ORIGINAL ARTICLE

The relationship between placental transfusion, and thymic size and neonatal morbidities in premature infants - A Randomized Control Tiral

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Abstract

Objectives: To compare the effect of umbilical cord milking and early cord clamping on thymic size, and neonatal mortality and morbidity in preterm infants.

Methods: This single-center, prospective, double-blind, randomised controlled study was conducted at Baskent University, Konya Education and Research Centre, Konya, Turkey, between October 2015 and April 2016. Pregnant women who delivered before 32 weeks of gestation were randomised to receive umbilical cord milking (group 1) or early cord clamping (group 2). Ultrasonographic evaluation was performed in each newborn by an experienced radiologist within the first 24 hours of life. Thymic size was estimated in line with literature. SPSS 15 was used for all data analyses.

Results: There were 38 subjects in group 1 and 37 in group 2. There were as many infants in the two groups (p>0.05) The haemoglobin levels was higher in group 1, but not significantly (p=0.213). The absolute neutrophil count in group 1 was significantly lower (p= 0.017) than group 2. In terms of neonatal mortaility and morbidity, there were no significant differences between the groups (p>0.05).

Conclusion: Umbilical cord milking was not associated with thymic size during the the first 24h of life.

Keywords: Placental transfusion, Thymic size, Premature infant, Perinatal moribidities.
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Introduction

The thymus is a retrosternally located lymphoid organ that plays an important role in the maturation of thymus lymphocytes (T-lymphocytes). Additionally, thymic size is thought to be associated with thymic and immune system functions. Aaby et al. showed that there is an association between thymic size, and infant mortality[1]. The mortality rate decreased as the thymic size increased. Thymic size is the greatest during the neonatal period and decreases with time [2]. The thymus can be measured via calculation of the thymic index (TI) [3], which is accepted as a reliable measurement of thymic volume[3].

Thymic size is affected by many factors, including the environment, feeding behaviour, prenatal toxin exposure, infections, and birth weight. Thymic size at birth is most likely determined by a combination of genetic factors, and gestational and perinatal environmental factors. Malnutrition, infectious diseases, exposure to toxins and stress are all associated with a small thymus [4-7]. The stress hormone cortisol often mediates this effect and can cause acute thymic involution with extensive cell death of developing T-lymphocytes in the thymic cortex [7]. Acute thymic involution during infection and malnutrition can have a long-term negative effect on cellular immune function[8].

The immune system in newborn infants, especially premature infants, is not fully developed. All the cells in the immune system of the newborn have decreased functional capacity. Inadequate polymorphonuclear leukocyte and monocyte chemotaxis in neonates, decreased phagocytosis and intracellular killing

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function complement the deficiencies, and insufficient antigen recognition of macrophages, decreased synthesis of antibodies, low quantities of fibronectin and adhesion molecules, and insufficient production of cytokines result in immunodeficiency, rendering newborns susceptible to infections [9].

Recent studies show that umbilical cord milking (UCM) or delayed cord clamping is beneficial in full-term and preterm infants. A study on the effects of placental transfusion full-term and late-preterm infants showed that in the placental transfusion group haemoglobin concentration was better, without polycythemia, jaundice or respiratory distress, compared to the non-placental transfusion group [10]. A meta analysis reported that there is less intraventricular haemorrhage of all stages, necrotising enterocolitis, and need for blood transfusion delayed cord clamping group than in the early cord clamping (ECC) group [11]. Umbilical cord blood has a large quantity of haematopoietic stem cells, versus few nucleated blood cells and immature T-cells. Infants born at 23-31 weeks of' gestations have a 3-fold higher number of haematopoietic progenitor cells (HPCs) than full-term infants [12]. Migration of haematopoietic stem cells through the blood, and across the endothelial vasculature to different organs and their bone marrow niches, requires active navigation, a process referred to as homing.

The current study was planned to test the hypothesis that UCM would improve immune system function via the homing process and that UCM might positively affect thymic size, resulting in lower incidence of neonatal morbidity compared to ECC.

Subjects and Methods

This single-center, prospective, double-blind, randomised controlled study was conducted at Baskent University, Konya Education and Research Centre, Konya, Turkey, between October 2015 and April 2016. Approval was obtained from the institutional review board. Written informed consent was taken from the parents of aa the enrolled infants. Inborn preterm infants with gestational age ≤32 weeks were included, while those with twin-to-twin transfusion syndrome, foetal and maternal bleeding, dysmorphic features and conotruncal heart disease were excluded.

Pregnant women admitted for delivery in the centre were randomised before delivery to two groups using random permuted blocks of 10; an independent statistician provided the randomisation sequence. Serially-numbered opaque envelopes contained arm bands signifying that a patient was assigned to the UCM or the ECC group. These arm bands were secured to each woman's wrist after randomisation to notify the obstetrical staff that the women were study participants. In order to ensure standardisation of the UCM technique, all delivering physicians were briefed before the initiation of the study.

Double-blinding ensured that neither the women nor the radiologists knew to which group each woman was assigned. A length of umbilical cord equal to an extended hand's width (from the tip of the thumb to the tip of the pinky [20±2 cm]) was used as the standard. Infants in the UCM group were placed at or below the level of the placenta if delivered vaginally or at the same level as the placenta if delivered by cesarean section (CS), and then ~20cm of the umbilical cord was gently milked towards the umbilicus 3 times before clamping the cord[13]. Uterotonic medications such as oxytocin were not given until the obstetrician had clamped the umbilical cord. In the ECC group, the umbilical cord was clamped within the first 10s of delivery and was immediately thereafter cut. The neonatologists and paediatric support staff were not blinded to the treatment group assignments, as they were required to be present for delivery. No detail of study participation was made in the neonate's chart in order to minimise the possibility that postnatal treatment decisions would be influenced by study participation.

Sample size was calculated using the open source calculator OpenEpi v.3.0. Assuming a two-sided α level of 0.05, 42 evaluable infants were needed in each group to achieve 80% power [14]. The aim was to enroll 45 women in each group to account for loss due to patient withdrawal and the expectation that some participants would become ineligible due to the development of bleeding suspicious for placental abruption or congenital anomalies.

Maternal data, including age, intrauterine growth restriction (IUGR), preterm premature rupture of membranes (PPROM), preeclampsia, antenatal steroid administration, and mode of delivery, was obtained from the medical records. Infant data at birth, including gestational age, gender, weight, respiratory distress syndrome (RDS), intraventricular haemorrhage (IVH), haemodynamic significant patent ductus arteriosis

(hsPDA), whole blood cell count, peak serum bilirubin level, frequency of sepsis, duration of hospital stay, were likewise obtained from the medical records.

All ultrasound examinations were performed within the first 24h of life by the same ultrasound device and by the same blinded radiologist. All newborns were on the nasal continues positive airway pressure (nCPAP) when measurements were performed. Thymic size was estimated as described in literature [3]. With infants in the supine position the maximum thymic transverse diameter (TTD) was measured twice, so as to avoid intraobserver variability. Perpendicular to the diameter of the thymus, the maximum thymus sagittal area (TSA) of the largest lobe was measured. The mean of the 2 diameter and 2 area measurements were multiplied to estimate TI. The TI/weight ratio (TIWR) was estimated as the thymic index divided by infant weight in kilogrammes. A transportable ultrasound scanner (Hitachi, Tokyo, Japan) and a 7.5 MHz linear transducer (Hitachi, Tokyo, Japan), were used for the examination.

Descriptive statistics of scale variables were presented as mean ± standard deviation (SD) or median (range) as appropriate. Demographic and clinical continuous variables were compared using the 2-independent Student's t test for normally distributed values and the Mann-Whitney U test for non-normally distributed values. Categorical variables were compared using Fisher's exact test. For all tests, the level of statistical significance was set at p=0.05. Data were analyzed using SPSS 15.

Results

Of the 90 women enrolled, 44(49%) were in the UCM group, and 46(51%) in the ECC group. In the UCM group, 38(86.36%) subjects and 37(80.43%) in the ECC group completed the study. Likewise, of the 75 newborns, 38(51%) were in the UCM group, and 37(49%) were in the ECC group.

Table-1: Baseline infant characteristics.

	ECC N: 37	UCM N: 38	P value
Sex, male, n (%)	19(45,2)	23(54,8)	0,424
Mode of delivery, C/S, n (%)	29(43,9)	37(56,1)	0.023*
GW <30, n (%)	15(50,0)	15(50,0)	0,925
Antenatal steroid administration n (%)	25(48,1)	27(51,9)	0,815
PPROM n (%)	2(28,6)	5(71,4)	0,281
Preeclampsia, n (%)	3(50,0)	3(50,0)	0,916
IUGR, n (%)	1(25,0)	3(75,0)	0,331

*P <0.05.

UCM: Umbilical cord milking: ECC: early cord clamping: GW: gestational week, IUGR: Intrauterine growth restriction, PPROM: Preterm premature ruptures of membranes

*Fisher exact Chi-square test for categorical data.

There were no significant differences in gestational age, birth weight, antenatal steroid administration, the occurrence of PPROM, or IUGR between the groups (Table-1). The patient distribution for gestational week was similar between the groups. Additionally, there were no significant differences in TTD, TSA, TI and TI/WR between the groups (Table-2). The mean absolute neutrophil count (ANC) was significantly lower in the UCM group (p=0.017). Other whole blood cell values, including the leukocyte count,

Table-2: Thymus and other parameters.

	ECC	UCM	p value
TTD cm, Mean (SD)	1.62 (0.287)	1.54 (0.202)	0.201(a)
TSA cm2 Mean (SD)	1.67 (0.436)	1.52 (0.337)	0.099(a)
TI cm3 Mean (SD)	2.80 (1.127)	2.40 (0.784)	0.077(a)
TIWR cm3/Kg, Mean (SD)	2.04 (0.890)	1.82 (0.744)	0.241(a)
Leucocyte, Median (Range)	10100 (1200 - 47300)	9200 (4640-63700)	0.820(b)
ALC , Median (Range)	5480 (1880 - 30200)	6660 (2880 -51900)	0.213(b)
ANC, Median (Range)	2370 (305 - 29800)	1440 (18-8200)	0.017(b)
Haemoglobin, gr/dl Mean (SD)	16.94 (2.20)	17.55 (2.23)	0.234(a)
Haematocrit, Mean (SD)	49.56 (6.11)	49.63 (8.31)	0.965(a)
Platelet counts , Mean (SD)	210713 (68592)	225210 (69982)	0.368(a)
Peak serum bilirubin, mg/dl,Mean (SD)	11.60 (3.48)	12,68 (3.29)	0.180(a)
Birth weight, gr , Mean (SD)	1454 (394)	1408 (387)	0.607(a)
Weight at discharge, gr, Median (Range)	1860 (820 -2640)	1885 (620 -2990)	0.343(b)
Duration of hospital stay, day, Median (Range)	28 (4- 96)	29.5 (3 - 81)	0.837(b)
RDS, N (%)	23 (52.3)	21 (47.7)	0.544(c)
HSPDA, N (%)	5(33.3)	10(66.7)	0.166(c)
IVH, N (%)	2(50.0)	2(50.0)	0.956(c)
Sepsis, N (%)	7 (46.7)	8(53.3)	0.863(c)
Discharge, N (%)	35(54.0)	29(46.0)	0.027(c)

Abbreviations: ECC: Early cord clamping, UCM: Umbilical cord milking, TTD: Thymus transverse diameter; TSA: Thymus sagittal area; TI: Thymic index; TIWR: Thymic index weight ratio; ALC: Absolute lymphocyte count; ANC: Absolute neutrophyle count; HSPDA: Hemodinamicaly significant patent ductus arteriosus; RDS: Respiratory distress syndorme; IVH: Intraventricullar hemorhage

*Mean(Std. Deviation), frequency (row percentage)

(a) 2-independent samples t-test for continuous data normally distributed

(b) 2-independent samples Mann-Whitney U test for continuous data not normally distributed

(c) Fisher exact Chi-square test for categorical data.

Table-3: The characteristics of exitus and discharge groups.

	Exitus	Discharge	P value
Birth Weight, gram, Mean (SD)	1120(407)	1480 (376)	0.014(a)
The number of infants less than 30 GW, N (%)	21(77.8)	6(22.2)	0.022(b)
IVH, N (%)	3(75.0)	1(25.0)	< 0.001(b)
Sepsis, N (%)	5(22. 7)	17(77.3)	0.041(b)

Abbreviations: IVH: Intraventricular haemorrhage
(a) 2-independent samples t-test for continuous data normally distributed
(b) Fisher exact Chi-square test for categorical data.

haemoglobin level, haematocrit level, absolute lymphocyte count and platelet count did not differ significantly between the groups (p>0.05 each). The mean haemoglobin level was higher in the UCM group than in the ECC group but not significantly (p=0.23). The mean peak serum bilirubin level was higher in the UCM group but not significantly (p=0.18). There were no significant differences in sepsis, IUGR), hsPDA, RDS and IVH between the groups (p>0.05). The mortality rate was significantly higher in the UCM group than in the ECC group (p=0.027), but there were significantly more infants born at <30 weeks of gestation in the UCM group than in the ECC group (p=0.02).

Gender, antenatal steroid administration, PPROM, preeclampsia and IUGR had no effect on TI, TSA, TTD, or TIWR (p>0.05). There wasn't correlation between gravidity and TSA, TTD, TI or TIWR, although gravidity increased. TI increased but not significantly (p=0.66). There was a significant difference between TIWR and less than 30 weeks gestational ages infants (p=0.001). There were no relationships between TSA, TTD, TI, TIWR and hsPDA, IVH, the existing sepsis, hospital discharge, duration of hospital stay, and weight at discharge (p>0.05). TIWR was significantly higher in the group of existing RDS (p=0.031). Higher mortality rates were observed in infants who had hsPDA, sepsis and IVH (Table-3).

Discussion

The International Liaison Committee on Resuscitation has recommend that delayed cord clamping for longer than 30 seconds is reasonable for both fullterm and preterm infants who do not require resuscitation at birth[15]. UCM is as effective and safe a procedure as delayed cord clamping [16,17]. Some studies observed an association between the TI and thymic function [18,19]. Thymic size is also known to be associated with infant mortality. The present study's primary aim was to evaluate interactions between UCM and parameters of thymic size and to evaluate the effect of UCM on perinatal mortalities and morbidities.

In contrast to expectations, the findings showed that the UCM had no effect on thymic parameters, including TSA, TTD, TI and TIWR in preterm neonates born at ≤32 weeks of gestation, during the first 24h of life. To the best of our knowledge, the study is the first to evaluate the interaction between UCM and thymic size.

The ANC was significantly lower in the UCM group than in the ECC group, which is line with an earlier study [20]. Some studies reported that neutrophil recovery occurs significantly later in infants in cord blood transplantation groups than in those in bone marrow transplantation groups [21,22]. As such, the higher frequency of neutropenia in the present study's UCM group might have been due to delayed neutrophil recovery.

In contrast to another expectation, there was no significant difference in the haemoglobin or haematocrit levels between the UCM and ECC groups. Kiliçdag et al [20] reported that first-day haemoglobin level was significantly higher in their UCM group, but not the haematocrit level. On d 3 and d 7, there were no significance differences in the haemoglobin and haematocrit levels between their UCM group and non-UCM group. The main reason of this may be related to the fact that obstetricians want to put the baby immediately in front of the neonatologist in order to be resuscitated. In particular, extremely immature infants are a concern for obstetricians because of the need for resuscitation, and as such, the UCM technique may be insufficient.

In all, 2 patients in the present study's UCM group and 2 in ECC group had IVH. For IVH, similar to Takami et al, there was no significant relationship with cord milking [17].

The mortality rate in the present study was higher in UCM group, but this was not thought to be related to UCM because there were more infants born <30 weeks of gestation (n = 6) infants in the UCM group. Moreover, no study has shown that UCM increases the mortality rate. Furthermore, higher mortality rates were observed in present study's infants that had hsPDA, sepsis and IVH. A study on the correlation between thymic size and infant mortality reported thymic size, especially measured at age 8 week, was associated with infant mortality, but that measurement of thymic size at birth was not done [15].

TIWR was higher in the present study's infants who had RDS which might have been due to the high number of infants born at 30 weeks of gestation who had RDS as TI in relation to body size is greatest at birth and inversely correlated with gestational age.

The present study has some limitations. The UCM was not sufficiently standardised, which we think is why there was no significant difference in the haemoglobin level between the groups. Based on this finding, we think that UCM procedure training should be provided to obstetricians and neonatologists. Repeated measurements of thymic size could be made in the growing-up phase. Despite these limitations, the present findings suggest that additional research with larger samples and repeated thymic measurements are warranted to clarify the effect of thymic size on morbidity and long-term outcomes in preterm infants.

UCM techniques remain to be standardised. Takemi et al reported that the velocity of milking is 20cm/s [17]. The speed of milking was reported approximately 20cm per two seconds in another study[13]. Either two or three milkings have been performed in previously mentioned studies [13,17]. It was recently reported that performing UCM only once after the cord is cut is as effective as multiple milking in infants born at <29 weeks of gestation [24].

Conclusion

UCM was not found to have any effect on thymic parameters in premature infants during first 24 h of life. How many times UCM should be performed remains a contentious issue. Further investigation should be performed about right and effective milking technique. Studies with larger sample size should be done, and methods or devices should be developed to assess how much blood is being transferred to the baby during the UCM procedure.

Disclaimer: Due to non-existence of randomised controlled trial (RCT) registration authority in Turkey, RCT trial number was not allotted. The approval to conduct the trial was taken from the institutional review board (IRB).

Conflict of interest: None.

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