Sains Malaysiana 47(10)(2018): 2491-2499
http://dx.doi.org/10.17576/jsm-2018-4710-26

Total Nucleated Cell Count and CD34+ Cell is Reduced in Preeclampsia and Gestational Diabetes Mellitus Pregnancies: Viable Affix Criteria for Cord Blood Banking

(Jumlah Sel Bernuklues dan Sel CD34$^+$ Berkurangan dalam Kehamilan Praeklampsia dan Gestati Diabetes Mellitus: Kriteria Viabel Afiks untuk Perbankan Darah Tali Pusat)

MOHD RAZIF MOHD IDRIS, FAZLINA NORDIN*, ZALEHA ABDULLAH MAHDY & S FADILAH ABD WAHID

ABSTRACT

The aim of this study to determine the numbers of CD34+ cells and total nucleated cell (TNC) in umbilical cord blood (UCB) collected from pregnant mothers with gestational diabetes mellitus (GDM) and preeclampsia (PE), following statistical analysis of both maternal and perinatal factors which affect UCB parameters. Most of studies explored the influence of obstetric factors on the number of UCB cell collection and only a few looked at the effects on UCB haematopoietic stem cell (UCB-HSC) of common disorders complicating pregnancy. A total of 112 UCB samples (32 PE, 42 GDM and 38 non-diseased) were collected. CD34$^+$ cell and NC count were enumerated using FACS Calibur. The TNC and CD34$^+$ cells were significantly reduced in both PE and GDM groups as compared to the control group. The PE group shows significantly lower birth weight and higher BP which led to a lower UCB volume and CD34$^+$ count. Gestational age shows significant correlation with nucleated cell count (NCC) and TNC. GDM group shows significantly lower systolic BP, NCC and TNC count, including low placental weight and birth weight. Conclusively, some obstetrics factors have significant influences to the numbers and quality of UCB-HSC in both PE and GDM groups, which could guide in the selection criteria for CB banking.

Keywords: CD34+ cells; gestational diabetes mellitus (GDM); haematopoietic stem cell (HSC); preeclampsia (PE); total nucleated cell (TNC); umbilical cord blood (UCB)

ABSTRAK

Tujuan kajian ini adalah untuk menentukan bilangan sel CD34$^+$ dan jumlah sel bernukleus (TNC) dalam darah tali pusat (UCB) ibu mengandung dengan diabetes mellitus (GDM) dan praeklampsia (PE), serta analisis statistik faktor maternal dan perinatal yang mempengaruhi parameter UCB. Kebanyakan kajian mengkaji pengaruh faktor obstetrik pada bilangan sel UCB dan hanya beberapa kajian yang melihat kesan penyakit semasa kehamilan pada kualiti UCB-HSC. Sebanyak 112 sampel UCB (32 PE, 42 GDM dan 38 normal) diperoleh. Bilangan sel CD34$^+$dan TNC dihitung menggunakan FACS Calibur. Bilangan TNC dan sel CD34$^+$ adalah lebih rendah dalam kumpulan PE dan GDM berbanding kumpulan normal. Kumpulan PE menunjukkan berat bayi yang lebih rendah dan BP yang lebih tinggi yang menyebabkan jumlah isi padu UCB dan bilangan sel CD34$^+$ yang lebih rendah. Faktor umur kehamilan menunjukkan korelasi yang signifikan dengan bilangan NC dan TNC. Kumpulan GDM menunjukkan BP sistolik, NC dan TNC yang lebih rendah, termasuk berat plasenta dan berat bayi yang rendah. Kesimpulannya, beberapa faktor obstetrik mempengaruhi bilangan dan kualiti UCB-HSC secara signifikans pada kedua-dua kumpulan PE dan GDM yang dapat membantu di dalam kriteria pemilihan untuk perbankan CB.

Kata kunci: Darah tali pusat (UCB); jumlah sel bernukleus (TNC); kehamilan diabetes mellitus (GDM); praeklampsia (PE); sel CD34+; sel stem hematopoitik (HSC)

INTRODUCTION

UCB has been used widely as an alternative source of HSC for transplantation. There have been over 30,000 cases of UCB transplantation since the first successful case to treat Fanconi's anemia in 1988 and the number is increasing worldwide. The unique characteristics of UCB have brought more advantages and are promising in transplantation. It is capable to proliferate more than marrow and peripheral blood to reconstitute the host Haematopoietic pool (Ballen et al. 2013; Gluckman et al. 1997; Rocha et al. 2004). Also, it is easy to procure, acceptable of human leukocyte antigen mismatch, poses no risk to donor, has low risk of transmitting infections and is fast accessible of a graft (Broxmeyer & Farag 2013; Fadilah et al. 2008; Rocha et al. 2000). Despite advantages, there is a major limitation to the UCB use in transplantation. Mostly, a single unit of UCB is insufficient in HSC numbers and results in failure of transplantation in adults (Scaradavou et al. 2013; Tse & Laughlin 2005).

Most data suggested that the dosage of cells considered suitable for transplantation is 1×10^7 to 3×10^7 nucleated

cell count/kg recipients (Ballen et al. 2001). Clinical analyses suggested that a CD34$^+$ cell dose of less than 1.7 × 10^5/kg had a high risk of death (Kögler et al. 1998; Wagner et al. 2002). From all these findings, an established policy have been developed by UCB banks in order to select high quality UCB with appropriate numbers of total nucleated and CD34$^+$ cells for transplantation purposes and better engraftment (Ballen et al. 2001; Broxmeyer 2011; George et al. 2006).

Most studies were performed to develop guidelines for selecting a high quality of UCB with a focus on healthy pregnancy. Little knowledge has been recorded about the effect of common disorders complicating pregnancy such as PE and GDM. With regard to healthy pregnancy, some obstetric factors such as long gestation, long labour, high infant and placenta weight, a short interval between delivery of the infant, cord clamping (Donaldson et al. 1999), preterm delivery, route of delivery and younger maternal age as factors associated with higher quality of UCB yield (Dimitriou et al. 2006; Surbek et al. 2000). Jones et al. (2003) suggested that the collected volume of UCB will improve when a longer length of cord is left with the placenta and a shorter time interval exists between the delivery of the placenta and the collection (Jones et al. 2003). Neonatal factors also have shown that female babies and bigger babies correlate with higher NCC (Ballen et al. 2001; Nakagawa et al. 2004; Abdul Wahid et al. 2012). Meanwhile, a higher CD34$^+$ cell count is observed in the male baby (George et al. 2006).

PE and GDM are among the most common medical disorders in pregnancy (Easterling & Benedetti 1989; Ismail et al. 2011). Not much information exists whether PE and GDM affect the quality of UCB HSC. PE was found to result in smaller numbers of circulating endothelial progenitor cells and depression of haematopoiesis in the foetal liver (Luppi et al. 2010; Stallmach et al. 1998). A few studies showed that UCB TNC and CD34$^+$ cells were significantly less in PE mothers compared to normal (Abdul Wahid et al. 2012; Surbek et al. 2001). Meanwhile, in GDM pregnancy, a previous study showed CD34+ cells in cord blood was significantly higher compared to control group (Hadarits et al. 2015). Another previous study has compared the concentration of CD34+ cells in peripheral circulation of mothers with GDM, hypertension and control. GDM mothers had lower CD34$^+$ compared to normal and hypertensive subjects (Buemi et al. 2007). In addition, comparing the endothelial progenitor cells (EPC) and CFU-EC in the umbilical cord blood of GDM mothers with controls, Acosta et al. (2011) found that GDM mothers had less EPC but there was no difference in the CFU-EC. EPC are not HSC but they co-express CD34$^+$.

Therefore, this study was performed to investigate the effect of PE and GDM in pregnancy on UCB HSC. This study will determine the quality of UCB HSC from mothers with either GDM or PE that may influence neonatal factors and their ability to proliferate *in vitro*. Outcome data from this study will be useful to decide whether the single unit of UCB from PE and GDM pregnancy are suitable for cryo banking and transplantation purposes in future, and this will lay a foundation for counselling mothers undergoing UCB banking.

Materials and Methods

Subjects

This prospective case-control study was approved by the Ethical Research Board of UKM Medical Centre. The study was conducted on 32 patients with PE, 42 patients with GDM and 38 healthy pregnant women who were admitted for delivery to the UKM Medical Centre. The diagnosis of PE followed the definition by the American College of Obstetricians and Gynaecologists (Desforges et al. 1992). The criteria for PE include blood pressure above 140/90 mmHg on two readings taken 4 h apart occurring after 20 weeks of gestation and proteinuria at least 0.3 g/L in a urine sample. GDM was defined according to the American College of Obstetricians and Gynaecologists guidelines with 2 h post prandial following a 75 g OGTT result and fructosamine during the third semester of pregnancy. The control group consisted of pregnant women of similar age, parity and gestational age without PE or GDM. All subjects must pass screening blood tests for hepatitis B, hepatitis C, cytomegalovirus, syphilis, human immunodeficiency virus 1-2 and haematological disorders, genetic diseases, pre-gestational diabetes mellitus, chronic hypertension, autoimmune diseases, renal or liver impairment, multiple pregnancies and fetal anomalies or infection. Figure 1 shows the summary of the experimental design of the present study.

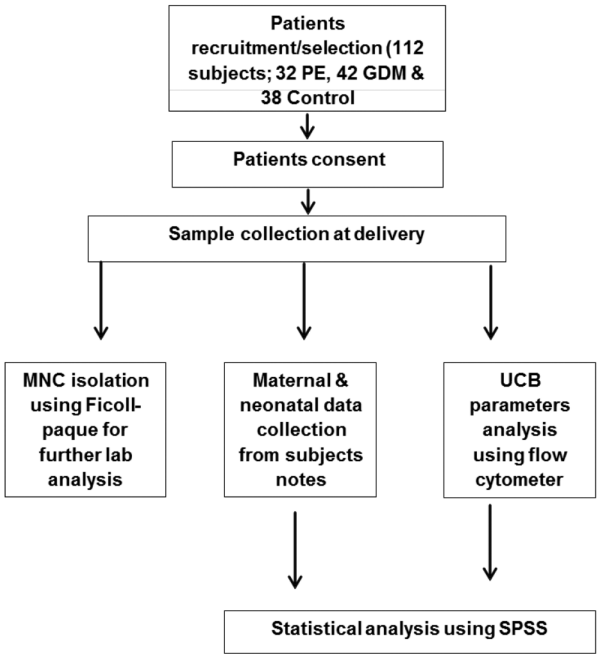


FIGURE 1. The summary of experimental design

SAMPLE SIZE CALCULATION

Sample size was estimated based on a study by Abdul Wahid et al. (2012) that looked at the effect of preeclampsia on the UCB hematopoietic progenitor cell by using the PS-Power and Sample Size Calculation Program version 2.1.31. The power of the study was 80%.

CORD BLOOD COLLECTION

At delivery, cord blood was collected from venous UCB by gravity. This collection was performed before placental expulsion in all patients to avoid interfering with the delivery of the baby while still preserving the sterility of the UCB. The cord was clamped and cut after the delivery of the baby. Before delivery of the placenta, a four to eight inches length of the cord was cleaned with alcohol and betadine. A 16-gauge needle from a standard cord blood collection bag set containing 22 mL of citrate phosphate dextrose (CPD) anticoagulant was inserted into the umbilical vein and cord blood was allowed to flow into a 157 mL bag. The needle was removed when blood flow stopped. Similar method was applied at caesarean section. Identical collection method was used for all three groups - PE, GDM and control. Collections were made by trained staff nurses accordance with hospital protocol. Blood samples were stored at 4°C immediately after collection before being collected by the lab outer personnel. Samples were processed within 24 h after collection.

NUCLEATED CELL COUNT AND CD34⁺ ANALYSIS

The volume of UCB was measured using a sterile tube. The final volume was determined by subtracting the volume of citrate phosphate dextrose (CPD) anticoagulant in the bag before collection from the total measured volume.

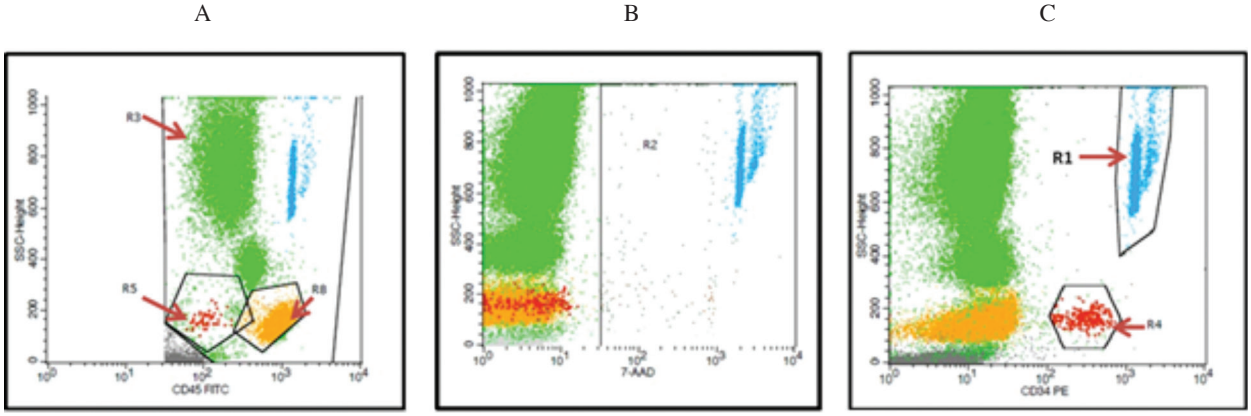
UCB nucleated cells and CD34⁺ cell counts were performed using FACS Calibur analyser in accordance with the *International Society of Hemotherapy and Graft Engineering* (ISHAGE) (Barnett et al. 1999). Briefly, 1 mL of well-mixed anticoagulant UCB was syringed out from the cord blood collection bag into 5 mL polypropylene tube. Then, a 20 µL of CD45/FITC (fluorescienisothiocyanate) + CD34/PE (phycoerythrin) was added to the TruCOUNT tube by using a pipette. The TruCOUNT tube contains a predisposed number of lyophilized 4.2 µm fluorescent beads. From the polypropylene tube, 50 µL of cord blood well mixed with CPD was added to the TruCOUNT tube using reverse pipetting technique. Another 20 µL 7-amino actinomycin D (7-AAD) solution was added to the sample as a viability dye. This allows discrimination between viable and non-viable cells. The mixture was gently mixed immediately after both reagents had been added. It was incubated for 15 min in the dark at room temperature (20° to 25°C). After incubation, the remaining red blood cells in the sample tube were lysed with 1 mL 1:10 dilution of lysing solution BD Pharm Lyse reagent (BD Biosciences) for 10 min. Then, 75000 events were acquired and analysed by three colour flow cytometry using Cell Quest Pro software (Becton Dickinson). Figure 2 shows an example of Dot Plot graph generated from FACS analysis of one control sample. The absolute and total numbers of nucleated (TNC) and CD34⁺ cell were counted using formulary according to manufacturer's protocol.

CLINICAL DATA COLLECTION

Clinical data for both mother and newborn will be recorded for further interpretation and statistical analysis. Maternal data includes age, gestational age, blood pressure, delivery, gravidity, cord pH, pre-delivery leukocytes and type of anti-diabetic and anti-hypertensive treatment. For the newborn, weight, gender, placental weight and 1 and 5 min Apgar scores will be recorded.

STATISTICAL ANALYSIS

The statistical software package SPSS version 16.0 was used to perform the data analysis. Data will be tested for



A) Threshold on FL1 channel of all events collected for this particular sample; R3 defines population of CD45⁺ leucocytes (except blue coloured dot defines as beads), R5 defines CD34⁺ population, and R8 defines the lymphocyte population of interest. B) Distinguished populations of live (selected population for further analysis), and dead cells (R2) by 7-AAD staining. C) CD45+ cells with distinguished CD34+ cells population (R4) and TruCOUNT beads (R1)

FIGURE 2. Dot plot graph generated from FACS analysis of one control sample

normality using the Shapiro-Wilk test. Continuous data were analysed using the analysis of variance (ANOVA) test, while categorical data were compared using the chi square test. Pearson's product-moment correlation and Spearman's rank order correlation were used to study the relationship between maternal and neonatal factors and CB parameters among PE and GDM subjects. A p-value < 0.05 was considered to be statistically significant.

Results and Discussion

The PE group shows significant correlations in both maternal and perinatal characteristics as compared to the control group

Maternal and perinatal characteristics data of the GDM, PE and control groups were summarized in Table 1. There were 32 PE, 42 GDM and 38 non-diseased subjects (control). There were no statistically significant differences between the groups in terms of maternal and perinatal factors including maternal leukocyte count, placental weight and neonatal gender. There was a significant difference in maternal age between the control group and the PE group (p=0.004), but not difference in GDM group. We predicated subjects with GDM were an elderly which is above 35 years old. However, the average maternal age for subject with GDM is 30.74 years old. This finding were contradicting with the previous study which reported, women aged 35 years and above are more likely to have GDM (Kalok et al. 2018). As expected, systolic and diastolic BP were significantly higher in the PE group compared to the control group (p=<0.0005), and the GDM group (p=<0.0005). We found that gestational age was also significantly different in the PE group compared to the control group (p=<0.0005) and the GDM group (p=0.031). In terms of route of delivery, the control group showed significant differences with the PE (p=<0.0005) and the GDM group (p=0.013), with spontaneous vaginal delivery (SVD) being more common in the control group. Gravidity 1-3 in the control group is higher compared to the PE group (p=0.028), and showed no significant difference with the

TABLE 1. Maternal & perinatal characteristics data

Parameters	Control group	PE group	GDM group	P value
Maternal age (y)	29.79± 0.53	32.69 ± 0.83	30.74 ± 0.61	Control vs GDM = 0.254 Control vs PE = 0.004* GDM vs PE = 0.060
Gestational age (wk)	38.55± 0.19	36.39 ± 0.48	37.89 ± 0.22	Control vs GDM =0.031* Control vs PE = <0.0005** GDM vs PE = 0.003*
Maternal leukocyte count (×10^9/L)	11.06± 0.43	11.22 ± 0.51	11.04 ± 0.53	Control vs GDM = 0.978 Control vs PE = 0.809 GDM vs PE = 0.814
Gravidity 1-3 4-6	 32 (94.1%) 2 (5.9%)	 19 (67.8%) 9 (32.2%)	 32 (82.1%) 7 (17.9%)	Control vs GDM = 0.268 Control vs PE = 0.028* GDM vs PE = 0.365
Systolic BP (mmHg)	120.21± 1.81	158.21 ± 3.49	120.74 ± 1.57	Control vs GDM = 0.825 Control vs PE = <0.0005** GDM vs PE = <0.0005**
Diastolic BP (mmHg)	69.38± 1.47	96.03 ± 2.44	70.94 ± 1.49	Control vs GDM =0.459 Control vs PE = <0.0005** GDM vs PE =<0.0005**
Delivery SVD LUSCS	 33 (97.1%) 1 (2.9%)	 16 (57.1%) 12 (42.9%)	 30 (76.9%) 9 (23.1%)	Control vs GDM = 0.013* Control vs PE = <0.0005** GDM vs PE = 0.088
Birth weight (g)	3.08± 0.58	2.72 ± 0.10	3.03 ± 0.07	Control vs GDM = 0.639 Control vs PE = 0.002* GDM vs PE = 0.011*
Placental weight (g)	600± 15.71	550.47 ± 22.77	582.70 ± 16.81	Control vs GDM = 0.459 Control vs PE = 0.07 GDM vs PE = 0.257
Fetal gender Male Female	 13 (38.2%) 21 (61.8%)	 17 (60.7%) 11 (39.3%)	 21 (53.8%) 18 (46.2%)	Control vs GDM = 0.185 Control vs PE = 0.080 GDM vs PE = 0.185

Values shown are mean ± SEM. p is significant if <0.05. ANOVA-test. Fisher's exact test. *Significant. **Highly significant

GDM group. Additionally, birth weight in the PE group is smaller compared to the control (p=0.002) and GDM groups (p=0.011) with significant differences. However, most parameters did not show differences between the control group and GDM groups.

TNC AND CD34+ CELLS WERE SIGNIFICANTLY REDUCED IN BOTH THE PE AND GDM GROUPS AS COMPARED TO CONTROL GROUP

Table 2 shows summary of UCB parameters of all patients' groups. All parameters in the control group including volume, nucleated cell count, total nucleated count, $CD34^+$ count and total $CD34^+$ count were significantly higher as compared to the GDM and PE groups.

We found that UCB parameters were significantly reduced in mothers affected by PE and GDM compared to the healthy pregnant mothers. These parameters include volume, TNC and $CD34^+$ count. In the PE group, our findings are consistent with previous published report. Stallmach et al. (1998) reported that PE led to less erythroid precursors and early granulopoietic cells in foetal livers and alteration of foetal haematopoiesis. This situation gives rise to alteration of cytokines and growth factor level in foetal blood. The long term alteration pattern of the haematopoietic growth factors and cytokines in the foetus might have influence on haematopoietic activity in the progenitor cell population (Surbek et al. 2001). The abnormalities in the placenta due to PE also may affect the ability of haematopoietic stem cells to grow and differentiate (Santillan et al. 2013). In the GDM group, the effects of GDM on UCB HSC have not been well studied. The mechanism by which this disease affects the UCB HSC is almost the same as in PE (Hadi & Al Suwaidi 2007). A few studies showed that such adverse conditions exist in pregnancies complicated by GDM, which tend to associate placental anatomy with physiological alterations. Mainly, the changes affect the micro-anatomical and molecular levels including aberrant villous vascularization. Angiogenesis facilitating functions might be altered and circulating haematopoietic progenitor cells will be reduced in the cord blood of GDM pregnancies (Gauster et al. 2011). However, this result is contradicts with Hadarits et al. (2015) that showed significantly higher CD34+ cells in mothers with GDM. This might be explained by different population of subjects, whereas Europe region compared to this study in Malaysia. Besides, Hadarits et al. (2015) cannot conclude the definite finding due to the small sample sizes. These results should be confirmed in other larger cohort study to come with absolute conclusion.

TNC is one of the parameters that should be considered before processing UCB unit. We showed that TNC obtained from healthy pregnant mothers is ($9.36 \pm 0.62 \times 10^8$ cells) 50% more than the medical disorders group. However, there were no significant differences between the PE and GDM groups. TNC content that requires UCB processing and storage is 6 to 10×10^8 cells (Jaime-Perez et al. 2011). In contrary, in a study by Nakagawa et al. (2004), the threshold for processing and cryopreservation was set at 4×10^8 TNC, hence it is unlikely that the UCB unit collected from PE and GDM mothers in our study will be selected for processing and storage. The TNC dose for transplantation is very important and significantly related to post-transplant survival and transplantation related mortality. Acceptable dose considered for transplantation is in the range of 1 to 3×10^7 TNC/kg recipient weights. Higher dose of TNC will reduce percentage of death (Donaldson et al. 1999). The cut-off value of 8×10^8 cells or more as standard TNC dose correlates with adequate numbers of $CD34^+$ for cryopreservation for grafting purposes.

$CD34^+$ count is another parameter that will influence the outcome of engraftment and transplantation related mortality (TRM) including survival post-transplantation.

TABLE 2. Summary of UCB parameters in all patients groups

Variable	Control Group	PE Group	GDM Group	P Value
Volume (mL)	102.16 ± 2.43	91.43 ± 2.55	80.36 ± 1.88	Control vs GDM = <0.0005** Control vs PE = 0.003* GDM vs PE = 0.001*
Nucleated Cell ($\times 10^6$ cells/mL)	9.03 ± 0.46	4.21 ± 0.41	4.74 ± 0.30	Control vs GDM = <0.0005** Control vs PE = <0.0005** GDM vs PE = 0.293
Total Nucleated Cell ($\times 10^8$ cells)	9.36 ± 0.62	3.96 ± 0.44	3.87 ± 0.29	Control vs GDM = <0.0005** Control vs PE = <0.0005** GDM vs PE = 0.853
$CD34^+$ (cells/μL)	125.60 ± 9.75	67.17 ± 5.47	61.18 ± 6.64	Control vs GDM = <0.0005** Control vs PE = <0.0005** GDM vs PE = 0.507
Total $CD34^+$ ($\times 10^6$ cells)	13.07 ± 1.11	6.28 ± 0.57	4.88 ± 0.55	Control vs GDM = <0.0005** Control vs PE = <0.0005** GDM vs PE = 0.086

Values shown are mean ± SEM. p is significant if <0.05. ANOVA test. *Significant. **Highly significant

High dose of CD34+ cells is crucial for hematopoietic engraftment (Fadilah et al. 2013). One study showed that patients who received 1.7×10^5 $CD34^+$/kg or higher will have lower TRM compared to those who receive lower than 1.7×10^5 $CD34^+$/kg (Wagner et al. 2002). By using this threshold, UCB collected from PE mothers in our study would be suitable for recipients with body weight of less than 40 kg and for UCB obtained from GDM mothers would be suitable for recipients with body weight of less than 28 kg. Henceforth, we suggest that UCB units from pregnancies complicated by such medical disorder should not be used in adult patients. These UCB units would only be beneficial for babies or children or young small built adolescents.

THE PE GROUP SHOWS SIGNIFICANTLY LOWER BIRTH WEIGHT AND HIGHER SYSTOLIC BP AND DIASTOLIC BP WHICH LED TO A LOWER UCB VOLUME

We further analysed the correlation between maternal and neonatal factors with UCB parameters in the PE group as shown in Table 3. Gestational age and birth weight were statistically significantly associated with NCC and TNC. The birth weight, Systolic BP and diastolic BP were the main significant factors affecting the low volume of UCB. Meanwhile, an increased systolic BP showed significant correlation with reduced counts of $CD34^+$ cell/μL (p=0.046) and total $CD34^+$ count (p=0.013) and increase diastolic BP also showed significant correlation with reduced counts of $CD34^+$ cell/μL (p=0.020). In addition, maternal age, maternal WCC, gravidity, route of delivery, placental weight and neonatal gender did not show any significant correlation with all UCB parameters.

Upon scrutinizing the relationship between maternal and neonatal factors and UCB parameters in PE pregnancies, we found that systolic BP and diastolic BP have significant effect on UCB volume and total $CD34^+$ count. PE mothers with higher systolic readings have reduced volumes, $CD34^+$ counts and total $CD34^+$ counts. Meanwhile, PE mothers with higher diastolic readings have reduced volumes and $CD34^+$ counts. These findings were consistent with a previous study that showed the severity of PE affects the volume, $CD34^+$ count and total $CD34^+$ count negatively (Abdul Wahid et al. 2012; Surbek et al. 2001). We found bigger birth weights increases cord blood volume, NCC and TNC. Our data is consistent with a previous study in normal pregnancy that bigger babies were associated with larger cord blood volumes and higher TNC counts (McGuckin et al. 2007). However, birth weight did not influence $CD34^+$ cell/μL and total $CD34^+$ cell count. This data was not consistent with prior studies on non-PE mothers, perhaps because of the high volume of cord blood in the PE group, which is almost similar to the control group in the earlier result. This high volume of cord blood contributes towards high total $CD34^+$ counts as it depends on the blood volume.

TABLE 3. The association between maternal & perinatal factors and UCB parameters in PE group

Factor	Volume (mL)	Nucleated cell (10^6 cells/ mL)	Total nucleated cell ($\times 10^8$ cells)	$CD34^+$ (cells/μL)	Total $CD34^+$ ($\times 10^6$ cells)
Maternal age (years)					
P value	0.716	0.320	0.229	0.701	0.629
r value	-0.092	-0.249	-0.298	-0.097	-0.122
Gestational age (weeks)					
P value	0.983	<0.0005**	<0.0005**	0.626	0.561
r value	-0.005	0.631	0.660	0.123	0.147
Maternal white cell count					
P value	0.894	0.709	0.619	0.286	0.232
r value	0.035	-0.098	-0.130	-0.275	-0.306
Gravidity	0.583	0.183	0.257	0.257	0.218
Route of delivery	0.704	0.399	0.223	0.925	0.925
Systolic blood pressure (mmHg)					
P value	<0.0005**	0.885	0.198	0.046*	0.013*
r value	-0.746	-0.031	-0.266	-0.380	-0.463
Diastolic blood pressure (mmHg)					
P value	0.009*	0.853	0.393	0.020*	0.172
r value	-0.487	-0.039	-0.179	-0.437	-0.282
Placental weight (g)					
P value	0.256	0.416	0.236	0.943	0.523
r value	0.259	0.227	0.326	0.020	0.179
Birth weight (g)					
P value	0.019*	0.001*	0.001*	0.406	0.232
r value	0.450	0.613	0.591	0.216	0.306
Baby gender	0.396	0.588	0.961	0.349	0.588

P is significant if <0.05. Spearman's rank order test. Mann-Whitney μ-test.*significant. ** Highly significant.

Another possible explanation is the small sample size (n=32) compared to other studies. But one study with a huge cohort sample size (n=>8000) reported that birth weight was not strongly related to HSC count (Cairo et al. 2005), thus supporting the theory that altered foetal haematopoiesis is a consequence of PE itself rather than growth restriction alone as the UCB volume and numbers of HSC was smaller in the PE group compared to healthy pregnancies, regardless of birth weight (Hiett et al. 1995).

GDM GROUP SHOWS SIGNIFICANTLY LOWER SYSTOLIC BP, LOW PLACENTAL WEIGHT AND BIRTH WEIGHT WITH LOWER NCC AND TNC

The correlation between maternal and neonatal factors with UCB parameters in the GDM group were further analysed as shown in Table 4. We found that the placental weight significantly lowered the volume of cord blood (p=0.015). Both NCC and TNC were significantly reduced with lower systolic BP (p=0.007; p=0.017), including a decreased of placental weight (p=<0.0005; p=<0.0005) and birth weight (p=0.017; p=0.011). Other factors showed no correlation with UCB parameters.

In the GDM group, we found that maternal age, gestational age, maternal white cell count, gravidity, delivery and diastolic pressure had no association with UCB parameters. However, systolic BP showed positive significant influence on NC and TNC only. Higher systolic BP increases NC and TNC counts. However, no previous studies reported any correlation between maternal factors and UCB parameters. In a study by Abdul Wahid et al. (2012), it was shown that in healthy pregnancies lower systolic BP correlated with an increase in NC and TNC counts. The exact mechanism involving GDM which leads to an increase in NC and TNC counts is not exactly understood but we believe that vascular endothelial dysfunction or inflammation may play a role. A larger sample size may serve to further unveil the patterns in UCB parameters.

Among neonatal factors, birth weight had significant correlations with NC and TNC counts and placental weight appeared to influence volume, NC and TNC. Our study found that there was no significant difference in placental weight between healthy mothers and GDM mothers. This finding is not in line with a previous study which reported that the placentae of diabetic women were larger, thicker, more plethoric, and associated with macrosomia (Radaelli et al. 2003). Good glycaemic control among our GDM subjects may be the reason for the normal placental and birth weight. This is in agreement with previous study which reported macrosomia is less common in GDM subject with good glycaemic control (Kampan et al. 2013). However, GDM affects the angiogenic factors in placentae with decreased UCB parameters. In addition, our data showed that higher placental weight will increase the volume, NC and TNC of UCB in GDM mothers, without any association with $CD34^+$

TABLE 4. The association between maternal & perinatal factors and UCB parameters in GDM group

Factor	Volume (mL)	Nucleated cell (10^6 cells/ mL)	Total nucleated cell ($\times10^8$ cells)	$CD34^+$ (cells/μL)	Total $CD34^+$ ($\times10^6$ cells)
Maternal age (years)					
P value	0.119	0.262	0.836	0.317	0.527
r value	0.254	-0.184	-0.034	-0.165	-0.104
Gestational age (weeks)					
P value	0.525	0.868	0.940	0.471	0.406
r value	-0.105	0.028	-0.013	-0.119	-0.137
Maternal white cell count					
P value	0.182	0.623	0.280	0.974	0.817
r value	-0.225	-0.083	-0.182	-0.005	-0.039
Gravidity	0.142	0.475	0.884	0.107	0.213
Route of delivery	0.520	0.368	0.424	0.351	0.194
Systolic blood pressure (mmHg)					
P value	0.226	0.007*	0.017*	0.484	0.290
r value	0.201	0.433	0.387	0.117	0.176
Diastolic blood pressure (mmHg)					
P value	0.512	0.886	0.635	0.720	0.563
r value	-0.110	-0.024	-0.080	-0.060	-0.097
Placental weight (g)					
P value	0.015	<0.0005**	<0.0005**	0.621	0.268
r value	0.382	0.549	0.583	0.084	0.187
Birth weight (g)					
P value	0.160	0.017*	0.011*	0.448	0.197
r value	0.229	0.381	0.401	0.125	0.211
Baby gender	0.253	0.143	0.139	0.384	0.196

p is significant if <0.05. Spearman's rank order test. Mann-Whitney μ-test.*significant. ** Highly significant

and total CD34+ cell count. Since volume and TNC are the current standard practice for UCB cryopreservation, we can consider placental weight in GDM mothers as an additional factor that contributes to the UCB parameters.

CONCLUSION

From this study, we can conclude that medical disorders in pregnancy, namely PE and GDM, affect the quality of UCB HSC. The numbers of NCC from pregnancies affected by these disorders are shown to be lower than the international standards. Still, this parameter remains as an important marker for UCB processing and cryopreservation. In addition, several factors such as gestational age, systolic BP, placental weight and birth weight should be taken into consideration when mothers with PE consider undergoing CB banking. Meanwhile, in GDM mothers, factors such as systolic BP, placental weight and birth weight should also be considered when selecting good quality UCB for CB banking. Future studies involving larger sample sizes may help to further confirm these findings for efficient CB banking.

ACKNOWLEDGEMENTS

We would like to express our gratitude to all members of Department of Obstetrics & Gynaecology, UKMMC for their assistance and contributions especially with cord blood collection. Also, Universiti Kebangsaan Malaysia (UKM) and Ministry of Higher Education Malaysia (MOHE) for the awarded grant FRGS/2/2013/SKK01/UKM/03/2. The authors declare no conflict of interest.

REFERENCES

Abdul Wahid, F.S., Nasaruddin, M.Z., Idris, M., Razif, M., Tusimin, M., Tumian, N.R. & Abdullah Mahdy, Z. 2012. Effects of preeclampsia on the yield of hematopoietic stem cells obtained from umbilical cord blood at delivery. *Journal of Obstetrics and Gynaecology Research* 38(3): 490-497.

Acosta, J.C., Haas, D.M., Saha, C.K., Dimeglio, L.A., Ingram, D.A. & Haneline, L.S. 2011. Gestational diabetes mellitus alters maternal and neonatal circulating endothelial progenitor cell subsets. *American Journal of Obstetrics and Gynecology* 204(3): 254.e8-254.e15.

Ballen, K., Broxmeyer, H.E., McCullough, J., Piaciabello, W., Rebulla, P., Verfaillie, C.M. & Wagner, J.E. 2001. Current status of cord blood banking and transplantation in the United States and Europe. *Biology of blood and Marrow Transplantation* 7(12): 635-645.

Ballen, K., Wilson, M., Wuu, J., Ceredona, A., Hsieh, C., Stewart, F., Popovsky, M. & Quesenberry, P. 2001. Bigger is better: Maternal and neonatal predictors of hematopoietic potential of umbilical cord blood units. *Bone Marrow Transplantation* 27(1): 7-14.

Ballen, K.K., Gluckman, E. & Broxmeyer, H.E. 2013. Umbilical cord blood transplantation: The first 25 years and beyond. *Blood* 122(4): 491-498.

Barnett, D., Janossy, G., Lubenko, A., Matutes, E., Newland, A. & Reilly, J. 1999. Guideline for the flow cytometric enumeration of CD34+ haematopoietic stem cells. Prepared by the cd34+ haematopoietic stem cell working party. *Clinical & Laboratory Haematology* 21(5): 301-308.

Broxmeyer, H. 2011. Cord blood: Biology, transplantation, banking, and regulation. *Bethesda: AABB*.

Broxmeyer, H.E. & Farag, S. 2013. Background and future considerations for human cord blood hematopoietic cell transplantation, including economic concerns. *Stem Cells and Development* 22(S1): 103-110.

Buemi, M., Allegra, A., D'Anna, R., Coppolino, G., Crascì, E., Giordano, D., Loddo, S., Cucinotta, M., Musolino, C. & Teti, D. 2007. Concentration of circulating endothelial progenitor cells (EPC) in normal pregnancy and in pregnant women with diabetes and hypertension. *American Journal of Obstetrics and Gynecology* 196(1): 68.e1-68.e6.

Cairo, M.S., Wagner, E.L., Fraser, J., Cohen, G., Van De Ven, C., Carter, S.L., Kernan, N.A., & Kurtzberg, J. 2005. Characterization of banked umbilical cord blood hematopoietic progenitor cells and lymphocyte subsets and correlation with ethnicity, birth weight, sex, and type of delivery: A cord blood transplantation (COBLT) study report. *Transfusion* 45(6): 856-866.

Desforges, J.F., Cunningham, F.G. & Lindheimer, M.D. 1992. Hypertension in pregnancy. *New England Journal of Medicine* 326(14): 927-932.

Dimitriou, H., Perdikogianni, C., Stiakaki, E., Vorgia, P., Hatzidaki, E. & Kalmanti, M. 2006. The impact of mode of delivery and gestational age on cord blood hematopoietic stem/progenitor cells. *Annals of Hematology* 85(6): 381-385.

Donaldson, C., Armitage, W.J., Laundy, V., Barron, C., Buchanan, R., Webster, J., Bradley, B. & Hows, J. 1999. Impact of obstetric factors on cord blood donation for transplantation. *British Journal of Haematology* 106(1): 128-132.

Easterling, R. & Benedetti, T.J. 1989. Preeclampsia: A hyperdynamic disease model Thomas. *American Journal of Obstetrics & Gynecology* 160(6): 1447-1453.

Fadilah, S., Mohd-Razif, M., Seery, Z., Nor-Rafeah, T., Wan-Fariza, W., Habsah, A. & Leong, C. 2013. Predictors of the yield of mobilized peripheral blood CD34+ cells in HLA-matched sibling donor. *Transfusion and Apheresis Science* 49(3): 583-589.

Fadilah, S.A.W., Leong, C. & Cheong, S. 2008. Stem cell transplantation in Malaysia and future directions. *Med. J. Malaysia* 63(4): 279.

Gauster, M., Hiden, U., van Poppel, M., Frank, S., Wadsack, C., Hauguel-de Mouzon, S. & Desoye, G. 2011. Dysregulation of placental endothelial lipase in obese women with gestational diabetes mellitus. *Diabetes* 60(10): 2457-2464.

George, T.J., Sugrue, M.W., George, S.N. & Wingard, J.R. 2006. Factors associated with parameters of engraftment potential of umbilical cord blood. *Transfusion* 46(10): 1803-1812.

Gluckman, E., Rocha, V., Boyer-Chammard, A., Locatelli, F., Arcese, W., Pasquini, R., Ortega, J., Souillet, G., Ferreira, E. & Laporte, J.P. 1997. Outcome of cord-blood transplantation from related and unrelated donors. *New England Journal of Medicine* 337(6): 373-381.

Hadarits, O., Zóka, A., Barna, G., Al-Aissa, Z., Rosta, K., Rigó Jr., J., Kautzky-Willer, A., Somogyi, A. & Firneisz, G. 2015. Increased proportion of hematopoietic stem and progenitor cell population in cord blood of neonates born to mothers with gestational diabetes mellitus. *Stem Cells and Development* 25(1): 13-17.

Hadi, H.A. & Al Suwaidi, J. 2007. Endothelial dysfunction in diabetes mellitus. *Vascular Health and Risk Management* 3(6): 853.

Hiett, A., Britton, K., Hague, N., Brown, H., Stehman, F.B. & Broxmeyer, H.E. 1995. Comparison of hematopoietic progenitor cells in human umbilical cord blood collected from neonatal infants who are small and appropriate for gestational age. *Transfusion* 35(7): 587-591.

Ismail, N., Aris, N.M., Mahdy, Z.A., Ahmad, S., Naim, N.M., Siraj, H. & Zakaria, S.Z.S. 2011. Gestational diabetes mellitus in primigravidae: A mild disease. *Acta Medica (Hradec Kralove)* 54(1): 21-24.

Jaime-Perez, J.C., Monreal-Robles, R., Rodriguez-Romo, L.N., Mancias-Guerra, C., Herrera-Garza, J.L. &Gomez-Almaguer, D. 2011. Evaluation of volume and total nucleated cell count as cord blood selection parameters: A receiver operating characteristic curve modeling approach. *Am. J. Clin. Pathol.* 136(5): 721-726.

Jones, J., Stevens, C.E., Rubinstein, P., Robertazzi, R.R., Kerr, A. & Cabbad, M.F. 2003. Obstetric predictors of placental/ umbilical cord blood volume for transplantation. *American Journal of Obstetrics and Gynecology* 188(2): 503-509.

Kalok, A., Peraba, P., Shah, S.A., Mahdy, Z.A., Jamil, M.A., Kampan, N., Sulaiman, S. & Ismail, N.A.M. 2018. Screening for gestational diabetes in low-risk women: Effect of maternal age. *Hormone Molecular Biology and Clinical Investigation*. DOI: 10.1515/hmbci-2017-0071.

Kampan, N., Azman, H., Hafiz, I., Mohammad, H., Yee, C.S. & Abdul Ghani, N. 2013. Outcome of pregnancy among Malaysian women with diabetes mellitus-a single centre experience. *Malaysian Journal of Public Health Medicine* 13(2): 1-10.

Kögler, G., Somville, T., Göbel, U., Hakenberg, P., Knipper, A., Fischer, J., Adams, O., Krempe, C., McKenzie, C. & Rüttgers, H. 1998. Haematopoietic transplant potential of unrelated and related cord blood: The first six years of the EUROCORD/ NETCORD Bank Germany. *Klinische Padiatrie* 211(4): 224-232.

Luppi, P., Powers, R.W., Verma, V., Edmunds, L., Plymire, D. & Hubel, C.A. 2010. Maternal circulating CD34+ VEGFR-2+ and CD133+ VEGFR-2+ progenitor cells increase during normal pregnancy but are reduced in women with preeclampsia. *Reproductive Sciences* 17(7): 643-652.

McGuckin, C., Basford, C., Hanger, K., Habibollah, S. & Forraz, N. 2007. Cord blood revelations: The importance of being a first born girl, big, on time and to a young mother! *Early Human Development* 83(12): 733-741.

Nakagawa, R., Watanabe, T., Kawano, Y., Kanai, S., Suzuya, H., Kaneko, M., Watanabe, H., Okamoto, Y., Kuroda, Y. & Nakayama, T. 2004. Analysis of maternal and neonatal factors that influence the nucleated and CD34+ cell yield for cord blood banking. *Transfusion* 44(2): 262-267.

Radaelli, T., Varastehpour, A., Catalano, P. & Hauguel-de Mouzon, S. 2003. Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. *Diabetes* 52(12): 2951-2958.

Rocha, V., Labopin, M., Sanz, G., Arcese, W., Schwerdtfeger, R., Bosi, A., Jacobsen, N., Ruutu, T., De Lima, M. & Finke, J. 2004. Acute leukemia working party of European blood and marrow transplant group; Eurocord-Netcord Registry. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N. Engl. J. Med.* 351(22): 2276-2285.

Rocha, V., Wagner Jr., J.E., Sobocinski, K.A., Klein, J.P., Zhang, M.J., Horowitz, M.M. & Gluckman, E. 2000. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. *New England Journal of Medicine* 342(25): 1846-1854.

Santillan, D.A., Hamilton, W.S., Christensen, A., Talcott, K.M., Gravatt, L.K., Santillan, M.K. & Hunter, S.K. 2013. The effects of preeclampsia on signaling to hematopoietic progenitor cells. *Proceedings in Obstetrics and Gynecology* 3(1): 1-11.

Scaradavou, A., Brunstein, C.G., Eapen, M., Le-Rademacher, J., Barker, J.N., Chao, N., Cutler, C., Delaney, C., Kan, F. & Isola, L. 2013. Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukemia. *Blood* 121(5): 752-758.

Stallmach, T., Karolyi, L., Lichtlen, P., Maurer, M., Hebisch, G., Joller, H., Marti, H.H. & Gassmann, M. 1998. Fetuses from preeclamptic mothers show reduced hepatic erythropoiesis. *Pediatric Research* 43(3): 349-354.

Surbek, D.V., Danzer, E., Steinmann, C., Tichelli, A., Wodnar-Filipowicz, A., Hahn, S. & Holzgreve, W. 2001. Effect of preeclampsia on umbilical cord blood hematopoietic progenitor-stem cells. *American Journal of Obstetrics and Gynecology* 185(3): 725-729.

Surbek, D.V., Visca, E., Steinmann, C., Tichelli, A., Schatta, S., Hahn, S., Gratwohl, A. & Holzgreve, W. 2000. Umbilical cord blood collection before placental delivery during cesarean delivery increases cord blood volume and nucleated cell number available for transplantation. *American Journal of Obstetrics and Gynecology* 183(1): 218-221.

Tse, W. & Laughlin, M.J. 2005. Umbilical cord blood transplantation: A new alternative option. *ASH Education Program Book* 2005(1): 377-383.

Wagner, J.E., Barker, J.N., DeFor, T.E., Baker, K.S., Blazar, B.R., Eide, C., Goldman, A., Kersey, J., Krivit, W. & MacMillan, M.L. 2002. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: Influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood* 100(5): 1611-1618.

Mohd Razif Mohd Idris, Fazlina Nordin* & S Fadilah Abd Wahid
Cell Therapy Centre (CTC)
Universiti Kebangsaan Malaysia Medical Centre
Jalan Yaacob Latif, Bandar Tun Razak
56000 Cheras, Kuala Lumpur, Federal Territory
Malaysia

Zaleha Abdullah Mahdy
Department of Obstetrics and Gynaecology
Universiti Kebangsaan Malaysia Medical Centre
Jalan Yaacob Latif, Bandar Tun Razak
56000 Cheras, Kuala Lumpur, Federal Territory
Malaysia

*Corresponding author; email: nordinf@ppukm.ukm.edu.my

Received: 6 March 2018
Accepted: 20 June 2018