Gestational diabetes mellitus affects the differentiation of hematopoietic stem cells in neonatal umbilical cord blood

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Research article

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Gestational diabetes mellitus affects the differentiation of hematopoietic stem cells in neonatal umbilical cord blood

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Abstract

Background There are abundant hematopoietic stem cells (HSCs) in cord blood. It is known that HSCs continue to differentiate to common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). Furthermore, the CMPs could differentiate to megakaryocyte erythroid progenitors (MEPs), and MEPs ultimately differentiated to platelets and erythrocytes. It has been reported that the proportion of HSCs in cord blood was higher than that in healthy pregnant women, so as the incidence of neonatal polycythemia in gestational diabetes mellitus (GDM) patients. We aimed to investigate the HSCs population and the change of their differentiation in the cord blood of neonates of GDM mothers.

Methods In this study, we collected cord blood from GDM and healthy pregnant women at delivery. Totally 75 cases were included, in which 52 were for healthy control group and 23 were for GDM group. Then the number and differentiation status of HSCs in cord blood of the two groups were investigated. HSC (CD45+ CD34+), CLP (CD45+ CD34+ CD7+) and CMP (CD45+ CD34+ CD33+) cells were identified and quantified by flow cytometry.

Results Compared to healthy controls, HSC number in cord blood from GDM group were significantly increased ($0.77 \pm 0.063\%$ vs.$1.28 \pm 0.176\%$, p=0.0113). The number of CMP cells which were differentiated from HSCs were higher than control ($28.69 \pm 3.223\%$ vs.$46.43 \pm 4.927\%$, p=0.0033), while CLP cell number were lower ($27.04 \pm 2.044\%$ vs.$21.11 \pm 2.406\%$, p=0.0475).

Conclusions In conclusion, comparing with non-diabetic pregnant women, there were more HSCs in the cord blood of GDM patients, and the differentiation of HSCs to CMP cells was increased, while the differentiation to CLP cells was decreased. These

findings were probably caused by the high glucose microenvironment and insulin medication in pregnant women with GDM, and the HSCs differentiation changes might be influencing factors of the high incidence of neonatal erythrocytosis in GDM patients.

Keywords gestational diabetes mellitus；umbilical cord blood；Hematopoietic stem cells；differentiation

1. Background

Gestational diabetes mellitus (GDM) refers to the absence of diabetes mellitus and impaired glucose tolerance before pregnancy, and the diabetes or impaired glucose tolerance that occurred or was first discovered during pregnancy. It is a polygenic inherited, endocrine and metabolic disease [1, 2]. As a high risk factor for obstetrics, the clinical experience of pregnant women with diabetes is complicated. Many complications may occurred in pregnant women with diabetes, which are more harmful to the mother and the fetus, and must be paid attention to [3-5].

Hematopoietic stem cells (HSCs) are the most primitive types of cells with high renewal ability and multi-directional differentiation potential in bone marrow, peripheral blood and umbilical cord blood[6, 7]. Medical research in the past three decades has found that umbilical cord blood contains very abundant HSCs. Therefore, umbilical cord blood has become an important source of HSCs, and thus has been widely used in clinical practice, and is a valuable human biological resource[8, 9].

It is known that HSCs have different levels of differentiation. HSCs may differentiate downward to form multipotent progenitors (MPP). MPP can be differentiated continually into common lymphoid progenitor (CLP) and common myeloid progenitor (CMP). The CLP cells may further differentiate to B cells, T cells and NK cells. The CMP cells may further differentiate to granulocyte macrophage progenitor (GMP) and megakaryocyte erythroid progenitor (MEP)[10]. GMP can differentiate into granulocytes and macrophages, while MEP can differentiate into erythroid progenitor cells, and finally into platelets and erythrocytes[11].

In recent years, studies have shown that the proportion of HSPC (hematopoietic stem cells and progenitor cells) in cord blood of newborns born to pregnant diabetic mothers has increased [12]. In addition, it has been reported that compared with normal pregnant mothers, neonatal polycythemia is more common in newborns to pregnant diabetic mothers. To a large extent, polycystic blood disease found in infants of diabetic mothers may be secondary to the stimulation of erythrocytes by the mother in the hyperinsulinemic environment developed in the womb[13]. Even for GDM patients with diet control, the incidence of hematocrit, which exceeds 60% value of the normal range of newborn, is 10% higher than that of the normal population. For GDM patients and T1DM pregnant women who need insulin treatment, the incidence of neonatal polycythemia is 30% and 40% higher than that of the normal people [14].

Generally speaking, the most commonly used hypoglycemic drug for pregnant women is insulin. In China's relevant clinical guidelines, only one kind of insulin is recommended for the drug treatment of pregnant women with diabetes. In addition, it has been reported that proinsulin can promote the self-renewal of hematopoietic progenitors in vitro [15]. Insulin like growth factor-2 (IGF2) can regulate the activity of adult stem cells [16]. Long-term fasting can reduce IGF-1/PKA, promote the regeneration of hematopoietic stem cells and reverse the immunosuppression [17]. Therefore, insulin may affect the differentiation of HSC in cord blood during gestational diabetes.

Although there have been published study has assessed the hematopoietic stem and progenitor cell (HSPC) population in the cord blood of neonates born to mothers with GDM. They proved the cord HSC/HSPC population may be altered in GDM. However, no data were available about the differentiation of cord hematopoietic stem cell (HSC) population in GDM before our study.

2. Methods

2.1. Patients and cord blood collection

Altogether, 23 pregnant women with GDM as well as 52 control pregnant women were enrolled immediately after their routine OGTT between the 24th and 28th gestational week on a voluntary basis after signing the informed consent.We designated a control group as individuals who carry a pregnancy and were neither diagnosed with GDM nor with overt diabetes between the 24th and 28th gestational week at OGTT or later throughout the course of their pregnancy. The diagnosis of GDM has been established according to the according to China's guidelines for the prevention and treatment of type 2 diabetes (2017) (At any time during pregnancy, 75 g OGTT was administered, 5.1 mmol/L≤fasting blood glucose < 7.0 mmol/L, 1 hour OGTT blood glucose≥10.0 mmol/L, 8.5 mmol/L≤OGTT 2 hours blood glucose < 11.1 mmol/L. GDM was diagnosed when one of the above blood glucose was up to standard.

After the birth of the newborn, the cord blood samples were collected by taking the 3-8cm umbilical cord at the baby's end and storing two hemostatic forceps to ligate and break the umbilical cord. The baby is taken away for treatment, disinfected near the hemostatic forceps at the mother's end, and the needle is inserted into the umbilical vein to collect the cord blood. The most important clinical maternal data were recorded including the age at delivery, 75 g OGTT at 0 and 120min values between the 24th and 28th gestational week, prepregnancy body mass index (BMI), weight gain during pregnancy, and third trimester HbA1c value in the GDM group.

2.2. Materials and equipment

Blood sample with anticoagulation (heparin),Ficoll, phosphate buffered saline (PBS), fetal bovine serum (FBS),Diamond CD34 isolation Kit,human(miltenyi,130-094-531), LS columns and miniMACS separator(miltenyi,130-042-401), PerCP-Cy5.5 CD33, APC-Cy7 CD7，FITC CD45 and PE CD34 antibodies(eBioscience),BD FACSCantoII flow cytometer, centrifuges,centrifuge tubes, pipettes, hemocytometer.

2.3. Ethical consideration and approval,informed consent

The research was performed with the approval of the institutional review board (IRB) of the Lu He Hospital Capital Medical University, Beijing, 101149, China; and the project was evaluated and approved by the assessment panel. The purpose of this research was explained to the participants, and when the oral consent was understood and agreed, in front of a nurse, a consent form was provided in English and Chinese. This process was done to obtain the informed consent as well as the legal identification. The individuals who gave consent were included in the project.

2.4. Data collection

We involved all participants from Lu He Hospital. We obtained 10 ml umbilical cord blood samples from all enrolled patients, aged between 18 and 55 years of age. These participants did not receive any medication for cancers or autoimmune diseases.

2.5. Preparation and storage of PBMCs

First of all, umbilical cord blood sample was obtained in 20 ml disposable syringes and anticoagulation(heparin) was added. It was then gently diluted in PBS (Good Bio, China) and peripheral blood mononuclear cells (PBMCs) were isolated through Ficoll-Paque^{TM}PREMIUM sterile solution(density 1.077,GE Healthcare, 17-5442-02,Sweden) gradient centrifugation based on the manufacturer's protocol.

2.6. Flow cytometric analysis

All data were acquired using the BD FACSCantoII Flow Cytometer and 100,000 events per sample were collected. Acquired data were subsequently analyzed with FACSDiva Analysis Software (BD, USA). In addition, we performed titration to identify optimal anti-body staining. Isotype controls were used for the purposes of gating out non-specific antibody binding during analysis. PBMCs were gated and differentiated from debris according to their forward and side scatter. Cell surface CD45 (label: FITC, isotype:IgG1, k, Clone: 2D1) was used to differentiate living from dead cells and CD34 (label: PE, isotype: IgG1, k, Clone: 4H11) were stained with specific fluorescent antibodies (BioLegend, San Diego, CA).

2.7. Identification of stem and progenitor cell populations

We identified CD34+ cells within the gate of the nucleated cells. Umbilical cord blood HSCs were defined according to the International Society of Hematotherapy and Graft Engineering(ISHAGE) criteria Our gating strategy is indicated on Fig. 1.

2.8. Statistical Analysis.

All data in the study were analyzed with Prism 5.0 software (GraphPad Software, San Diego, CA, USA) and are presented as the means ± standard deviations (SDs).Statistical significance was assessed by unpaired two-tailed Student's t-tests ($*p < 0:05$; $**p < 0:01$).

3. Results

3.1. Clinical data

The most important clinical data of the pregnant population are summarized in Table 1. No significant difference was found in age or red cells levels between the two groups. However, weight gain,1h and 2 h plasma glucose in the oral glucose tolerance

test (OGTT) during pregnancy were significantly lower in the GDM group compared to the controls.

Table1. Clinical Characteristics of the Pregnant Populations Studied

Characteristic	**Control(n=22)** **（mean±SEM）**	**GDM(n=21)** **（mean±SEM）**	***p*-value**
Age at delivery (years)	31.65±1.134	32.95±0.9104	0.4735
Weight gain during pregnancy (kg)	14.78±1.043	11.94±1.081	0.0551
Plasma glucose OGTT -0h	4.887±0.2318	5.047±0.1686	0.5427
Plasma glucose OGTT -1h	7.831±0.4379	10.3±0.5299	0.0008
Plasma glucose OGTT -2h	6.608±0.3035	8.717±0.4488	0.0024
Red Cells (×10^12/L)	3.853±0.08452	4.041±0.05406	0.1468

3.2. Flow cytometric dyeing method and condition setting

Umbilical cord blood from gestational diabetes patients and healthy pregnant women was collected clinically. The frequency and differentiation of hematopoietic stem cells in umbilical cord blood were detected by flow cytometry. After fresh cord blood was separated from peripheral blood mononuclear cells (PBMCs) by Ficoll method, anticoagulated neonatal umbilical cord blood was diluted with PBS in equal amounts. The Ficoll-Hypaque lymphocyte layered solution (specific gravity 1.077 g/mL) was centrifuged at 2000r/min to take the lymphocyte layer. CD45 FITC, CD34 PE, CD7 Apc-cy7, CD33 percp-cy5.5 antibody staining, HSC (CD45+ CD34+), CLP (CD45+ CD34+CD7+), CMP (CD45+ CD34+CD33+) cells were identified and quantified by flow cytometry (Figure 1)[18].

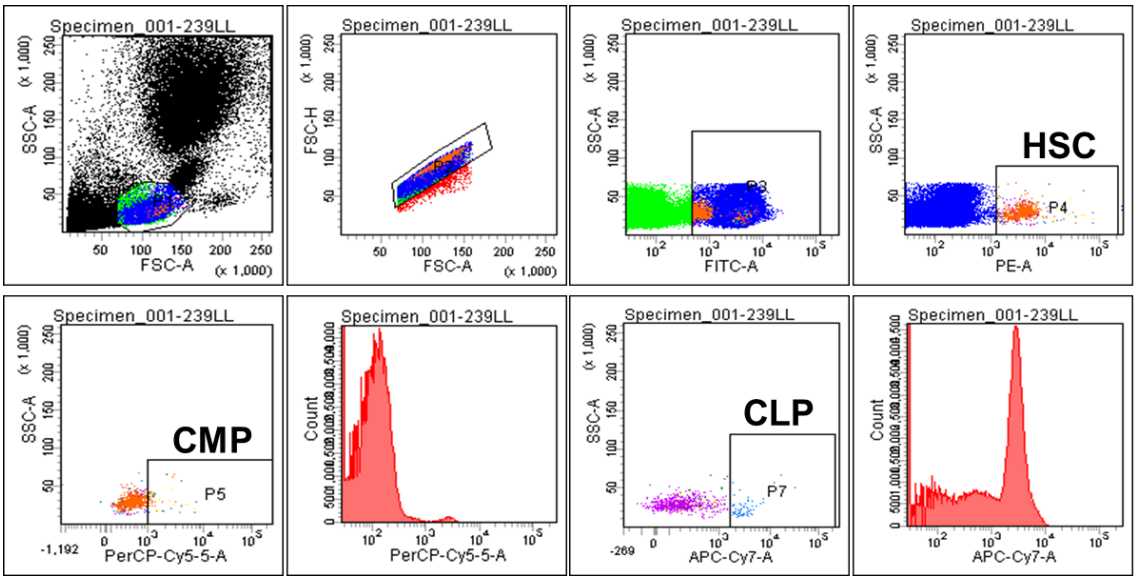

Figure 1: Flow cytometric analysis of HSC, CMP and CLP cells in cord blood

3.3. Proportions of hematopoietic stem cells, CLP and CMP

The frequency and differentiation of HSCs in cord blood of GDM group and normal group were detected by flow cytometry. There were 75 cases in total, 52 in normal control group and 23 in GDM group (Figure 2). The results showed that compared with the control group, the proportion of HSC in GDM group did not change significantly, but the number of CMP differentiated from HSC was increased and CLP was decreased. This suggests that GDM promotes the differentiation of HSC to CMP in neonatal umbilical cord blood.

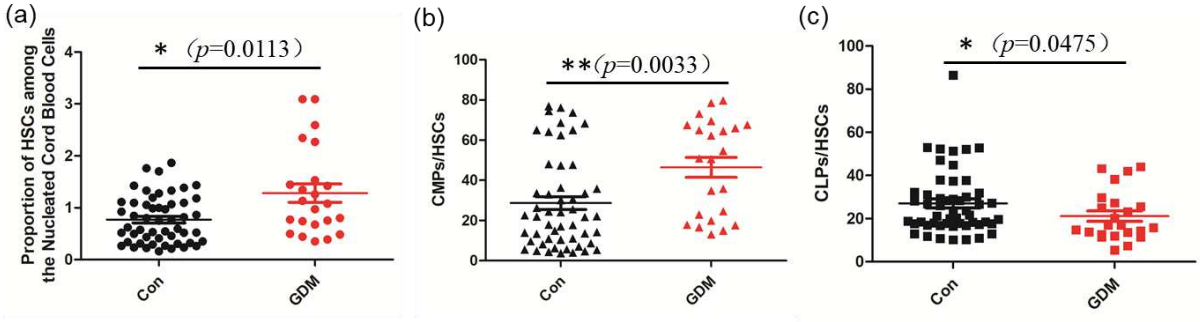

Figure 2. Compared with normal control group, HSC cells in cord blood of GDM group were increased (a), HSC cells were more differentiated to CMP cells (b) and less differentiated to CLP cells(c).

3.4. Comparison of blood glucose level and RBC level between the two groups.

We first compared the blood glucose levels of the two groups of pregnant women. Because routine neonatal full blood count, hemoglobin level, and serum bilirubin measurements were not feasible in all participating neonates as per guidelines. We analyzed the RBC in maternal blood routine of two groups (Figure 3).

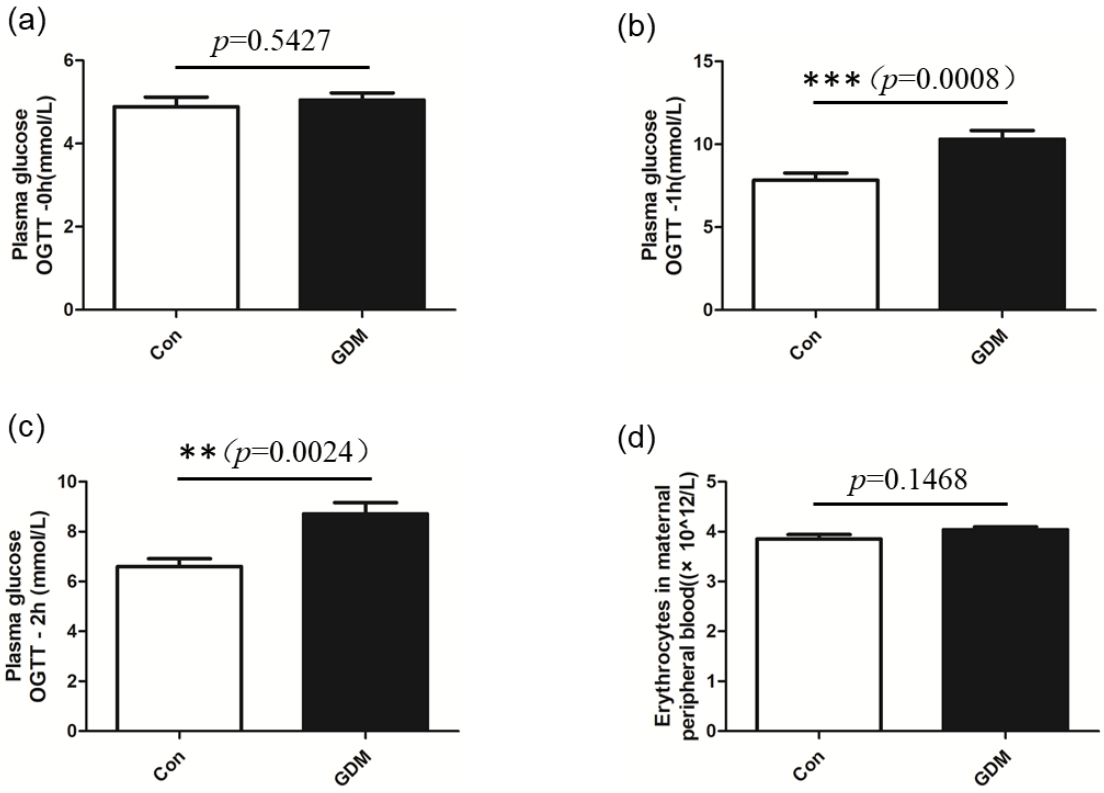

Figure 3. Compared with normal control group, there was no significant difference in fasting blood glucose(a), but OGTT 1h(b) and 2h(c) blood glucose were increased, RBC cells in cord blood of GDM group were increased (d).

4. Discussion

The self-renewal and lineage differentiation of hematopoietic stem cells (HSCs) is a subtle regulation process, which is regulated by cytokines, transcription factors, cell-cell contact, cytokine signals and their microenvironment. Stem cell microenvironment refers to all factors that affect the physiological functions of stem cell proliferation and differentiation. Studies have confirmed that high glucose microenvironment can affect the proliferation and directional migration ability of bone marrow mesenchymal stem cells [19]. Cell metabolism is also a key factor, and the utilization of substrate also restricts the fate of cell differentiation.

It has been reported that cell metabolism plays an important role in the proliferation and differentiation of HSC into erythrocytes. The utilization of glutamine and glucose in the synthesis of de novo nucleotides is a necessary condition for HSC to differentiate into erythroid [20], and glucose metabolism affects the start and degree of HSC induction in vivo [21]. Therefore, understanding the metabolism of cell proliferation and differentiation is helpful to optimize the culture conditions for the proliferation, differentiation and maturation of hematopoietic stem cells and progenitor cells (HSPCs). However, recent studies have shown that resting HSCs have relatively inactive mitochondria, which mainly rely on glycolysis, but when they are activated and differentiated, they rapidly change into mitochondria, with oxidative phosphorylation as the main energy supply [22]. In addition, it has been reported that

the production of mitochondrial energy involves various cellular processes. Mitochondrial respiratory defects can regulate the expression and differentiation of HSPC cells, making them into progenitor cells limited by lineage [23]. Therefore, it is very important to study the mitochondrial energy metabolism of HSC cells.

Therefore, we speculate that the high glucose microenvironment and insulin control of blood glucose during pregnancy have an impact on the energy metabolism of hematopoietic stem cells in cord blood, and further promote the differentiation of HSC into erythroid progenitor cells. Of course, a clearer mechanism needs further study.

5. Conclusions

First of all, the research work of this topic is helpful to deepen the understanding of whether gestational diabetes mellitus affects the differentiation of cord blood stem cells into CLP and CMP. In addition, in-depth study on the effect of differentiation of hematopoietic stem cells in cord blood of GDM patients will further determine whether high glucose environment or insulin affects the mitochondrial energy metabolism of hematopoietic stem cells and the differentiation to erythroid cells. This study will provide a reference for the quality of hematopoietic stem cells in cord blood of GDM patients.

Abbreviations

GDM: Gestational diabetes mellitus; HSC: Hematopoietic stem cells; PBMC: Peripheral blood mononuclear cells; HSPC: Hematopoietic stem cells and progenitor cells; OGTT: oral glucose tolerance test; PBS: phosphate buffered saline; FBS: fetal bovine serum; MPP: multipotent progenitors; CLP: common lymphoid progenitor CMP: common myeloid progenitor; GMP: granulocyte macrophage progenitor; MEP : megakaryocyte erythroid progenitor; BMI: body mass index.

Declarations

Ethics approval and consent to participate

All experimental protocols for this study are in accordance with the national guidelines for the use of human blood sample in scientific research. Additional approval was granted by the Ethics Committee of Lu He hospital (Ethics approval No.2019LH-KS-050).

Availability of data and materials

Data and materials related are available upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

ZD and YLY conceived and designed the experiments. ZLJ, ZYY and WLL performed the experiments. ZLJ analyzed experimental data. ZD wrote the manuscript. All authors read and approved the manuscript. ZLJ contributed most to this work.

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Figures

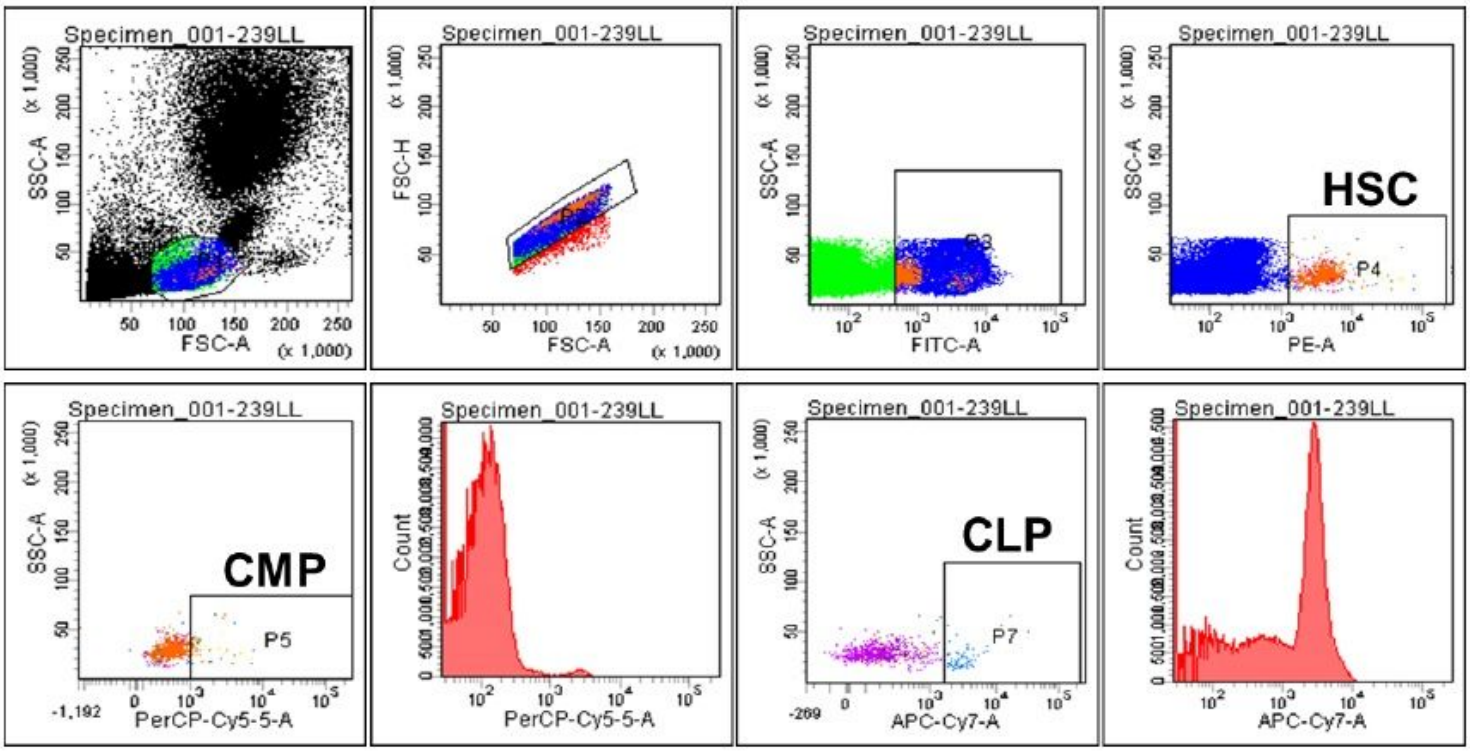

Figure 1

Flow cytometric analysis of HSC, CMP and CLP cells in cord blood

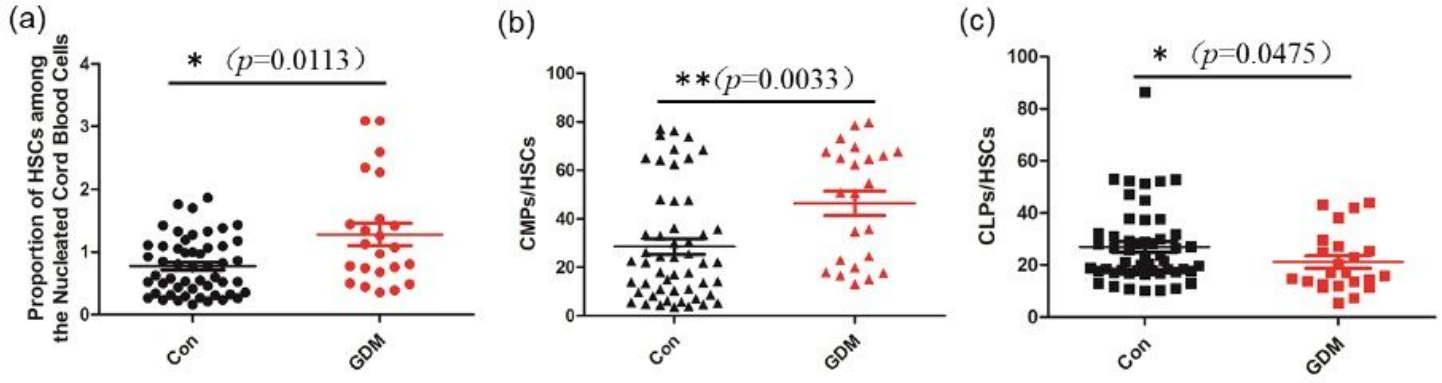

Figure 2

Compared with normal control group, HSC cells in c ord blood of GDM group were increased (a), HSC cells were more differentiated to CMP cells (and less differentiated to CLP cells)

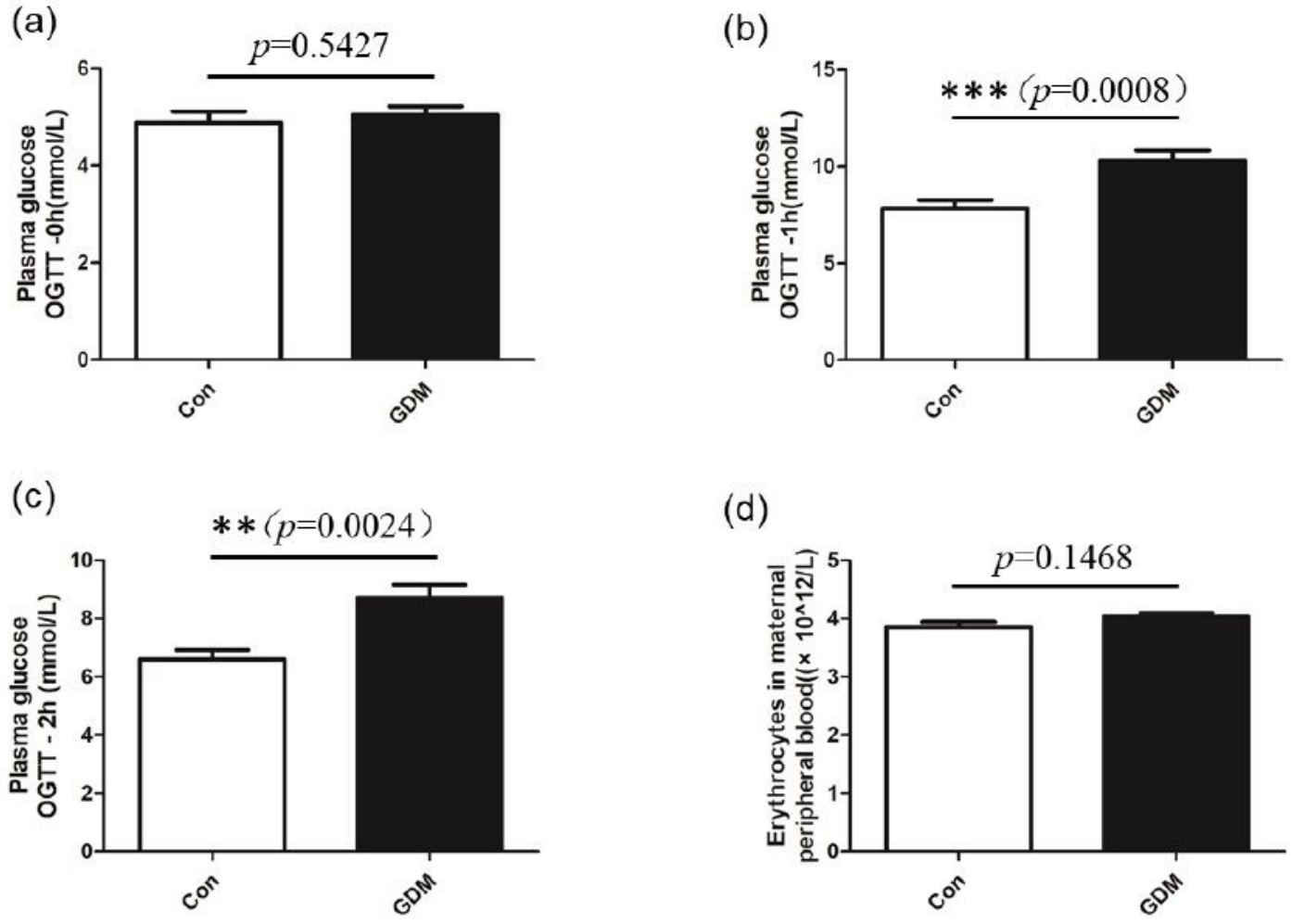

Figure 3

Compared with normal control group, t here was no significant difference in fasting blood glucose (a), but OGTT 1h (and 2 h(c) blood glucose were increased , RBC cells in cord bloo d of GDM group were increased (d)