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Regular paper

Comparative evaluation of a local anesthetic effect between lignocaine and lignocaine administered with epinephrine in healthy children

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Lignocaine a first amino amide-type local anesthetic, when combined or co-administered with epinephrine, a sympathomimetic amine, allows to administer larger doses for numbing, to decrease bleeding, and to make the numbing effect last longer. The study presented here focuses on measures to prove this activity in patients with abdominal surgery. Liposomal formulations of lignocaine and lignocaine plus epinephrine were prepared by a thin film evaporation method. This formulation was injected successfully as liposomal infusion. Thus prepared liposomes were found to be fit for drug delivery when evaluated as per physicochemical parameters. The smooth, even surfaced liposomes with PDI of 0.298 (p<0.05) were found to be efficient in delivering the drug when tested *in-vitro* (lignocaine as a single drug was at 93.78%, and from combined dosage lignocaine was at 96.29% with 94.62% of release of epinephrine). The randomized, controlled trial was conducted with a population of children that had undergone abdominal surgery and who were grouped into three groups depending upon the type of formulation they received. The three groups of subjects were first one receiving lignocaine liposomal infusion only; second one with lignocaine plus epinephrine liposomal infusion; the third group served as control and received a saline solution only. The serum Cortisol concentration was found to be the least in Group II when compared to Group I. Similarly, end point measurements such as the cool sensation, warm sensation, hot pain, and the sensory blockade test had indicated the superiority of combination of lignocaine with epinephrine in lowering the pain threshold. The result obtained from the above study has shown that epinephrine markedly enhances the local anesthetic activity of lignocaine.

Key words: child anesthesia, epinephrine, lignocaine, liposomal infusion, local anesthetic

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Abbreviations: ACF, Lignocaine + Epinephrine; $EtCO_2$, end tidal CO_2 (carbon dioxide); EPC, egg phosphatidylcholine; FLACC, the face, legs, activity, cry, consolability scale; LA, local anesthetic; PACU, post-anesthetic care unit; PDI, polydispersity index; SOP, standard operating procedure; VAS, visual analogue scale

INTRODUCTION

Acute pain in abdominal regions, especially in the case of children, is reason that leads to dilemmas while diagnosing the disease. Despite the fact that most of abdominal pains are benign, a rapid diagnosis with suitable treatment can be a great help to reduce the mortality rate. Age, symptoms and the pain site are some of the factors affecting pain intensity in the abdomen. The most often encountered surgical and non-surgical conditions associated with the abdominal pain are appendicitis and gastroenteritis, respectively (Herroeder *et al.*, 2002). Here, the age, symptoms and location of pain are important factors to consider. Patients history and reports of physical examination greatly help to determine the root cause of an acute pain of the abdomen, as well as to identify a surgical condition. Better results can be obtained by efficient acquisition of the children's history with physical examination accompanied by laboratory, as well as radiological studies. There is a tendency of children with atypical symptoms to interfere with diagnosis and good decision making. Pediatric patients are less able to provide the desired information which makes the treatment somewhat tedious (Herroeder *et al.*, 2007).

After a major abdominal surgical procedure, the major concern is often pain associated with operation. Pain is often considered as the reason for increased hospital stay after surgery and is a component of the inflammatory response. The aftermath of pain is often associated with delayed activities related to bowel movement and development of ileus as a result of activation of nociceptors by inflammatory mediators (Vigneault *et al.*, 2011). Therefore, pain management is a big issue and traditionally was dealt with by administering opiates, although this has some serious risks associated specifically with the pediatric groups. On one hand, it blunts the stress response, provides rapid mobilization, and leads to early extubation with rapid recovery of the bowel function. On the other hand, insertion of an epidural catheter carries its own risks, especially in the pediatric population. Therefore, seeking for alternative and/or adjunct drugs and techniques should continue, especially in the era of fast track surgery and enhanced recovery programs (Herroeder *et al.*, 2007; Vigneault *et al.*, 2011).

Lidocaine has been shown to have analgesic, antihyperalgesic and anti-inflammatory effects when administrated intravenously. Several studies have shown a role of intravenous lidocaine administration during abdominal surgery in improving postoperative analgesia, reducing postoperative opioid requirements, accelerating the postoperative recovery of bowel function, decreasing postoperative fatigue, reducing the duration of hospitalization, and enhancing acute rehabilitation in patients undergoing major abdominal surgery (Kaba *et al.*, 2007). However, all of these studies were carried out in an adult popula-

tion and did not involve the pediatrics' one. In the study presented here, as a primary outcome we aim to evaluate the role of systemic lidocaine administration in children undergoing elective major abdominal surgery in regard to the length of hospital stay. We also aim at studying its effect on the hormonal response, opioid requirement and return of bowel function (Marret *et al.*, 2008; Dirks *et al.*, 2000).

After administration of a local anesthetic (LA), multiple actions on the muscles and nerves take place that lead to a net increase or decrease in circulation, which may removes the drug from the site of action. Furthermore, the blood flow changes can be reversed over time, thereby naturally decreasing a local distribution in the body tissues, lowering concentration of plasma LA. The vasomotor effects of lignocaine are concentration dependent (Niemi *et al.*, 2002).

Epinephrine, a sympathomimetic amine interacting with adrenergic receptors, shows unequivocal relationship between concentration and pain after surgery. When administered exogenously, it shows induction in pain. The epinephrine added to LA in order to prolong the action via vasoconstrictor effect resulting in reduction of tissue perfusion and oxygen availability (Gaumann *et al.*, 1992; Bernards *et al.*, 1999).

Thus, anesthesiologists prefer the combination of epinephrine with lignocaine during peripheral nerve block procedures, as this system offers advantages in reduction of LA plasma concentration, nullifying the chances of systemic toxicity and enhancing the quality and duration of anesthesia. A concept has been generally accepted which states that epinephrine exerts this effect via its vasoconstrictor action through adrenergic receptors on neural vasculature. The observed effects, such as smooth muscle contraction and decreased blood flow, result in reduced lignocaine clearance. An increased LA block by epinephrine when combined with lignocaine (by enhancement of submaximal LA doses) could result from pharmacokinetic factors that increase the intraneural LA concentration or pharmacodynamic actions on the nerve membrane (Catherine *et al.*, 2003).

MATERIALS AND METHODS

Materials. ignocaine and epinephrine was generously gifted by Shouguang Fukang Pharmacy Factory (Shandong, China). Egg phosphatidylcholine (EPC) and dipalmitoyl phosphatidylcholine were kindly gifted by Lipoid GmbH, Ludwigshafen Germany; disodium hydrogen phosphate, potassium dihydrogen phosphate, sucrose and chloroform were purchased from Shanghai Chemical Co. (Shanghai, China). All other materials were of analytical or reagents grade.

Methods. Fabrication of liposomes of lignocaine and lignocaine + epinephrine. Thin film hydration method was used to formulate both types of liposomes. In a round bottom flask, EPC and cholesterol were dissolved in chloroform with different molar ratios. 100 mg of ACF dissolved in methanol (5 ml) was added to the lipid solution. The rotary evaporator (Heidolph) was used to remove the organic solvent under reduced pressure at 40°C, to get very thin film of the dry lipids on the inner surface of the round bottom flask. This dry film was slowly hydrated with 15 ml of saline phosphate buffer (pH 7.4). The resulting suspension was mechanically shaken for 1 h at room temperature, using a shaker to form multilamellar liposomes. The liposomal dispersion was left overnight at 40°C to ensure complete lipid hydration. The drug loaded liposomes were separated from the unentrapped ACF by centrifugation at 30000 rpm for 3 hr at –5°C, using an ultracentrifuge with cooling. The cryoprotectant trehalose was dissolved in phosphate buffered saline at 5 g/g of dry phospholipids. The liposomal suspensions in the buffer alone or after mixing with an equal volume of each cryoprotectant buffered solution, were first freeze dried where the liquid was frozen at –50°C, and then freeze dried for 40 h under vacuum at –10°C to get the ACF loaded liposomal powder. Either the liposomal suspension or the powder were used for further analysis (Vyas *et al.*, 2013).

In this method of preparation, the drug concentration for lignocaine was taken as 20 mg/ml and for epinephrine 0.02 mg/ml. Thus prepared liposomes were stored in a well closed container and reconstituted with sterile water for injection when required.

Evaluation of Liposomes. Morphology of liposomes. Morphology, including shape, size and surface of the liposomes were studied with a field emission scanning electron microscopy (FESEM-S4800, Hitachi, Japan). A drop of liposomal suspension was made electrically conductive by mounting it on abrass stub using a double sided adhesive tape under vacuum in an ion sputter (Vyas *et al.*, 2013).

Drug loading. The percentage of drug loading in liposome was evaluated by using 3.0 ml of suspension. The unentrapped (free) drug was separated by using Sephadex G-50 mini column presaturated with empty liposomes and centrifugation at 7000 rpm for 10 min. Eluate was digested using Triton- X-100 solution (0.1% V/V) and the resulting solution was analyzed using UV spectrophotometer to estimate the drug loading (Vyas *et al.*, 2013).

Particle size determination. The particle size and PDI of the liposomes were determined by a laser scattering technique using nanozeta-sizer (ZS 90, Malvern Instruments, UK) at 25°C. The obtained liposomal suspension was diluted to 10 times in distilled water as a dispersion medium and sonicated before analysis for 1 min. The sample SOP was generated at a refractive index of 1.52, 243.8 of count rates (kcps) and 0.8872 (mp s) of viscosity (Vyas *et al.*, 2013; Pankaj *et al.*, 2009).

Encapsulation efficiency in liposomes. About 100 g of liposomal powder was taken and added to absolute alcohol to carry out the lysis of liposomes. This dispersion was further sonicated for 10 min to complete removal of the drug from the liposomes and then filtered through a 0.45 μm membrane filter and estimated for drug content by UV spectrophotometer. The drug entrapment efficiency was calculated using the formula

$$\text{Drug Encapsulation Efficiency} = \frac{\text{Estimated \% drug content}}{\text{Theoretical \% drug content}} \times 100$$

***In-vitro* release study**. Dialysis membrane diffusion technique was used to determine the drug release from the liposomes. In brief, an accurately measured amount of solutions of epinephrine Lignocaine and drug loaded liposomal formulations; equivalent to 20 mg was placed in dialysis tubing. The tube was tied at both ends. This dialysis tubing was suspended in buffer solution (pH 6.8, 200 ml, 37±2°C). The whole set was placed on the magnetic stirrer adjusted to 150 rpm speed. The samples (5 ml) were collected every 1 h and fresh 5 ml buffer was added to maintain the sink condition for the period of 10 h and drug release was estimated spectrophotometrically (Vyas *et al.*, 2013; Pankaj *et al.*, 2009).

Protocol for experiment. For the experimental period of about 6 months, all subjects (children 2–6 years of age) who were expected to undergo a major abdominal surgery were selected for this study. Children who suffered from any kind of disease, such as hepatic, renal, or cardiac disease, and had any kind of allergy to local anesthetics, were not included in the study. The whole experimental pattern was approved by an ethical committee. The full experimental design was explained to the parents/legal guardians of the children enrolled for trial and the informed consent was taken in a written form . All of the procedures performed in this trial were conducted by or under supervision of expert medical professionals, especially the pediatric surgeons.

The patients were randomly divided into three groups. Solutions were prepared based on the patient allocation. The liposomal injections of lignocaine (20 mg/ml), lignocaine + epinephrine (0.02 mg/ml), and the saline solution were prepared. Patients in group one were administered with liposomal injection of lignocaine (2%), while patients in group two were treated with liposomal injection of 2% lignocaine and epinephrine at 0.02 mg/ml. Plain saline solution was given to patients in the third group. Twenty five minutes before induction of anesthesia, the patients had received an i. v. bolus (0.1 ml/kg), followed by an i. v. liposomal injection at a rate of 0.1 ml/kg/h; the infusion then continued for 6 h postoperatively.

Blood pressure, heart rate, and end tidal carbon dioxide concentration were monitored. Target ventilation was maintained at $EtCO_2$ at 4–4.5 kPa. Ketoprofen at a dose of 2 mg/kg of body weight given intravenously and 15 mg/kg paracetamol was given intra-operatively if required.

Collection of samples for analysis. Blood samples were collected pre-operatively and at 10 minutes after start of infusion. Serum level of lidocaine was checked at periodic intervals. Samples were centrifuged immediately and stored at –60°C and analyzed by a radio-immune assay technique.

End point determination. Patients were then moved into post-anesthetic care unit (PACU) in order to measure the level of pain post-operation every 15 minutes, by specially trained nurses. The use of FLACC scale was employed here in order to assess the pain. The FLACC scale rates on basis of Face, Legs, Activity, Cry, and Consolability. The intensity of pan is rated from 0 to 2 by a trained observer and information is given by a volunteer. When the score of 9–10 is reached by patients, they are allowed to leave the PACU and relocated to the ward. The postoperative pain was assessed in them at intervals of 1, 2, 5, 10, 24, 36 h using the same FLACC scale. At the same time, the degree of sedation was measured using the Ramsay sedation scale. The team of physiotherapists was kept for assessment of the patient's activity based on a standardized hospital protocol. Reporting any abnormal body function, such as seizures, was done immediately. Children were discharged once they were able to tolerate a light diet, were pain free or tolerated pain with analgesics and were ambulate unaided.

Thermal threshold testing. The thermal thresholds (3) were established in the central portion of the treated area; warm, cool and hot pain. The order of stimuli was kept the same as it progresses from the lowest to the highest stimulus. The meander electrode was used to measure the warm and cool sensations; it consists of electrodes of alternating polarity and the gap is filled with an insulating material. The temperature of the thermode was either increasing or decreasing at a rate of 1.0°C/second, depending on the direction of the current flow through the device. The patient holds a switch that is pressed at the first sensation of warmth or coldness; pressing the switch reverses the temperature change, returning to a neutral temperature of 32°C. The warm pain measurements also used the Thermal Sensory Analyzer, but the end point was pain instead of the temperature change sensation (Yarnitsky *et al.*, 1991).

Depth of anesthesia (pain intensity score measurement). The test was performed in blindfold subjects by mounting a sterile 27-gauge short needle perpendicular to the skin. The needle was pinned gently in one direction with controlled and continuous movement to the targeted site. The needle was pinned in slowly and smoothly. The intensity of pain was measured at first sensation of pain felt by the subject and recorded as a Visual analogue scale (VAS) read out, as 'no pain' (0) to 'worst pain' (10). The needle was removed at a first sensation of pain. The needle was changed each time for each individual subject. To avoid human error, the same investigator performed all of the measurements for all groups.

The same end point was measured using depth of the needle insertion. After completion of treatment, a sterile 27 gauge short bevel needle was inserted into the forearm of the patient and thumb roll knob on gauge scale was rotated downwards which inserts the needle downwards. The readings were recorded at increments of 0.001 mm. The needle was removed immediately after the first sensation of pain and reading was noted as shown on a readout device (Yarnitsky *et al.*, 1991; Gasser *et al.,* 1929; Gissen *et al.,* 1985).

Visual effects on the skin. Surface of the skin was examines visually for any observable signs, such as redness, swelling, etc.

RESULTS

The film evaporation method was found to be successful for the preparation of both types of liposomal formulations. The surface morphology study using SEM (Fig. 1) had revealed a spherical nature with smooth surface for lignocaine alone and lignocaine +epinephrine combined liposomes. It also confirmed a uniform and un-cracked surface which is important in order to prevent leakage of the drug from the liposome and increases the encapsulation efficiency. The zeta potential, particle size and the polydispersity index (PDI) were measured using light scattering technology. The particle size was found to be in the range of 147.34 nm to 184.23 nm, with an average particle size 165.78 nm for lignocaine liposomes, while combined drug liposomes

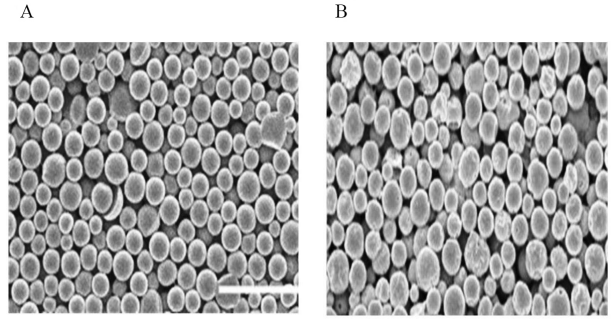

Figure 1. Scanning electron microscope image of liposomal formulations of A: Lignocaine, B: Lignocaine + Epinephrine.

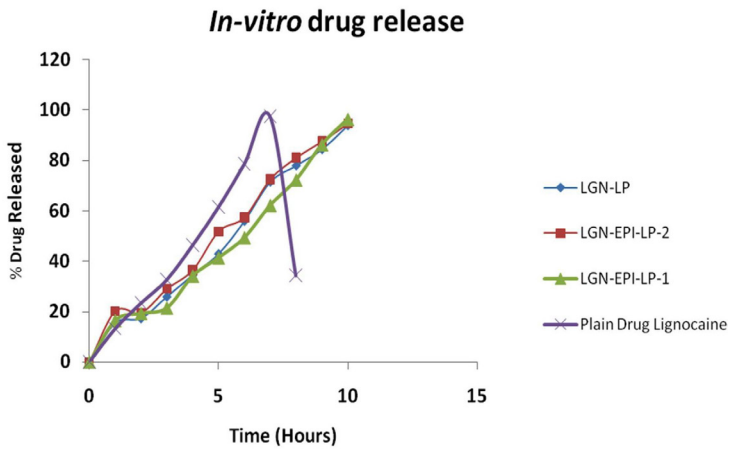

Figure 2. ***In-vitro*** **release study of lignocaine formulation showing that epinephrine can have a release enhancing effect on lignocaine when co-administered.**

formed particles in the range of 150.5 nm to 180.35 nm, with an average particle size of 165.80 nm. The polydispersity index for the system with single drug was found to be 0.298 ($p<0.05$), while for the drug combination it was 0.347 ($p<0.05$), indicating a uniformed, monodispersed and narrow distribution in size in both liposomal systems. The entrapment efficiency for lignocaine in liposomes was found to be at 78.45% for the single drug system, while in combination with epinephrine it was raised to 81.23%, with 79.74% of entrapped epinephrine in the combined drug liposomal system.

The *in-vitro* release study for both formulation was observed for the period of 10 hrs by using dialysis tubing. The lignocaine release from liposomes was found to be at 93.78%, while when combined with epinephrine the release of lignocaine was found to be at 96.29%, with 94.62% of epinephrine release. Figure 2 explains the release pattern of both formulations.

In total, 61 subjects were entered in the trial, out of which 3 were eliminated due to a reason of hepatic insufficiency and one subject denied to participate in the trial. Thus, out of 61 only 57 subjects were enrolled in the experimental trial. All of the subjects that were enrolled in the trial, had successfully completed it, with no in between dropouts. The baseline characteristics were the same through the study between the experimental groups. All subjects underwent a similar type of surgery (Table 1). These 57 subjects were randomly divided into three groups. The groups were named after the type of medication they received: all of the subjects in group I were administered with the liposomal injection of lignocaine only, whereas those in group II received combination of lignocaine and epinephrine, and group III served as control was only administered with a saline solution. The results obtained for group I were compared with those in group II and group III.

Table 1. Demographic and surgical details of the population enrolled in the trial.

Factors	Group I	Group II	Group III
Age (year)	3.4±1.2	3.7±0.9	3.6±1.4
Weight (kg)	15±2.1	17±0.7	16±2.0
Gender Male Female	 57.7% 42.3%	 61.45% 39.55%	 58.00% 42.00%
Surgical time (min)	95±10.7	96±9.67	99±8.6
Surgery performed	Splenoctomy	Splenoctomy	Splenoctomy

The serum cortisol concentrations were found to be in the range of 25–30 µg/dl as a basal value which was found to be increasing with increasing time in case of the control group where no drug treatment was done. About 40 µg/dl in the cortisol level was found for the control group at 30 minutes, where children with lignocaine liposomes maintained that level around 33 µg/dl. Lignocaine and epinephrine liposomal formulation had shown the best results in maintaining the level of cortisol close to normal as shown in Fig. 3.

All of the subjects who underwent the IP treatment experienced a very mild type of skin irritation which was completely subdued in 2–5 min after completion of treatment No patient entered in the trial had reported an swelling, itching or edema.

The time courses of mean pain intensity after the treatment to all groups is shown in Fig. 4.

The post treatment pain intensity of group II was numerically found to be much greater just after treatment i.e. at 0 hr, when compared with group I and controls. The pain was found to be subdued as time passed in all 3 formulations. In case of all formulation groups, the intensity of pain at baseline or at 0 hr was found to be numerically the same, but the as the time passed, the pain of group II patients a was relieved at a greater extent. In fact, after 20 min, no patient felt the pain at all. Whereas in case of group I and control formulation the intensity of pain was lowered, but the score was lesser when compared with group II. The patients felt the pain even after 30 min in case of group I and control formulations. Thus, this statistical difference in the pain scores proves the superiority of group II formulation over the other.

Comparison of baseline values or values at 0 time course indicates that there is no significant difference between the treatment conditions for any end point, i.e. for each end point the response at 0 hr is nearly the same for all treatment groups.

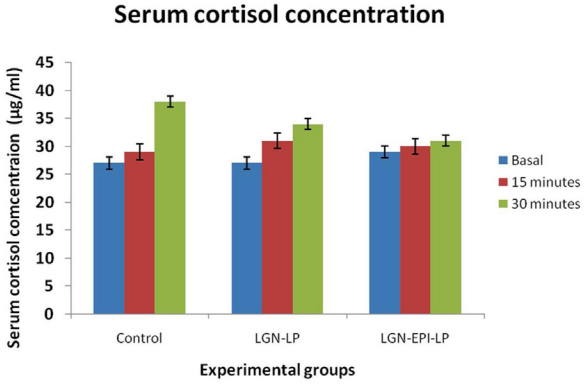

Figure 3. Serum cortisol concentration (ug/dl) calculated post-operatively.

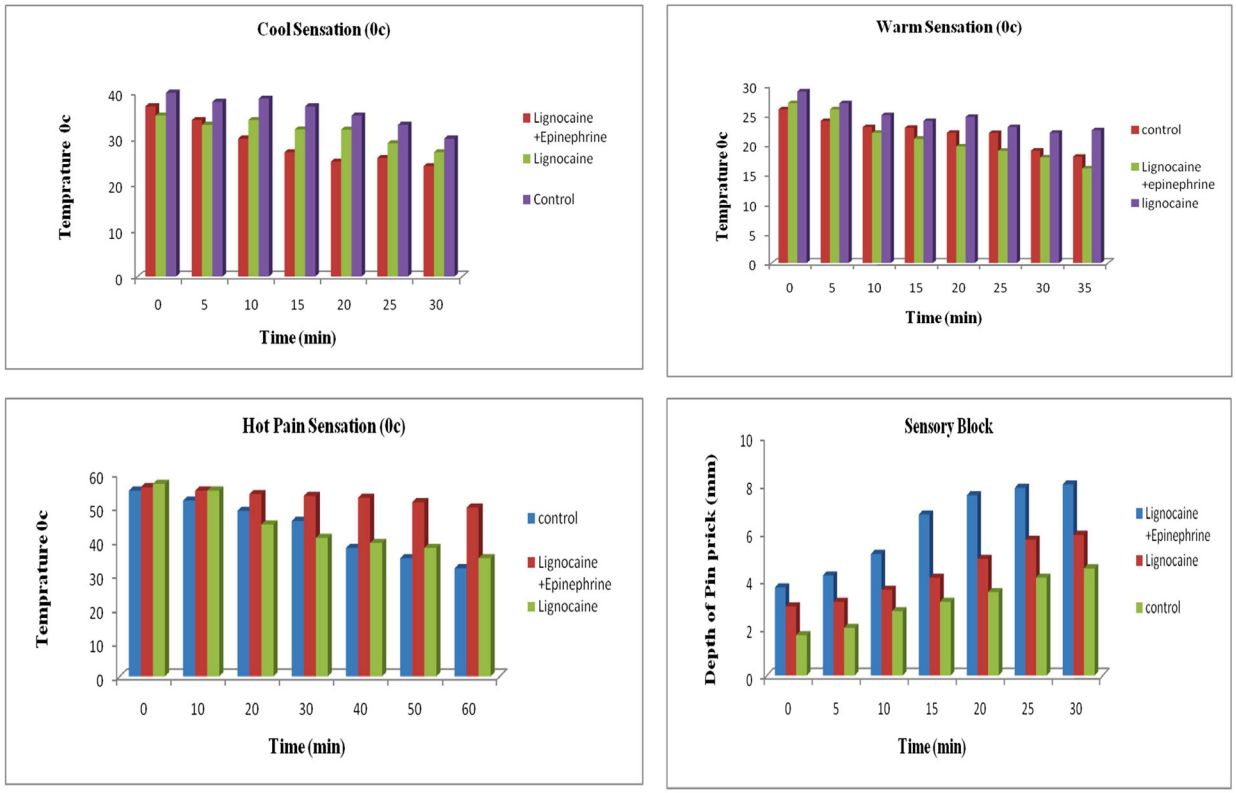

Figure 4. End point measurements showing superiority of combining epinephrine with lignocaine liposomes.

As can be seen in Fig. 4, in case of cool sensation there was no significant difference between the 3 test groups at 0 hr, but as the treatment time had passed the ability of group II to combat the cool sensation had increased, i.e. anesthetic effect had increased. This enhanced effect helped to reduce the cool sensation. This ability had increased in the case of group II when compared to group I and the control group.

As shown in Fig. 4, the difference in sensation for all groups is lesser at the baseline or at 0 hr, but it increases as the treatment proceeds. Formulation administered to group II was found to be superior in decreasing the warm sensation when compared to group I and the control group. Figure 4 also depicts the effect of epinephrine and lignocaine concentration on hot pain. As can be seen**,** the baseline values for all groups are almost same. As the time passed from 0 min to 30 min, the hot pain sensation had sharply decreased for group II. The effect on depth of anesthesia is also presented in Fig. 4. It measures the distance in mm up to which the needle can be inserted. From Fig. 4 one can clearly state that the distance of the needle prick is the greatest for group II group, i.e patients are more anesthetized in this group, which indicates that depth of anesthesia is greater in group II than in others.

DISCUSSION

The current investigation focuses on the dose response relationship and effect of concentration of epinephrine on the anesthetic activity of lignocaine. The results obtained from current investigation suggest that adrenaline, if administered exogenously, possesses a pain reducing capacity when co-administered with lignocaine. As stated earlier, the sympathomimetic amine exerts its action *via* interaction with adrenergic receptors. They are thought to be delaying the absorption velocity of a local anesthetic from the site of injection. The physical responses obtained from patients (like inflammation) involves a complex set of cellular reactions which may be biochemical or cellular. Several researchers had made hypotheses regarding the effect of adrenaline on various local anesthetics. It is possible that direct receptor mediated pharmacodynamic effects of adrenaline contribute to the soothing effect (i.e. reduction in pain intensity) of adrenaline.

Physical responses obtained from the patients in the trial present here had shown variation and distinct patterns of physical responses. Patients in the treatment group without epinephrine (control) had observed the highest pain intensity in the procedure time course, as there was no effect of either epinephrine or lignocaine itself. The initial mean pain intensity observed for treatment group II shows approximately the same level of pain intensity as group I or the control group, but rapidly decreases as the time of treatment passes. When compared to treatment group II, group I and control had experienced a comparatively lesser decrease in the pain intensity.

Our study suggests that treatment group I and the control group had initially experienced an early similar degree of local anesthesia as group II in case of warm sensation and hot pain threshold, but the depth of anesthesia experienced by group II became greater as the treatment time had passed. The effect on cool sensation was also greater for group II than for group I or control. For the skin, the temperature above 34°C and below 32°C produces the sensation of warmth and coolness, respectively. Politei and others (Politei *et al.*, 2016) had stated that the cool sensations are results of a response by small myelinated fibers, while warmth and pain sen-

sations are due to small unmyelinated fibers. The study undertaken here supports the fact that myelinated fibers are more sensitive to local anesthetic blockade as cool threshold.

Thus, it can be stated that the effects observed for group II are significantly higher than for the other treatment groups i.e. group I or control, and confirm the fact that co-administration of epinephrine with local anesthetic lignocaine significantly increases its anesthetic activity in a concentration or dose dependent manner. Thus, we can state that increasing concentrations of epinephrine increase the anesthetic activity of lignocaine. The total physical responses obtained in group II are of a relatively low intensity.

CONCLUSIONS

Lignocaine when combine with relative doses of Epinephrine offers great advantages for relieving pain associated with major abdominal surgeries in children concentration of Epinephrine increases the pain relieving effect also increases. So, from the above investigation it can be concluded that co-administration of epinephrine with Lignocaine possesses pain relieving potential which influence the physical responses like cool, warm threshold and hot pain.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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