Randomised controlled trial of eutectic mixture of local anaesthetics cream for venepuncture in healthy preterm infants

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Abstract

***Aim*—To assess the safety and efficacy of EMLA cream (eutectic mixture of local anaesthetics) used to induce surface anaesthesia for venepuncture in healthy preterm infants.**

***Methods*—Nineteen infants, median gestational age 31 weeks (range 26–33 weeks) were assessed in a randomised, double blind, placebo controlled, cross-over trial. Changes in physiological variables (heart rate, blood pressure, oxygen saturation) and behavioural responses (neonatal facial coding system score, crying time) before and after venepuncture with EMLA cream were compared with those obtained with a placebo cream to assess efficacy. Toxicity was assessed by comparing methaemoglobin concentrations at 1 hour and 8 hours after application.**

***Results*—There was no significant difference in efficacy between EMLA and placebo creams in physiological and behavioural responses. There was no significant difference in methaemoglobin concentrations one hour after the cream had been applied. At eight hours, however, concentrations were significantly higher after EMLA than placebo (p=0.016). There was no evidence of clinical toxicity.**

***Conclusion*—This study does not support the routine use of EMLA for venepuncture in healthy preterm infants.**

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Keywords: methaemoglobin; surface anaesthesia; EMLA; venepuncture

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Venepuncture and intravenous cannulation are painful procedures commonly performed in the neonatal intensive care unit. In infants below 30 weeks of gestational age the number of procedures performed is higher, reflecting the severity and length of their illness.[1] A recent review of practice in the United States of America showed that analgesic agents were used for 2% of venepunctures and 10% of intravenous cannulations.[2]

The long term effects of such practice are unknown, but may make preterm babies hypersensitive after injury and affect the family's mental and physical wellbeing and their attitudes towards medical and nursing staff.[3–4] It has also been suggested that the responses to pain can increase the risk of hypoxic episodes and intraventricular haemorrhage.[5] Recent work suggests that neonatal circumcision may result in long term changes in an infant's pain behaviour due to altered central neural processing of painful stimuli.[6] Very preterm infants studied at 4½ years showed a higher incidence of somatic complaints of unknown origin than children who had been born at term.[7]

EMLA is an oil in water emulsion of an eutectic mixture of prilocaine and lignocaine. It is an effective local anaesthetic cream for procedures such as venous cannulation, venepuncture, lumbar punctures and subcutaneous drug reservoir punctures in older children.[8–10] There is evidence that it may be effective in younger children and infants over 3 months of age for venepuncture.[11 12]

Very few reports have looked at the use of EMLA in infants younger than 3 months, mainly due to concern about possible toxicity from methaemoglobinemia. This arises because metabolites of prilocaine oxidise haemoglobin to methaemoglobin. Methaemoglobin reductase, which converts methaemoglobin back to haemoglobin, is low in neonates (40–60% of adult values), rising to adult values by 3 months.[13–14] Increased percutaneous drug absorption can also occur in infants below 32 weeks, particularly in the first fortnight, as a result of poor development of the stratum corneum of the skin.[15] This may increase the risk of methaemoglobinemia by increasing absorption of prilocaine.

EMLA has been used in neonates as surface anaesthetic before circumcision, heel prick, lumbar puncture and percutaneous line insertion, arterial puncture, venepuncture and suprapubic aspiration, but its efficacy has not been consistently demonstrated.[16–20] A Medline search from 1966 and a manual search of library journals did not reveal any previous randomised controlled trial for measuring the efficacy of EMLA for venepuncture in preterm infants.

A recent study of toxicity documented no clinically significant rise in methaemoglobin concentrations after application of EMLA to the heel of preterm infants.[21] It is not known if application of EMLA to other sites—dorsum of the hand or foot—is as safe. We therefore investigated the safety and efficacy of topical EMLA cream for use as surface anaesthesia before venepuncture in healthy preterm infants.

Methods

Preterm infants with gestational ages between 26 and 36 weeks, admitted to the Special Care

Baby Unit, Peterborough District Hospital, were eligible for inclusion into the study. Twenty patients were included in the study. Nineteen patients completed the study (one patient was discharged early and had venepuncture with only one cream. Hence these data were excluded). Ten were boys, 18 were caucasian, and one Asian. The median (range) gestational age was 31 (26–33) weeks and median (range) postnatal age at inclusion into the study was 21 (3–65) days. The median (range) birthweight was 1.564 (0.916–2.246) kg.

The study was approved by the local hospital ethics committee. Written parental consent was obtained for each infant.

All infants were clinically well at the time of entry into the study and were expected to stay in hospital for at least two weeks. Exclusion criteria included those requiring oxygen, those receiving any analgesia or sedation, those receiving drugs causing methaemoglobinemia. Infants with renal or hepatic insufficiency, neuromuscular dysfunction, and dysmorphic features were also excluded. The study was carried out between December 1995 and July 1996.

Each infant was studied during routine blood sampling. Infants were randomly assigned to receive either EMLA or a placebo cream (aqueous cream) on the first occasion. The alternate cream was used on the second occasion. The creams were dispensed in 1 ml syringes in 0.5 ml aliquots. Randomisation and blinding were done by one of the authors (JP) in the hospital pharmacy.

The procedure was performed between 7 and 9 am. All babies had been fed within two hours of the venepuncture. The doctor performing the procedure (AA or PB) applied 0.5 ml of cream over a prominent vein on the dorsum of the hand or foot, in a thick layer, and covered it with occlusive dressing (Tegaderm 3M; Ontario, Canada). After 60 minutes the dressing was removed and the cream wiped off with sterile gauze. A blood pressure cuff was applied to the arm and a pulse oximeter sensor to the foot. The infant was then allowed to settle for 10 minutes. Recordings were begun at the end of this period. The infant's state of arousal, as described by Prechtl, was noted.[22]

Each infant was bled by the same doctor on both occasions. AA performed 22 and PB performed 16 of the venepunctures. Gentle, steady pressure, applied with the thumb and index finger encircling the hand or foot, was used to make the chosen vein prominent while the remaining fingers of the same hand helped stabilise the hand or foot.

A 21 gauge needle was inserted into the vein to obtain steady blood flow and samples were collected. The needle was removed at the end of blood sampling and cotton wool applied to obtain haemostasis.

The procedure was divided into three phases. The pre-procedure phase, from beginning of recordings to needle insertion, lasted 2.5 minutes. The procedure phase lasted from needle insertion to needle removal, and the post-procedure phase, from needle removal to end of recordings, lasted 2.5 minutes.

Heart rate and oxygen saturation were recorded at 30 second intervals through all the three phases from a Nellcor Escort Pulse Oximeter (MDE Arieta California) connected to the infant. Blood pressure (systolic/diastolic) was recorded twice during each phase of the procedure using a Graesby Ossilomate model 901(CAS medical systems Inc. Brentford, CT USA) neonatal blood pressure monitor.

The infants' facial action and cry were recorded from a distance of 1 metre, using a Yashica Samurai Hi 8 KXH1 colour video camera. Verbal cues were given by the doctor performing the procedure at the point of limb holding, needle insertion, and needle removal. Recordings were transferred to a videotape using a standard videocassette recorder at normal speed.

Thirty seconds of the tape before needle insertion and 30 seconds of the tape after needle insertion for all infants with each cream were analysed by two of the authors (RB) and (PB) on separate occasions. Both were blinded to the type of creams used. Each gave a neonatal facial coding system score (NFCS) for the two periods—maximum score 10 and minimum score 0. The scores of the two were averaged and used for analysis.

Each infant's cry on the videotape after needle insertion was timed using a digital stopwatch to the nearest tenth of a second and rounded off to the nearest second.

The following definitions were used: duration of first cry: from onset to end of first cry (cessation of crying for 5 seconds). Total duration of crying: from onset of fist cry to cessation of all crying for 30 seconds.

Difficulty of venepuncture was recorded on a 4 point scale:1= first attempt successful, 2= minor adjustment needed, 3= second insertion required, 4= failure of two attempts.

Local changes at the site of cream application were recorded as pallor, redness, or no change.

To measure methaemoglobin concentrations 0.2 ml of blood was collected in an EDTA container one hour after cream application, along with other routine samples for full blood counts and electrolyte estimation. A similar sample was obtained by a separate venepuncture 8 hours after cream application for methaemoglobin estimation. Infants were observed carefully for clinical toxicity for the next 24 hours. Samples were sent to the laboratory soon after collection and methaemoglobin concentrations (percentage of total haemoglobin) measured in an IL482 co-oximeter (Instrumentation Laboratory Warrington UK).

STATISTICAL ANALYSIS

Tabulation and statistical analysis were performed using Microsoft excel and Minitab computer software packages. The median difference between averaged pre-procedure and procedure phase recordings of heart rate, systolic and diastolic blood pressure, oxygen saturation; NFCS score; and duration of first

Table 1 Median (range) pre-procedure and procedure phase values for physiological and behavioural parameters and methaemoglobin levels with placebo and EMLA cream

	Placebo		*EMLA*	
Variable	*Pre-procedure phase*	*Procedure phase*	*Pre-procedure phase*	*Procedure phase*
Heart rate(bpm)	157 (126–170)	159 (127–181)	158 (134–175)	163 (144–179)
Oxygen saturation %	97 (92–99)	96 (91–99)	98 (95–100)	96 (93–98)
Systolic BP mm Hg	61 (45–85)	69 (56–87)	63 (45–88)	67 (51–86)
Diastolic BP mm Hg	36 (23–51)	41 (29–50)	39 (27–54)	39 (25–62)
NFCS*	1 (0–6)	5 (0–8)	0 (0–4)	6 (0–7)
DFC^ (s)	NA	13 (0–411)	NA	6 (0–260)
TDC** (s)	NA	43 (0–411)	NA	6 (0–361)
Methaemoglobin % 1 h	NA	1.2 (0.7–2.0)	NA	1.6 (0.7–4.4)
Methaemoglobin % 8 h	NA	1.5 (0.8–2.1)	NA	1.7 (1.2–6.7)

*NFCS=neonatal facial coding system score.
^DFC=duration of first cry.
**TDC=total duration of crying.

cry and total crying times for each cream was compared using the Wilcoxon signed rank test. This test was used to compare the state of infant arousal at the time of the study between the two creams. Interobserver variation of the NFCS score was assessed using the Bland and Altman method.[23]

Comparison of local skin change between EMLA and placebo was done using the McNemar's test for paired binary data. The same test was used to compare the difficulty of venepuncture with the two creams.

A two sample *t* test was performed for each criteria to look for any carry over effect.

Results

Nineteen patients completed the study. No adverse reactions were seen. Table 1 shows the median averaged pre-procedure and procedure phase recordings of heart rate, systolic blood pressure, diastolic blood pressure and oxygen saturation obtained with placebo and EMLA cream.

There was no significant difference between the creams (table 2).

Table 1 shows the median values of the averaged neonatal facial coding system score for pre-procedure and procedure phases with each cream. There was no significant difference in treatment effect between the creams (table 2).

There was good agreement in the neonatal facial coding system score given by the two observers: 95% range for the pre-procedure phase; interobserver difference mean (± 2 SD) = −0.4 (−2.8 to 2.0) and 95% range for the procedure phase interobserver difference mean (± 2 SD) = −0.9 (−3.6 to 1.9).

Table 1 shows the median duration of first cry and total duration of crying with placebo and EMLA. The median within patient difference between the creams was not significant (table 2).

Table 2 Estimated median differences between EMLA and placebo

Variable	*95% Confidence intervals**	*P value (non parametric)*
Heart rate	−2.5 (−8.8 to 3.2)	0.376
Systolic BP	−3.5 (−8.2 to 1.25)	0.126
Diastolic BP	−3.0 (−7.5 to 1.75)	0.220
Oxygen saturation	0.11(−1.2 to 1.22)	0.825
NFCS score	0 (−2.00 to 1.75)	0.984
Duration of first cry	−6.5 (−22.5 to 11.5)	0.446
Total duration of crying	−22 (−96 to 24)	0.240
Methaemoglobin 1 h	0.3 (−0.1 to 0.7)	0.112
Methaemoglobin 8 h	0.5 (0.1 to 2.3)	0.016

*Note: negative value means that the variable median is lower with EMLA than placebo cream.

Table 1 shows the median methaemoglobin percentages at 1 and 8 hours after application of placebo and EMLA. Four 8 hour placebo samples were not available (two not collected as patients had been transfused and two patients discharged home early) and one 1 hour EMLA sample was missing.

The median 1 hour methaemoglobin percentages were not significantly different between the two creams. The median 8 hour methaemoglobin percentages were significantly higher after EMLA than after placebo (table 2).

There was no significant difference between the creams in terms of local reactions or difficulty of venepuncture. The state of arousal was not different between the creams.

Discussion

Using a combination of physiological and behavioural responses to venepuncture, we have shown that EMLA is no better than a placebo cream for use in preterm infants. This could be due either to there being no treatment effect, or there being a small treatment effect that was not detectable in a study of this size.

The limb holding required before needle insertion may have been painful and therefore reduced the beneficial effect of EMLA on the venepuncture. Review of the pre-procedure neonatal facial coding system scores showed no response (score =0) to hand holding in 23 of the 38 venepunctures and minimal response (score =1-2) in nine. In all the six venepunctures where a moderate response was noted (score 3–6), a further increase in the score after needle insertion was found.

There was no within patient difference in pre-procedure scores with the two creams when assessed by Wilcoxon signed rank test; p=0.72; 95% confidence intervals −0.75 to 0.50. Overall, the effect of limb holding was minimal and no different between the two creams.

EMLA cream can substantially reduce pain in infants older than 3 months and in older children during procedures like intravenous cannulation, venepuncture, lumbar puncture and intramuscular injection.[8 10–12 24 25]

Use of EMLA in neonates has produced variable results. During circumcision in male term infants, application of 0.05 ml to the prepuce 45–60 minutes before the procedure significantly reduced pain responses.[16] The results

are surprising as the dose of EMLA is very small and application beyond 10–15 minutes over the genital skin is said to reduce efficacy.[14] The data were also collected by an observer who was aware of treatment assignment.

A recent randomised controlled trial of EMLA for circumcision used a dose of 1g applied for 60–80 minutes under an occlusive dressing. EMLA was said to decrease pain based on significantly less facial activity, crying time, and smaller increases in heart rate. Methaemoglobin concentrations were not significantly increased. However, it was less effective during phases associated with extensive tissue destruction such as lysis of adhesions and clamp tightening.[26] This effect of EMLA did not diminish pain responses at 4 to 6 months during immunisation and it may not be the best method of anaesthesia for circumcision.[27]

A study applying 0.5 ml of EMLA cream over the heel in term infants before a heel prick did not show any benefit.[28]

In preterm infants of 27–32 weeks the flexion reflex threshold was reduced by repeated tissue damage caused by heel pricks. This was almost completely reversed by the application of EMLA cream to the damaged area.[3] The dose of EMLA used in this study was too small as a tube of EMLA cream lasted about 2 weeks (approximately 0.35 g per day in divided doses). No occlusive dressing was used. The reason for the beneficial effect is unclear.

McIntosh *et al* found that EMLA cream was ineffective in reducing distress from heel prick in preterm infants 26–34 weeks of gestation whereas using a spring loaded heel lance was most effective.[17]

In 116 preterm and full term neonates EMLA was used for 120 skin punctures (venepunctures and arterial punctures) after application times of 90–120 minutes. Twenty punctures were performed without local anaesthesia. A reaction score (0–5) was documented at the time of skin puncture. EMLA applied for at least 90 minutes resulted in low scores in 57% compared with only 18% with low scores without anaesthesia. Methaemoglobin concentrations estimated at 18 hours to 3 days on 47 occasions were all below 5%.[29] This study was not randomised, placebo controlled, and the observer was aware of the treatment group of each patient. The reaction score used has not been validated as a measure of pain in neonates.

Studies on preterm infants published only as abstracts have suggested no benefit from EMLA for lumbar puncture (1 g applied for one hour), but useful for percutaneous line insertions (1.25 g applied for one hour).[18–19] The latter study showed benefit only in terms of a reduced heart rate.[18] These studies suggest that the efficacy of EMLA in neonates is still not proved.

Reasons for the mostly negative response to EMLA cream in our study are unclear but may be related to variability in skin perfusion and thickness, needing a different dose and application time in neonates. Areas with thin skin or increased vascularity need a shorter application time as longer application may result in rapid clearance of the drug from the dermis and prevent sufficiently high concentrations of the drug around the nerve endings.[28 30] No clinical toxicity was noted in our study as in all previous studies on term and preterm infants.

We have shown that median methaemoglobin percentages after EMLA application were significantly higher at 8 hours than after the application of placebo cream, but not significantly different at 1 hour. All concentrations were still within the normal range for methaemoglobin percentage for preterm infants 0.08–4.7%,[31] except one infant in the 8 hour EMLA group who had a concentration of 6.7%.

In a recent study methaemoglobin concentrations were measured in preterm infants after EMLA application to the heel. Thirty preterm neonates, mean gestational age 32.8 weeks, had methaemoglobin estimated at baseline, 4, 8, or 12 hours after EMLA application. There was no significant rise in methaemoglobin concentrations and the concentration of prilocaine and lignocaine measured were well below toxic concentrations.[21]

The higher 8 hour methaemoglobin concentrations in our study may reflect greater absorption of prilocaine through the skin over the dorsum of the hand and foot than the heel and suggest that repeated use of EMLA over a short time span may lead to a cumulative rise in methaemoglobin cocentrations with clinical toxicity.

Clinically significant methaemoglobinaemia (15%) was reported after two applications of EMLA a few hours apart in a septic preterm infant.[32] In another report on the use of EMLA in 500 neonates, 10 of 158 methaemoglobin percentages estimated were above 3% and 3/158 were above 5%. The highest methaemoglobin recorded was 6.2%.[20]

Though pallor was noted in several infants at the site of application, this was no different from the effect of the placebo cream. Pallor, redness, and occasionally purpura have been noted at the site of application of EMLA in previous reports.[20–22 23–26] The use of EMLA cream did not increase the difficulty of venepuncture any more than placebo.

There are no previous data to suggest an appropriate dose of EMLA cream for venepuncture in preterm infants. Two previous studies have not mentioned a dose.[3–17] Doses used in other studies vary from 0.5 to 1.25 g.[18 19 21 28] We found that 0.5 ml of cream weighing 0.5 g covered an adequate area (about 5 cm^2) of the skin over a vein on the dorsum of the hand or foot of the preterm infant. This dose is within the recommended range for older children (Astra Pharmaceuticals, 1991). Different doses may be needed in term and preterm infants.

There is no gold standard for the measurement of pain in neonates. A combination of physiological and behavioural responses are generally used.[33] The physiological and behavioural criteria we used have been validated

and used in earlier studies of pain in neonates.[5 28 33–36]

In conclusion, our study does not show any significant benefit from the use of EMLA cream. Although a significantly higher concentration of methaemoglobin was found 8 hours after its application, no clinical toxicity was observed.

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1 Barker DP, Rutter N. Exposure to invasive procedures in neonatal intensive care unit admissions. *Arch Dis Child* 1995;**72**:F47-F8.
2 Baucher H, May A, Coated E. Use of analgesic agents for invasive medical procedures in paediatric and neonatal intensive care units. *J Pediatr* 1992;**121**:647-9.
3 Fitzgerald M, Millard C, McIntosh N. Cutaneous hypersensitivity following peripheral tissue damage in new-born infants and its reversal with topical anaesthetics. *Pain* 1989;**39**:31-6.
4 Koren G. Use of the eutectic mixture of local anaesthetics in young children for procedure related pain. *J Paediatr* 1993;**122**:S30-S5.
5 Gonsalves S, Mercer J. Physiological correlates of painful stimulation in preterm infants. *Clin J Pain* 1993;**9**:88-93.
6 Taddio A, Katz J, Ilerisch AL, Koren G. Effect of neonatal circumcision on pain response during subsequent routine vaccination. *Lancet* 1997;**349**:599-603.
7 Grunau RVE, Whitfield MF, Petrie HJ, Fryer LE. Early pain experience, child and family factors, as precursors of somatisation: a prospective study of extremely premature and full term children. *Pain* 1994;**56:**353-9**.**
8 Maunuksela EL, Korpela R. Double blind evaluation of a lignocaine-prilocaine cream (EMLA) in children. *Br J Anaesth* 1986;**58**:1242-5.
9 Halperin DL, Koren G, Attias D, Pellegrini E, Greenberg MC, Wyss M. Topical skin anaesthesia for venous subcutaneous drug reservoirs and lumbar punctures in children. *Pediatrics* 1989;**84**:281-4.
10 Joyce TH. Topical anaesthesia and pain management before venepuncture. *J Pediatr* 1993;**122**:S24-S9.
11 Hopkins CS, Burkley CJ, Bush GH. Pain free injection in infants. *Anaesthesia* 1988;**43**:198-201.
12 Robieux I, Kumar R, Krishnan SR, Koren G. Assessing pain and analgesia with a lidocaine prilocaine emulsion in infants and toddlers during venepuncture. *J Pediatr* 1991;**118**:971-3.
13 Nilsson A, Engberg G, Henneberg S, Danielson K, De Verdier CH. Inverse relationship between age dependent erythrocyte activity of methaemoglobin reductase and prilocaine induced methaemoglobinaemia during infancy. *Br J Anaesth* 1990;**64**:72-6.
14 Burtin P, Ito S. Clinical pharmacology and toxicity of EMLA. In: *EMLA: a breakthrough in skin anaesthesia*. Toronto: Marcel Dekker, 1995:17-36.
15 Harpin AV, Rutter N. Barrier properties of the new-born infants skin. *J Pediatr* 1983;**102**:419-25.
16 Benini F, Johnston CC, Faucher D, Aranda JV. Topical anaesthesia during circumcision in new-born infants. *JAMA*;1993;**270**:850-3.
17 McIntosh N, Veen LV, Brameyer H. Alleviation of the pain of heel prick in preterm infants. *Arch Dis Child* 1994;**70**:F177-F81.
18 Garcia OC, Reichberg S, Brion LP, Schulman M. Topical anaesthesia with EMLA during percutaneous line insertion in very low birth weight infants. *Pediatr Res* 1995;**37**:205A.
19 Enad D, Salvador A, Brodsky NL, Hurt H. Safety and efficacy of eutectic mixture of local anaesthetics for lumbar puncture in new-borns. *Paediatr Res* 1995;**37**:204A.
20 Gourrier E, Karoubi P, El Hanache A, Merbouche S, Mouchnino G, Leraillez J. Use of EMLA cream in a department of neonatology. *Pain* 1996;**68**:431-4.
21 Taddio A, Shennan AT, Stevens B, Steven J, Koren G. Safety of lidocaine prilocaine cream in the treatment of preterm neonates. *J Pediatr* 1995;**127**:1002-5.
22 Prechtl HFR. The behavioural states of the newborn infant; a review. *Brain Res* 1974;**76**:185-212.
23 Bland MJ, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;**i:**307-11.
24 Uhari M. A eutectic mixture of lidocaine and prilocaine for alleviating pain in infants. *Paediatrics* 1993;**92**:719-21.
25 Taddio A, Nulman I, Goldbach M, Ipp M, Koren G. Use of lidocaine prilocaine cream for vaccination pain in infants. *J Pediatr* 1994;**124**:643-7.
26 Taddio A, Stevens B, Craig K, *et al*. Efficacy and safety of Lidocaine-Prilocaine cream for pain during circumcision. *N Engl J Med* 1997;**336**:1197-201.
27 Taddio A, Katz J, Ilersich L, Koren G. Effect of neonatal circumcision on pain response during subsequent routine vaccination. *Lancet* 1997;**349**:599-603.
28 Larsson BA, Jyli L, Lagercrantz H, Olsson GL. Does a local anaesthetic cream(EMLA) alleviate pain from heel lancing in neonates? *Acta Anaesthesiol Scand* 1995;**23**:1028-31.
29 Gourrier E, Karoubi P, El Hanache A, *et al*. Utilisation de l' EMLA chez le nouveau-né à terme et premature. Etude d'efficacité et de tolerance. *Arch Pediatr* 1995;**2**:1041-6.
30 Larsson BA, Norman M, Bjerring P, Egekvist H, Lagercrantz H, Olsson GL. Regional variations in skin perfusion and skin thickness may contribute to varying efficacy of topical local anaesthetics in neonates. *Pediatr Anaesth* 1996;**6**:107-10.
31 Kravitz H, Elegent LD, Kaiser E, Kagan BM. Methaemoglobin values in preterm and mature infants and children. *Arch Paediatr Adolesc Med* 1956;**91**:1-5.
32 Nioloux C, Floch-Tudal C, Jaby-Sergent MP, Lejeune C. Anesthesie locale par creme EMLA et risque de methemoglobinemia chez le premature. *Arch Pediatr* 1995;**2**:291-2.
33 Craig KD, Grunau RVE. Neonatal pain perception and behavioural measurement: In: Anand KJS, McGrath PJ, eds. *Pain in neonates*. Amsterdam: Elsevier Science BV,1993: 66-99.
34 Johnston CC , Stevens BJ, Yang F, Horton L. Differential responses to pain by very preterm neonates. *Pain* 1995;**61**:471-9.
35 Craig KD, Whitfield MF, Grunau RVE, Linton J, Hadjistavropoulos HD. Pain in the preterm neonate: behavioural and physiological indices. *Pain* 1993;**52**:287-99.
36 Rushforth JA, Levene MI. Behavioural responses to pain in healthy neonates. *Arch Dis Child* 1994;**70**:F174-F6.