Gestational diabetes mellitus in pregnancy increased erythropoietin level affecting differentiation potency of haematopoietic stem cell of umbilical cord blood

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Research article

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Abstract

Background The in utero environment has many factors that can support cell differentiation. Cytokines, chemokines and growth factors play big roles in haematopoietic mechanisms. Some diseases like gestational diabetes mellitus (GDM) might affect the environment and haematopoietic stem cell (HSC) quality. The aim of this study is to investigate the adverse effects of GDM on umbilical cord blood (UCB) HSC in terms of differentiation potency including the UCB parameters used for banking and transplantation purposes.

Methods UCB-HSC was isolated and further enriched using immuno-magnetic separation beads (MACS). The UCB-HSC were cultured in methylcellulose media to investigate the differentiation potency. The level of erythropoietin (EPO) and insulin in the UCB plasma was measured using enzyme linked immunoassay (ELISA) technique.

Results The UCB parameters; volume, total nucleated count (TNC) and total CD34+ cells were significantly reduced in the GDM group compared to the control group. The number of HSC progenitors' colonies were significantly reduced in the GDM group except for progenitor BFU-E, which was significantly increased (GDM=94.19 ± 6.21, Control=73.61 ± 2.73, p=0.010). This data was associated with higher EPO level in GDM group. However, the insulin level in the GDM group was comparable to the Control group.

Conclusion Our results suggest that the changes in the in utero environment due to abnormalities during pregnancy such as GDM affect the differentiation potency of UCB-HSC. These findings can be considered as an additional parameter for the inclusion and exclusion criteria for UCB banking, particularly for mothers with GDM.

Background

UCB has been used as an alternative source of haematopoietic stem cells (HSCs) for transplantation. Over 30,000 UCB transplants have been performed worldwide for the treatment of various diseases. Although the volume of UCB is small at around 100 mL per unit, it is capable of growing faster and enabling it to regenerate the entire of HSC population compared to bone marrow (Gluckman et al. 1997). UCB offers the advantages of easier acquisition, lack of risk for donors (mothers), less risk of infection, immediate availability and less stringent criteria for the human leucocyte antigen (HLA) matching and low incidence of graft versus host disease (Rocha et al. 2000). However, insufficient number of HSCs in one unit UCB causes the slowdown in haematopoietic recovery, especially in adult and child patients

(Chao et al. 2004). The successful outcome of transplantation is closely correlated to the nucleated cell count (NCC) and the number of $CD34^+$ cells infusion (Gluckman et al. 1997; Rocha et al. 2000; Wagner et al. 2002).

The ideal number of NCC for transplantation is $1x10^7$ to $3x10^7$ cells / kg of recipient weight, whereby the higher the dose of NCC, the shorter the engraftment period (Gluckman et al. 1997). Meanwhile for $CD34^+$ cells, the best dose for transplantation is $>1.7 \times 10^5$ cells / kg. One finding showed that only 22% of patients were able to survive more than one year after receiving NCC $<3.7 \times 10^7$ cells / kg, while 41% of patients receiving at least 3.7×10^7 cells / kg had more than one year of survival (Gluckman et al. 1997) . While one study found that patients receiving $CD34^+$ cells $<1.7 \times 10^5$ cells / kg and NC 1×10^7 cells / kg had a higher mortality rate (Kogler et al. 1999). Based on these findings, most UCB banks have set policies for selecting high quality UCB units for storage purposes (George et al. 2006).

To date, very few studies have been conducted to examine the effects of gestational diabetes mellitus (GDM) on the quality of UCB-HSC. GDM is defined as glucose intolerance with onset or first recognition during pregnancy. Previous study on a single centre experience at Hospital Universiti Kebangsaan Malaysia (UKM), reported an overall poor outcome of GDM pregnancy with high level of HbA1c, but the effect on the quality of UCB-HSC was not further investigated (Kampan et al. 2013). Data from developed countries suggest that the prevalence of GDM is increasing, being almost 10% of pregnancies and probably reflecting the global obesity epidemic. In our centre, the prevalence can be as high as 24.9% (Shamsuddin et al. 2001). While considering longer term outcomes for the baby, evidence is gradually mounting that GDM adds an intrauterine environmental risk factor (Kaaja & Rönnemaa 2008). GDM may affect placental haemodynamics with abnormal umbilical artery blood flow. The impaired oxygenation in the umbilical vein can alter the *in utero* environment such as cytokines and growth factors (Gauster et al. 2012) resulting in foetal hypoxia. These cytokine factors are EPO and insulin.

Most studies examined the association between EPO with foetal polycythaemia or hypoxia during pregnancy (Teramo 2010; Cetin et al. 2011). Besides, EPO levels are often correlated with BFU-E colony formation units. A study by Shannon et al, 1986 found BFU-E colonies did not show any difference between the control and GDM groups. The study concludes that the production of erythrocytes is normal in GDM infants and supports the hypothesis that polycythaemia in GDM infants is caused by a secondary phenomenon, not due to abnormalities in primary haematopoietic stem cells (Shannon et al. 1986). EPO is a hormone that plays a role in regulating foetal erythropoiesis (Teramo & Widness 2009). A pathology has been detected in the process of erythropoiesis in infants of diabetic mothers, attributable to abnormalities in the metabolic system and intra-uterine hypoxia (Di Cianni et al. 2003; Teramo & Widness 2009).

To date, no studies have been conducted to examine the correlations between UCB insulin concentration and UCB-HSC quality. In a study conducted by Gauster et al. (2012), it was concluded that high levels of insulin in the UCB plasma could interfere with foetal growth and subsequently alter the process of angiogenesis and vascularization that could affect the quality of UCB-HSC (Gauster et al. 2012). These

two cytokines, EPO and insulin, may provide a good microenvironment for cell growth and haematopoiesis (Santillan et al. 2013), thus producing good quality HSC. High EPO levels always correlated with diabetic pregnancies and hypoxia, whilst higher insulin might impair the match between supply and demand from the placenta. This study aims to investigate whether GDM might influence the quality and *in vitro* differentiation of UCB-HSC and its correlation with EPO and insulin levels.

Methods

Subjects

This prospective case-control study was conducted on 42 pregnancies with GDM, and 38 healthy pregnancies, which were on monthly follow-up and admitted for delivery at Universiti Kebangsaan Malaysia Medical Centre (UKMMC). The study was approved by the Research Ethics Committee of UKMMC. GDM was defined according to the American College of Obstetricians and Gynaecologists guidelines with abnormal glucose values at fasting and 2 hours post-prandial (75g OGTT test) (Randel 2014). Healthy subjects (the Control group) consisted of pregnant women of similar age, parity and gestational age without any serious disease, neither preeclampsia nor GDM. All subjects must have blood screening for hepatitis B, hepatitis C, cytomegalovirus, syphilis, human immunodeficiency virus 1-2 and haematological disorders, genetic diseases, pregestational diabetes mellitus, chronic hypertension, autoimmune diseases, renal or liver impairment, multiple pregnancies and foetal anomalies or infection. **Additional File 1** shows the summary of the experimental design of the present study.

Umbilical cord blood (UCB) collection

UCB collections were performed by trained staff nurses in accordance with hospital protocol during delivery. It was performed before placental dismissal in all patients to avoid interfering with the delivery of the baby while still preserving the sterility of the UCB. Briefly, the cord was clamped and cut after delivery of the baby. 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 freely into a 157 mL collection bag. The needle was removed when blood flow stopped. An identical collection method was used for the control group. Blood samples were stored at 4°C immediately after collection before being collected by the laboratory personnel. Samples were processed within 24 hours after collection.

Mononuclear cell (MNC) collection and CD34+ cells selection

The UCB mononuclear cells (MNCs) were extracted through density centrifugation using Lymphoprep solution (Stem Cell Technologies, USA). Briefly, the MNC layer was aspirated into new tubes and suspended with PBS (1:3 ratios) and centrifuged at 1000 rpm for 10 minutes. The pellet was washed twice with PBS and resuspended in 300 uL of Iscove's Modified Dulbeccos's Medium (IMDM). CD34+ cell selection was performed according to the manufacturer's protocol (MACS CD34 MicroBead Kit Human).

Briefly, 100 uL of blocking reagent and 100 uL of $CD34^+$ microbeads solution was added, followed by 30 minutes' incubation at 2-8°C. The microbeads-conjugated cells were separated using a magnetic column following cell counting by haemocytometers.

Colony Forming Unit (CFU) Assay

CFU assay was performed to observe the differentiation potency of the umbilical cord blood-haematopoietic stem cell (UCB-HSC) enriched CD34+ cells and to quantify the numbers of each differentiated haematopoietic progenitor cells. The assay was performed using a Methocult™ Kit (Stem cell Technologies, USA). Briefly, a total of $5x10^3$ of enriched CD34+ cells were seeded in 35 mm petri dish with differentiation media, and further incubated at 37°C for 14 days. The HSC differentiated colonies; *colony-forming unit granulocyte, erythrocyte, monocyte, megakaryocyte* (CFU-GEMM), *colony-forming unit granulocyte, monocyte* (CFU-GM), *burst-forming unit erythrocyte* (BFU-E), *colony-forming unit monocyte* (CFU-M), and *colony-forming unit granulocyte* (CFU-G) were counted under an inverted microscope. Experiments were done in duplicate for each sample. **Additional File 2** shows the morphology of UCB-HSC progenitor cell differentiation from a control subject as reference.

Insulin Assay

The plasma insulin level was determined using Quantikine® ELISA Human Insulin (R&D Systems™ a bio-techne brand, USA) according to the manufacturer's protocol. Briefly, the plasma taken during MNC density centrifugation, control and standard samples were added to the coated wells to allow antigen-antibody binding. The plate was incubated at room temperature, and washed four times. The biotinylated antibody was added to each well. The excess detection antibody was removed, and an enzyme (Streptavidin-HRP) was added and incubated to complete the four-member sandwich binds (*antibody-antigen-detection antibody-enzyme*). After the second incubation, the plate was washed four times to remove all unbound enzymes, and the final solution (stabiliser substrate solution) was added to produce the colour of the substrate-enzyme linked product. The intensity of the coloured product (optical density, OD) was compared to the concentration of human insulin, which can be determined using an ELISA plate reader at 450 nm wavelength. The concentration of human insulin in tested samples can be obtained by comparing the OD of the samples to the standard. Overall incubation time was four hours in total with 30 minutes' hands-on time. Each sample was run in duplicate.

Erythropoietin (EPO) Assay

The plasma EPO level was determined using pre-coated Human EPO ELISA Kit (Elabscience, Biotechnology Inc. USA) according to manufacturer's protocol. Briefly, standards and samples (plasma) were added to a microplate and combined with the specific antibody. After the first incubation, the plate was washed four times and the biotinylated antibody was added, following Streptavidin-HRP in each well. The plate was further incubated, washed and the enzyme-substrate reaction was terminated by adding the stop solution. The OD was measured using an ELISA plate reader at 450 nm wavelength. The OD is proportionate to the concentration of human EPO. The concentration of human EPO in tested

samples can be obtained by comparing the OD of the samples to the standard. Overall incubation time was 3 hours in total with 30 minutes' hands-on time. Each sample was run in duplicates.

Clinical data and statistical analysis

The clinical data for each subject was obtained from the Medical Records Department, UKMMC. The statistical analysis was calculated using SPSS 17.0 software. The clinical and laboratory parameters were calculated using One-way ANOVA test. The correlation of maternal and UCB parameters between GDM and control groups were calculated using Pearson's correlation test and Spearman's rank order. Data is shown in mean±standard deviation (SD) and considered statistically significant if *p* value is equal or less than 0.05 (*p<0.05*).

Results

Clinical Data Analysis

Overall, the mean age of women with GDM in this study was 30.81 years (range between 28-34 years old (**Table 1**). Body mass index (BMI) was significantly higher in women with GDM compared to the Control group (*p<0.0005*). The average gestational age at delivery was 37.89 in the GDM group, which was significantly lower compared to the Control group (*p=0. 031*) (**Table 1**). This could be the reason for the higher rate of caesarean section in theGDM group compared to the Control group (*p=0.028*). This data correlated with the relatively high percentage of caesarean birth in the GDM group compared to the Control group (23.81% vs 5.26%) (**Table 1**).

TABLE 1 A comparison of maternal characteristics in GDM and Control groups.

Parameter	GDM (n=42)	Control (n=38)	*P* Value
Mother's age	30.81 ± 0.58	29.89 ± 0.54	0.534
Body mass index (BMI)	26.7 ± 0.4	22.8 ± 0.5	**<0.0005**
Pregnancy duration (week)	37.89 ± 0.22	38.55 ± 0.19	**0.031**
Leucocyte count (x10^9/L)	11.04 ± 0.53	11.06 ± 0.43	0.978
***Gravidity**			
1-3	35 (83.33%)	36 (94.74%)	0.159
4-6	7 (16.66%)	2 (5.26%)	
Systolic pressure (mmHg)	120.74 ± 1.57	120.21 ± 1.81	0.825
Diastolic pressure (mmHg)	70.49 ± 1.49	69.38 ± 1.47	0.459
***Delivery method**			
SVD	32 (76.19%)	36 (94.74%)	**0.028**
LUSCS	10 (23.81%)	2 (5.26%)	

Notes: *p* values based on ANOVA statistical analysis.
p value is significant < 0.05
*Shown as frequency
Data shown in mean±SD.

UCB Volume and UCB Parameters Analysis

The correlation between UCB volume and parameters such as nucleated cell count (NCC), total nucleated cell (TNC), $CD34^+$ cell count and total $CD34^+$ cell was further analysed (**Table 2**). Our data showed all UCB parameters were significantly decreased in the GDM group compared to the Control group (*p<0.0005*) (**Table 2**). The TNC and CD34+ cell counts in the GDM group were two times lower than the Control group (**Table 2**).

TABLE 2 A comparisons of UCB Parameters in GDM and Control groups.

Parameter	GDM (n=42)	Control (n=38)	*P* Value
Volume (mL)	80.36 ± 1.88	102.16 ± 2.43	<0.0005
NCC (x10^6 cell/mL)	4.74 ± 0.30	9.03 ± 0.46	<0.0005
TNC (x10^8 cell)	3.87 ± 0.29	9.36 ± 0.62	<0.0005
$CD34^+$ count (cell/µL)	61.18 ± 6.64	125.60 ± 9.75	<0.0005
Total $CD34^+$ Count (x10^6 cell)	4.88 ± 0.55	13.07 ± 1.11	<0.0005

Notes: *p* values based on ANOVA statistical analysis.
p value is significant < 0.05
Data shown in mean±SD.

EPO analysis

The average EPO concentration for the GDM and Control groups were 1562.9pg/mL, and 601.8pg/mL respectively (**Figure 1**). The EPO level was significantly higher in the GDM group compared to the Control group (*p<0.0005*) (**Figure 1**). Overall, there was no correlation between UCB parameters with the EPO level in both GDM and the Control group, except for the reduced TNC counts, which was significantly correlated with a higher EPO level in the GDM group (p=0.033) (**Table 3**).

TABLE 3 A correlation between UCB parameters and EPO level in GDM and Control groups.

Group	EPO	Volume (ml)	NCC (x10^6)	TNC (x10^8)	$CD34^+$ (cell/µL)	Total $CD34^+$ (x10^6)
Control (n=38)	Nilai *p*	0.859	0.896	0.956	0.215	0.202
	Nilai r	-0.030	-0.022	0.009	-0.206	-0.212
GDM (n=42)	Nilai *p*	0.087	0.243	**0.033**	0.072	0.612
	Nilai r	0.268	0.184	0.329	0.281	0.080

Nota: *p* is Spearman's rank order test
p is significant if < 0.05.

Insulin analysis

The average insulin concentrations for the GDM and Control groups were 62.28pmol/L and 54.2pmol/L respectively (**Figure 2**). There was no significant difference between the two groups studied. Overall, there was no significant correlation between UCB parameters and insulin level in the GDM and Control groups (**Table 4**).

TABLE 4 A correlation between UCB parameters and insulin level in GDM and Control groups.

Group	Insulin	Volume (ml)	NCC ($x10^6$)	TNC	($x10^8$)	$CD34^+$ (cell/µL)	Total $CD34^+$ ($x10^6$)
Control (n=38)	Nilai *p*	0.558	0.465	0.674		0.959	0.646
	Nilai r	-0.098	0.122	0.071		0.009	-0.077
GDM (n=42)	Nilai *p*	0.730	0.456	0.658		0.411	0.908
	Nilai r	-0.055	0.118	0.070		-0.130	-0.018

Nota: *p* is Spearman's rank order test
p is significant if < 0.05.

The haematopoietic differentiation of CD34+ enriched cell by CFU assay

We observed the colony forming units of all types of progenitors at 14 days' post-incubation. The CFU-GEMM (*p<0.0005*), CFU-GM (*p<0.0005*), CFU-G (*p=0.001*), and CFU-M (*p<0.0005*) colonies were significantly reduced in the GDM group compared to the Control group (**Table 5**). Interestingly, this study showed a high number of BFU-E colonies forming in the GDM group (94.19±6.21) compared to the Control group (73.61±2.73) (*p=0.01*) (**Table 5**).

TABLE 5 A comparison of progenitors counts in GDM and Control groups.

Types of progenitor	GDM (n=42)	Control (n=38)	*P* value
CFU-GEMM	65.98 ± 2.98	87.45 ± 1.46	**<0.0005**
CFU-GM	49.50 ± 3.06	70.92 ± 1.93	**<0.0005**
BFU-E	94.19 ± 6.21	73.61 ± 2.73	**0.010**
CFU-G	70.55 ± 4.00	89.21 ± 1.46	**0.001**
CFU-M	62.79 ± 3.93	81.39 ± 1.76	**<0.0005**

Notes: *p* values based on ANOVA statistical analysis.
p value is significant < 0.05
Data shown in mean±SD.

Discussion

Statement of principle findings

This study has demonstrated that GDM in pregnancy affected the UCB volume, TNC and total CD34+ cell counts. Furthermore, the CFU assay has confirmed the adverse effect of GDM in terms of the ability of UCB-HSC to differentiate into progenitor colonies. The GDM group has shown a significantly reduced number of CFU colonies except for high BFU-E suggesting an active erythropoiesis process due to abnormal placental blood flow and hypoxia. These data also demonstrated a high EPO level in the GDM group. The EPO level was significantly higher in GDM, however the insulin level was comparable to that of the Control group.

Findings in context of existing research

UCB volume decreased in GDM pregnancy probably due to placental microvascular pathology

The UCB volume in the GDM group was significantly lower than the Control group (p<0.0005) (**Table 2**). This difference was as expected based on the pathophysiology of diabetes mellitus. It has been reported that the production of prostacyclin, a blood vessel development agent, is reduced in DM patients, thus leading to vasoconstriction of utero-placental blood vessels (Ylikorkala et al. 1981; Hadi & Suwaidi 2007). Therefore, a smaller volume of UCB will be obtained. For UCB banking, the UCB volume is a crucial

criterion in order to enable further processing and retrieval, as this will determine the number of CD34+ cell needed for downstream treatment.

NCC and TNC counts were reduced in GDM pregnancy and did not meet the international cell transplant standards for processing and cryopreservation

The NCC and TNC values in the GDM group were twice lower than the Control group. According to the standards of UCB banks, the minimum value of TNC for processing and cryopreservation is 4-6 x 10^8 cells (Nakagawa et al., 2004; Jaime-Pérez et al., 2011). However, the average value of TNC in the GDM group was 3.87 x 10^8 cells (**Table 2**), which may be considered for processing and cryopreservation. TNC count is an important parameter for prediction of the survival and mortality outcomes following HSC transplantation. The TNC transplant required a standard TNC count of 1-3 x 10^7 cell / kg weight of the receiver. A higher TNC count will give better transplant outcomes including reduced mortality (Donaldson et al. 1999).

Total $CD34^+$ cell count reduced in GDM group and only sufficient for child recipients

The GDM group had significantly lower total CD34+ cell counts compared to the Control group. The number of $CD34^+$ cells is an important parameter in determining the success of transplantation in terms of post-transplant survival and mortality. A study that was conducted in patients who received a higher $CD34^+$ cell count (> 1.7x10^5 $CD34^+$ / kg) showed a reduced mortality rate (Wagner et al. 2002). Our data showed a mean total of 4.88 x 10^6 CD34+ cells in the GDM group (**Table 2**), suggesting that children aged between 5-11 years (with less than 28 kg weight) would be suitable recipients.

The insulin concentration of UCB in the GDM group is comparable to the Control group and does not affect HSC quality

Early and intensive treatment provided by the clinical team at UKMMC to pregnancy mothers' with GDM likely contributed to a controlled insulin level. This was done through a dietary plans and insulin control. The UCB insulin assessment of the GDM group is very useful in monitoring the level of foetal exposure to glucose *in utero*. The foetus exposed to hyper-insulinemia has the potential to develop illnesses such as diabetes, abnormal glucose tolerance and metabolic complications later on (Westgate et al. 2006). However, the association between the level of insulin and HSC quality is not well studied. It was also reported that angiogenesis and vascularization changes may affect the quality of HSC (Gauster et al. 2012).

GDM group shows reduced number of differentiated haematopoietic progenitor colonies as compared to Control group

These findings are in line with previous studies that reported pregnant mothers with preeclampsia (PE) (Stallmach et al. 1998; Surbek et al. 2001). However, there is as yet no such published research on GDM patients. The generation of HSC in placenta and cord during pregnancy is quite complex and not well

understood. Gekas et. al. suggested that the placental microenvironment is quite rich with cytokines and growth factors, properties that support the expansion of HSC (Gekas et al. 2010). In theory, GDM could alter placental microenvironment properties (Gekas at al. 2010), thus suggesting a reduced progenitors count in the GDM group. In addition, the higher number of progenitor cells in the Control group may be due to the decent quality of $CD34^+$ cells. The $CD34^+$ cell is a haematopoietic cell that undergoes haematopoiesis in clonogenic assay (Sarı et al. 2010). The high number of colonies in the Control group indicates that these unobstructed $CD34^+$ cells are able to differentiate and sprout.

The number of BFU-E colonies is higher in the GDM group compared to the Control group

BFU-E is a progenitor cell colony formed from over 200 erythroblasts that undergo erythropoiesis. This finding is similar to the previous study on UCB of premature infants (Wisgrill et al. 2014). However, the study of Wisgrill et al. (2014) showed a high number of $CD34^+$ cells in premature UCB, and suggested stress in these premature infants causing instability of both cytokine and chemokine levels. This phenomenon gives impact to the haematopoiesis process (Wisgrill et al. 2014). However, in this study, the $CD34^+$ cell count in the GDM group was lower than the Control group, thus suggesting that the high number of colonies of BFU-E is likely due to an active erythropoiesis process. Erythropoiesis is the process mediated by EPO which produces red blood cells. In addition, high oxygen demand by foetal hyper-insulinaemia syndrome also affects the process of aerobic metabolism that causes changes in the erythropoiesis process. In this study, the EPO assay was performed to further investigate the correlation between EPO level and HSC quality, predominantly with the high number of BFU-E colonies in the GDM group.

The high number of BFU-E colonies is probably due to an increased level of EPO in the GDM group

Generally, the quality of HSC is translated as the HSC's ability to differentiate and form various haematopoietic colonies observed through the CFU assay. A high EPO level in the GDM group may also explain the high number of BFU-E colonies. According to literature studies, most cases of GDM show increased foetal oxidative stress, which is thought to play a role in maternal and foetal complications of diabetic pregnancies (Brown et al. 1990; Fadda et al. 2001). Oxidative stress is also related to the degree of increased EPO production and hence polycythaemia in the infant. This leads to an increase in EPO levels in UCB following activation of erythropoiesis processes (Madazlı et al. 2008) and occurrence of polycythaemia in the newborn (Hadarits et al. 2015). This suggests that an increased number of BFU-E colony in the GDM group corresponds to an increased EPO level, probably due to hypoxia affecting the placenta.

Limitations and recommendation of the study

We are aware of some limitations of our study. First, the study was performed on a limited sample size. A larger sample in future may help confirm our findings in relation to EPO and CFU assays. Another limitation is that the study was performed in a single hospital, possibly leading to poor representation of the general population. We suggest extension of such a study to other hospitals in future.

Conclusion

Preliminary findings from this study suggest that GDM influenced the quality of UCB HSC. These results would be useful for physicians and mothers in relation to decision on UCB banking. In addition, studies on cytokines such as osteopontin and other growth factors should also be considered in order to better comprehend their effect on the quality of UCB-HSC and their association with placental development.

Implication of the findings

To date, no studies have reported the correlation of EPO and insulin level with the quality of UCB-HSC. Thus, this study serves as a foundation to further investigate the effect of these cytokines on the quality of HSC especially in UCB, as it is suggested that these abnormalities might interfere with the quality of HSC production.

Abbreviations

UCB-Umbilical cord blood

HSC-Hematopoietic Stem Cell

GDM-Gestational Diabetes Mellitus

TNC-Total Nucleated Cell

CFU-Colony Forming Unit

NCC-Nucleated Cell Count

CFU-GM-Colony forming unit granulocyte monocyte

CFU-G-Colony forming unit granulocyte

CFU-GEMM-Colony forming unit granulocyte, erythrocyte, monocyte, megakaryocyte

CFU-M-Colony forming unit megakaryocyte

BFU-E-Burst forming unit erythroid

MNC-Mononuclear cell

LUSCS-Lower uterine segment caesarean segment

SVD-Spontaneous vaginal delivery

BMI-Body mass index

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Research Ethics Committee of UKM (UKM 1.5.3.5/244/FRGS/2/2013/SKK01/UKM/03/2). All participants provided written informed consent to participate.

Consent for publication

All participants provided written informed consent for publication.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors Contributions'

MRI conducted the study; FN, ZAM and SFAW contributed to the design of the study; MRI and FN wrote this manuscript and involved in the collection of the data and analysis. All authors have read and approved the final version of the manuscript.

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Additional Files

ADDITIONAL FILE 1: The experimental design of the present study.

ADDITIONAL FILE 2: The UCB-HSC progenitors cell morphology. The scoring for each progenitor types were performed under inverted microscope after 14 days of incubation. A) CFU-GEMM, B) CFU-GM, C) BFU-E, D) CFU-G, and E) CFU-M. 4X magnifications.

Figures

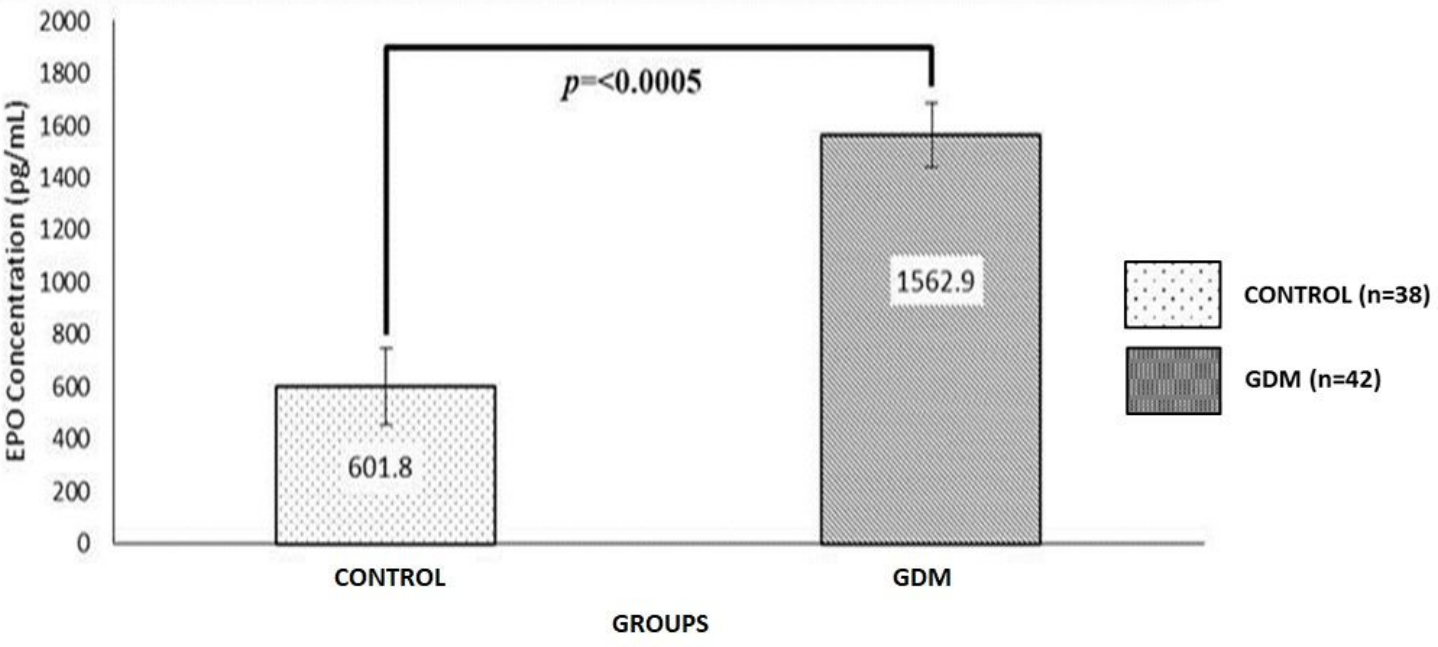

Figure 1

Comparison of EPO UCB concentration (pgmol/mL) between GDM and Control groups. The EPO level was significantly higher in GDM as compared to the Control group (p=<0.0005).

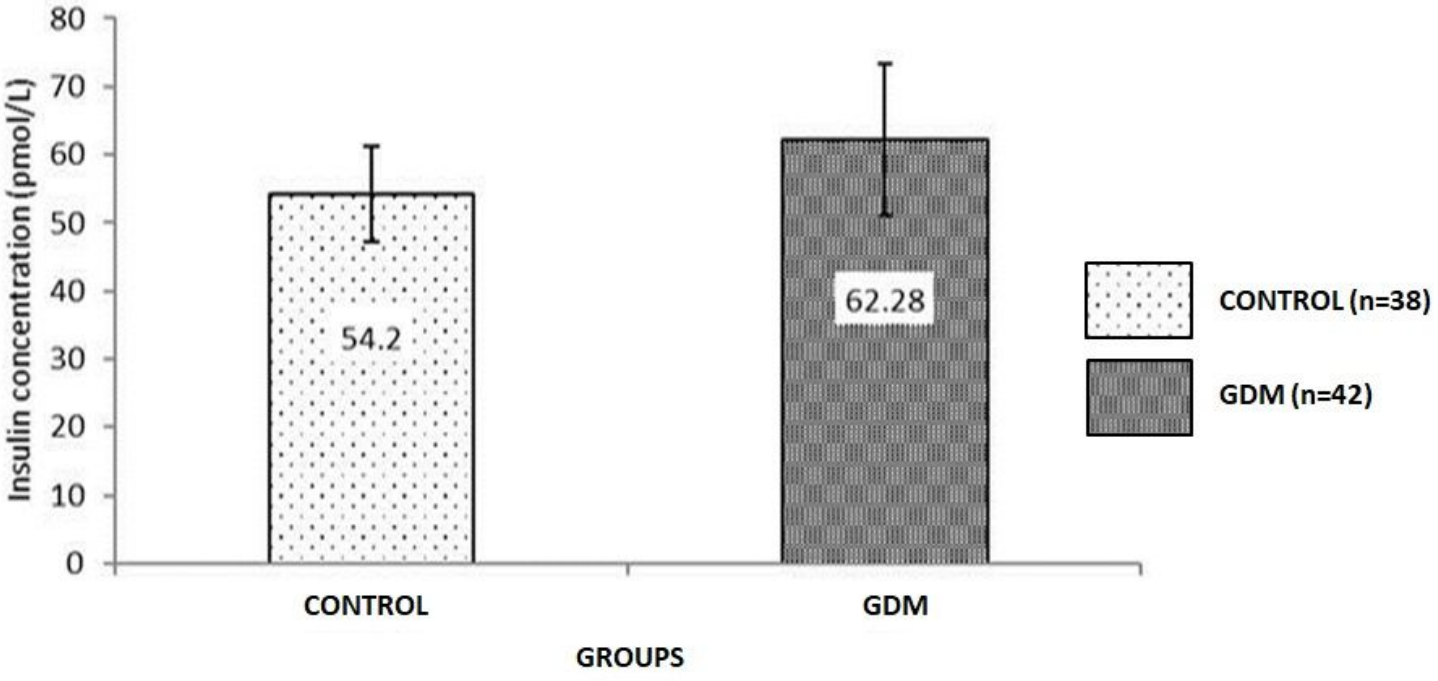

Figure 2

Comparison of insulin UCB (pmol/L) concentration between GDM and Control groups. The insulin level in the GDM group is comparable with the Control group. Data shown is mean±SEM.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ADDITIONALFILE1.GDM.pptx
- ADDITIONALFILE2.GDM.pptx