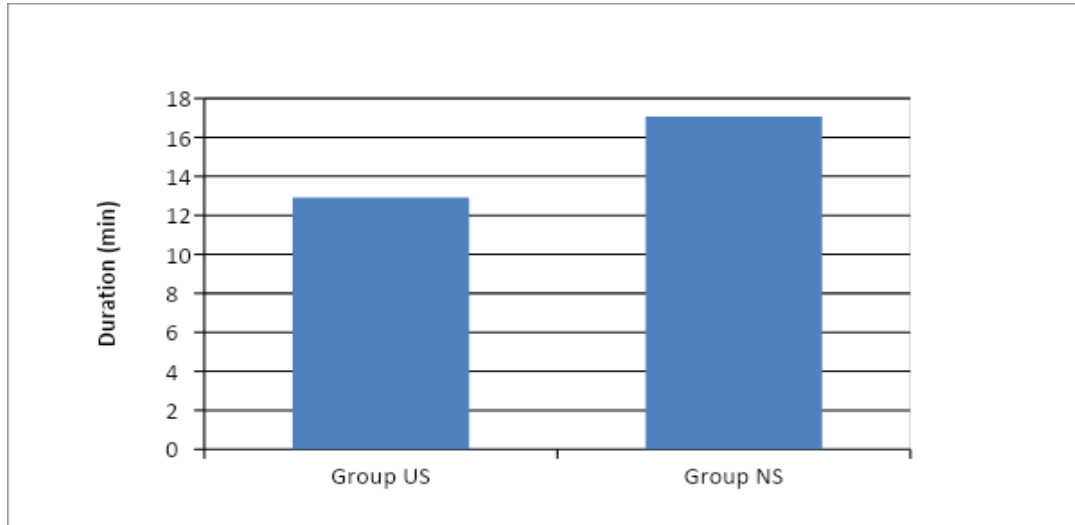
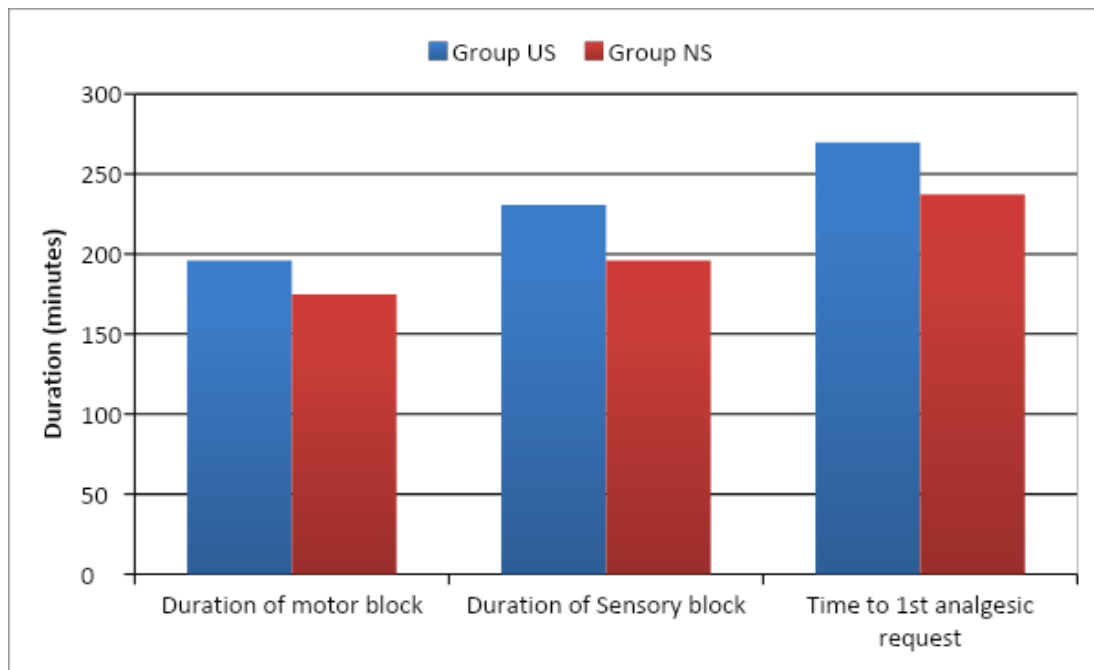


Chart-III: Time to achieve complete block

Table-V Duration of anaesthesia and analgesia

Time (Minutes)	Group US n=49	Group NS n=46	P value	Inference
Duration of motor block	196.04±19.81	174.76±15.45	<0.0001	Highly Significant
Duration of sensory block	230.57±19.73	195.85±15.00	<0.0001	Highly Significant
Time to 1 st analgesic request	269.59±17.01	237.32±15.99	<0.0001	Highly Significant



This table V shows that mean duration of sensory block and motor block and time to 1st analgesic

request are significantly longer in group US as compared to group NS(p<0.0001).



Chart-IV: Duration of anaesthesia and analgesia

There were 2 cases of vessel puncture observed in Nerve stimulator guided supraclavicular brachial plexus block, while no complications were noted in usg guided block.

In our study, each patient was monitored for **vitals** in each group. All patients were **hemodynamically stable** intraoperatively and postoperatively.

V. DISCUSSION

Supraclavicular block provides a rapid, dense, and predictable anaesthesia of the entire upper extremity.

A successful brachial plexus block depends not only on the technique, but also on the anaesthetists experience, patient's body habitus, amount and type of drug injected, level of motivation of the patient.^[11] With the advances in imaging and wider availability, ultrasound made its application in peripheral nerve blocks with the advantage of optimizing the spread of the local anaesthetic solutions around the nerves, shortening of latency of the block and improving the onset and completeness of sensory and motor blockade.

The use of ultrasound to perform peripheral nerve blocks is a relatively new technique rapidly gaining popularity over the traditional techniques of peripheral nerve stimulators and paraesthesia. The objective of this study is to discuss which technique offers more advantages: Ultrasound or Nerve Stimulator.

DEMOGRAPHIC VARIABLES:

In our study both groups were comparable with respect to age, gender, weight and ASA grade of the patients. No significance difference was found in between two groups. ($p > 0.05$)

HEMODYNAMIC PARAMETERS:

In our study both groups were comparable in terms of heart rate, systolic and diastolic blood pressure, respiratory rate and oxygen saturation of the patients and found to be stable.

Our data correlated with studies done by M.Veeresham et al^[12] and Singh G et al^[13].

BLOCK EXECUTION TIME:

The mean block execution time was significantly less in group US, 4.02 ± 0.96 min as compared to group NS, 7.56 ± 1.05 min. ($p < 0.0001$)

RuperaKB et al^[14] also found that procedure time in US group was 4.55 ± 0.74 minutes and in group PNS, it was 5.71 ± 0.92 minutes.

Williams SR et al^[11] also found that the average procedure time in nerve stimulator guided group was 9.8 minutes and 5.0 minutes in US guided group for supraclavicular brachial plexus block.

Mani KV et al^[15] found that mean time required for performing ultrasound guided technique was 2.58 minutes and for PNS it was 5.82 minutes.

The possible reasons for the less time taken in performing US guided technique could be due to direct visualization of the structures and accuracy of needle placement. The less time taken to perform the procedure can also be attributed to a fair amount of expertise and readiness with all the equipment and drugs as and when needed^[16,17].

ONSET OF SENSORY AND MOTOR BLOCK:

The mean onset time for sensory and motor block was found significantly less for group US, 2.72 ± 0.99 min and 6.06 ± 1.41 min as compared to group NS, 6.05 ± 0.90 min and 11.30 ± 0.84 min respectively. ($p < 0.0001$)

Rupera KB et al^[14] also found that onset time of sensory and motor block was 2.97 ± 0.72 min and 4.55 ± 0.78 min in US group and in NS group, it was 3.63 ± 0.76 min and 5.13 ± 0.71 min.

Ratnawat A et al^[18] found that onset of sensory and motor block was 6.46 ± 1.02 min and 8.10 ± 1.02 min in US group and in PNS group, it was 7.68 ± 1.33 min and 9.94 ± 1.28 min respectively.

The likely explanation for faster onset of sensory and motor blockade could be that ultrasound can determine the size, depth and exact location of the brachial plexus and its neighbouring structures. Also with US guidance, positioning and if required repositioning of the needle is performed under direct vision and in real time as opposed to blind redirection and repositioning of needle with nerve stimulator^[18,19].

TIME TO ACHIEVE COMPLETE BLOCK:

In our study, we found that time to achieve complete block was 12.92 ± 1.12 min in group US which was shorter as compared to 17.07 ± 1.08 min in group NS. ($p < 0.0001$)

Rupera KB et al^[14] also found that time to achieve complete block was 13.17 ± 1.54 min in group US and 16.93 ± 1.83 min in group PNS.

Ratnawat A et al^[18] also found that time to achieve complete block was 13.74 ± 1.11 min in group US and 16.11 ± 1.54 min in group PNS.

SUCCESS RATE OF BLOCK:

The block was successful in 98% of patients in group US compared to 92% in NS group. These



were comparable both clinically and statistically. This was not statistically significant. ($p > 0.05$) Ratnawat A et al^[18] also found that the block was successful in 90% in group PNS and 97.5% in group US.

DURATION OF SENSORY AND MOTOR BLOCK:

The mean duration of sensory and motor block was 230.57 ± 19.73 minutes and 196.04 ± 19.81 minutes in US group was found significantly prolonged compared to 195.84 ± 15.00 minutes and 174.76 ± 15.45 minutes in NS group. ($p < 0.0001$)

Rupera KB et al^[14] found that mean duration of sensory and motor block in US group was 5.29 ± 0.82 hours and 5.05 ± 0.67 hrs. And in PNS group, it was 4.73 ± 0.81 hours and 4.58 ± 0.73 hours.

Ratnawat A et al^[18] also found that mean duration of sensory and motor block in US group was 8.13 ± 1.63 hours and 7.13 ± 1.63 hours and in PNS group, it was 6.14 ± 2.36 hours and 5.14 ± 2.36 hours respectively.

Kapral S et al^[20] (2008) found that sensory, motor, and extent of blockade was significantly better in the ultrasound group when compared with the nerve stimulation group.

It could be due to accurate deposition and spread of local anaesthetics around the nerve plexus in ultrasound guided group^[21]. The reason for delay in onset of action in nerve stimulator guided blocks can be attributed to distant spread of injected drugs away from perineural tissues thus limiting the duration of sensory and motor blockade.

TIME TO FIRST ANALGESIC REQUEST:

In our study, time to first analgesic request in group US was 269.59 ± 17.01 minutes which was more than 237.33 ± 15.99 minutes in the group NS. This was statistically significant ($p < 0.0001$)

William SR et al^[11] also conducted similar study using the same drug combination and the duration was 846 ± 531 min and 652 ± 473 min in the groups US and NS, respectively.

Raghove P et al^[21] found that duration of analgesia in group USG was 312 ± 54 min and in blind group it was 232 ± 47 min.

COMPLICATIONS:

In our study not a single complication was identified in US group as compared to group NS; in which incidence of artery puncture was 4%.

Ratnawat A et al^[18] also found no complications in US group as compared to group PNS; in which incidence of artery puncture was 10%.

Singh G et al^[13] also found 10% incidence of vessel puncture/hematoma in CB group compared to 3.33% in US group.

Kapral S et al^[22] (1994) observed no complications such as pneumothorax, puncture of a major blood vessel, paresis, or irritation of the plexus, the recurrent laryngeal nerve, or the phrenic nerve in his study of ultrasound guided supraclavicular approach brachial plexus blockade.

There was no incidence of nerve injury and pneumothorax in both the groups. This could be because ultrasound facilitates the identification and avoidance of important structures, and direct visualization of local anaesthetic spread resulting in selective blocks with higher accuracy and fewer complications^[23].

VI. CONCLUSION

Ultrasound guided technique was more effective than nerve stimulator guided technique for supraclavicular brachial plexus block in terms of block execution time, onset and duration of sensory and motor block, time to achieve complete block, success rate, time to first analgesic request and incidence of complications.

BIBLIOGRAPHY

- [1]. A Hadzic and J. D. Vloka, Eds., Halstead. Peripheral Nerve Blocks: Principles and Practice, The New York School of Regional Anaesthesia, New York, NY, USA, 2004.
- [2]. Kulenkampff D, Persy MA. Brachial plexus anesthesia: its indications, technique and dangers. *Ann Surg.* 1928;87:883-91.
- [3]. Suzanne Carty, Barry Nicholls, Ultrasound guided regional anaesthesia, oxford journals, medicine BJA; CEACCP; 2007; 7; 1:20-24
- [4]. Greenblatt GM, Denson JS. Needle nerve stimulator-locator: nerve blocks with a new instrument for location of nerves. *AnaesthAnalg.* 1962;41:599-602.
- [5]. Fanelli G, Casati A, Garancini P, Torri G. Nerve stimulator and multiple injection technique for upper and lower limb blockade: failure rate, patient acceptance, and neurologic complications. *AnesthAnalg* 1999; 88: 847-52.
- [6]. Haleem S, Siddiqui AK, Mowafi HA, Ismail SA, Ali QA, nerve stimulator evoked motor response predicting a successful supraclavicular brachial plexus block, *Anesth Analg*;2010;110(6)1745-6
- [7]. Mak P H, Irwin M G, Ooicg B F; Incidence of diaphragmatic paralysis following supraclavicular brachial plexus block and its



- effects on pulmonary function, *Anesthesiology* 2001;56(4):352-6
- [8]. Brown DL, Cahill DR, Bridenbaugh LD. Supraclavicular nerve block: Anatomic analysis of a method to prevent pneumothorax. *AnesthAnalg* 1993; 76:5304
- [9]. Sites, Brian D.; Chan, Vincent W.; Neal, Joseph M. ; Weller, Robert; Grau, Thomas MD; Koscielniak-Nielsen, Zbigniew J.; Ivani, Giorgio. The American Society of Regional Anesthesia and Pain Medicine and the European Society of Regional Anaesthesia and Pain Therapy Joint Committee Recommendations for Education and Training in Ultrasound-Guided Regional Anesthesia, *Regional Anesthesia and Pain Medicine*, Volume 34, issue 1, January-February 2009: 40-46.
- [10]. Singh R, Gupta D, Jain A. The effect of addition of intrathecal clonidine to hyperbaric bupivacaine on postoperative pain after lower segment caesarean section: A randomized control trial. *Saudi J Anaesth*. 2013 Jul-Sep; 7(3): 283–90.
- [11]. Williams SR, Couinard P, Arcand G, Harris P, Ruel M, Boudreault D, et al. Ultrasound guidance speeds execution and improves the quality of supraclavicular block *AnesthAnalg*. 2003;97:1518-23
- [12]. M. Veeresham, Upender Goud, P. Surender, Pavan Kumar. “Comparison between Conventional Technique and Ultrasound Guided Supraclavicular Brachial Plexus Block in Upper Limb Surgeries”. *Journal of Evolution of Medical and Dental Sciences* 2015; Vol. 4, Issue 37, May 07; Page: 6465-6476, DOI: 10.14260/jemds/2015/939
- [13]. Singh G, Mohammed YS. Comparison between conventional technique and ultrasound guided supraclavicular brachial plexus block in upper limb surgeries. *IJSS*. 2014;2(8):169-76.
- [14]. Rupera KB, Khara BN, Shah VR, Parikh BK. Supra-Clavicular Brachial Plexus Block: Ultra-Sonography Guided Technique Offer Advantage Over Peripheral Nerve Stimulator Guided Technique. *Natl J Med Res*. 2013;3(3):241-4.
- [15]. KarthiVellakalpatti Mani, ArunpandiyarVeerapandiyar, Shankar Radhakrishnan. A study on comparison between ultrasound guided technique and peripheral nerve stimulator guided technique in performing brachial plexus block (supraclavicular approach) for upper limb surgeries *International Journal of Medical and Health Research* Volume 3; Issue 7; July 2017; Page No. 61-64
- [16]. Anthony R. Brown. Ultrasound versus peripheral nerve stimulator for peripheral nerve blockade: Pros and cons. *Anaesthesiologia* 2008; 31:S79-84.
- [17]. Perlas A, Chan VW, Simmons M. Brachial plexus examination and localization using ultrasound and electrical stimulation: a volunteer study. *Anesthesiology* 2003 Aug;99(2):250-1.
- [18]. Ratnawat A, Bhati FS, Khatri C, Srinivasan B, Sangwan P, Chouhan DP. Comparative study between nerve stimulator guided technique and ultrasound guided technique of supraclavicular nerve block for upper limb surgery. *Int J Res Med Sci* 2016; 4:2101-6.
- [19]. Simpson G, Nicholls B. Use of ultrasound in chronic pain medicine. Part 1: neuraxial and sympathetic blocks. *Contin EducAnaesthCrit Care Pain*. 2013; 13:145-51.
- [20]. Kapral S, Greher M, Huber G, et al. Ultrasonographic guidance improves the success rate of interscalene brachial plexus blockade. *Reg Anesth Pain Med* 2008; 33: 253–8
- [21]. Raghove P, Singh K, Taxak S, Ahlawat M, Hooda S. Comparison of Ultrasound Guided Technique with Conventional Landmark Technique for Supraclavicular Brachial Plexus Nerve Block in Patients Undergoing Upper Limb Surgery. *Int J Pharmacol and Clin Sci*. 2016;5(1):1-4.
- [22]. Kapral S, Krafft P, Eibenberger K, Fitzgerald R, Gosch M, Weinstabl C. Ultrasound-guided supraclavicular approach for regional anaesthesia of the brachial plexus. *AnesthAnalg*. 1994;78:507-13
- [23]. Yuan JM, Yang XH, FU SK, Yuan CQ, Chen K, LI Ji, et al. Ultrasound guidance for brachial plexus block decreases the incidence of complete hemi-diaphragmatic paresis or vascular punctures and improves success rate of brachial plexus nerve block compared with peripheral nerve stimulator in adults. *Chin Med J*. 2012; 125:1811-6.