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EFFECTS OF MILD GESTATIONAL DIABETES MELLITUS ON QUALITY OF UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELLS PROCURED AT DELIVERY

SARA. M.EL. AHMED

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By

SARA. M.EL. AHMED

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Chairman : Maiza Tusimin, PhD
Faculty : Medicine and Health Sciences

Hematopoietic stem cell (HSC) transplantation persists as the most successful and effective curative therapy for many haematological and non-haematological diseases, and cord blood HSCs have considerable advantages compared to other sources. The low number of cells harvested restricts their use and hence successful engraftment is highly dependent on the quality and quantity of stem cells and nucleated cells in cord blood. Forasmuch, the higher costs of cryopreservation that is needed to store the blood units, it is important to determine the maternal and neonatal factors, which include maternal illnesses that influence the higher yield of hematopoietic stem cells obtained from umbilical cord blood. Hence, a wise decision of storing UCB for the definitive aim of successful cells engraftment can be made. This study evaluated the effect of mild Gestational Diabetes Mellitus (mGDM) on the quality and quantity of UCB parameters in terms of $CD34^{+}$ cell count and viability, a total number of nucleated cells and cord blood volume.

130 samples of umbilical cord blood collected from Malaysian women who delivered their babies at Hospital Serdang between May 2016 and April 2017 were collected; 63 were mGDM and 67 were from healthy women. The harvesting of the umbilical cord blood was performed via the in utero method. The UCB samples analysis include the total UCB collected volume, total nucleated cell (TNC) count, and CD34+ cell count by flow cytometry following the ISHAGE protocol and the measurement of the viability resorting to the nucleic acid marker 7-amino actinomycin D.

There was a significant statistical association between the healthy group and mGDM women group in terms of UCB unit volume and TNC number and viability. The mean UCB volume for healthy women was 60.88 ± 18.16, while it was 54.06 ± 19.40 in mGDM women ($p = 0.041$). The mean of TNCs was 54.84 ± 26.79, 44.42 ± 24.13 and viability of TNCs was 53.71 ± 25.52, 42.88 ± 24.12 ($p = 0.022$ and 0.014) for healthy women and mGDM women respectively. Nevertheless, the analysis of $CD34^+$ count and its viability between the two groups yielded no significant statistical difference. On the other hand, the means of $CD34^+$ percentage in mGDM (0.16 ± 0.19) was statistically significant when compared to healthy group (0.11 ± 0.10) with $p = 0.027$, which reflect that mGDM, yielded a higher % of $CD34^+$. The correlation coefficients for CD34+ indicated a significant and positive relationship between placental weight and CD34+ cells counts ($r = 0.572$, $p < 0.001$), $CD34^+$ viability ($r = 0.279$, $p = 0.027$), and $CD34^+$ percent ($r = 0.422$, $p = 0.001$). UCB volume and TNCs count and viability are highly significantly related to the placental weight when r correlation coefficient values were 0.438 ($p < 0.0010$), 0.3810 ($p = 0.002$), and 0.382 ($p = 0.002$) respectively. On contrary, in the healthy group, placental weight only correlates to CB volume and TNC number with $r = 0.288$ ($p = 0.019$) and 0.246 ($p = 0.047$) respectively. The study revealed that gender has no significant impact on all UCB parameters regardless mothers of mGDM group or healthy group as ($p > 0.05$). Infant's birth weight in the mGDM group has a positive correlation with UCB volume, TNC count and viability, but not $CD34^+$ parameters (count, viability and percentage) ($p > 0.05$). As compared to neonates with normal birth weight (NBW) have higher blood volume, TNC count and viability, than low birth weight (LBW) neonates ($p = 0.007$, 0.010, 0.013). In the healthy group, all UCB parameters are not affected whether neonates are NBW or LBW ($p > 0.05$).

In conclusion, mGDM has a negative impact on the UCB volume and TNC count and viability obtained from cord blood at delivery, but not on the yield of HSC. Placental weight and neonatal birth weight are the most important factors influencing the numbers of cord blood HSC. These findings would provide guidance to mothers who are GDM on diet control and to their health care providers in making the right decision regarding UCB stem cell collection and banking.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN DIABETES MELLITUS GESTASI RINGAN KE ATAS SEL PUNCA HEMATOPOIESIS DARAH TALI UMBILIKUS YANG DIDAPATI SEMASA BERSALIN

Oleh

SARA M. EL. AHMED

Oktober 2018

Pengerusi : Maiza Tusimin, PhD
Fakulti : Perubatan dan Sains Kesihatan

Pemindahan sel punca hematopoiesis (HSC) masih merupakan terapi pemulih yang paling berjaya dan berkesan untuk banyak penyakit hematologi dan bukan-hematologi, dan HSC darah tali umbilikus mempunyai kelebihan yang banyak berbanding dengan sumber lain. Penggunaannya adalah terhad kerana bilangan sel tuaian yang sedikit dan oleh itu kejayaan cantuman sangat bergantung pada kualiti dan kuantiti sel punca dan sel-sel bernukleus di dalam darah tali umbilikus. Oleh kerana kos pengawetan kriogenik yang lebih tinggi yang diperlukan untuk menyimpan unit-unit darah tersebut, adalah penting untuk menentukan faktor-faktor ibu dan neonat yang termasuk penyakit-penyakit ibu yang boleh mempengaruhi hasil sel punca hematopoiesis yang lebih tinggi daripada darah tali umbilikus dan justeru itu suatu keputusan bijak menyimpan UCB untuk matlamat khas pencantuman sel yang berjaya. Kajian ini menilai kesan Diabetes Mellitus Gestasi Ringan (mGDM) terhadap kualiti dan kuantiti parameter-parameter UCB dari segi kiraan dan daya maju sel CD34+, jumlah bilangan sel bernukleus dan isi padu darah tali umbilicus.

130 sampel darah tali umbilikus yang dikumpulkan dari wanita Malaysia yang melahirkan bayi mereka di Hospital Serdang antara Mei 2016 dan April 2017 dikumpulkan; 63 mengidap mGDM dan 67 adalah dari ibu-ibu yang normal. Penuaian darah tali umbilicus dilakukan melaluI kaedah in utero. Analisis sampel-sampel UCB termasuklah jumlah UCB yang dikumpulkan, jumlah sel bernukleus (TNC), dan kiraan sel CD34+ secara sitometri aliran mengikut protokol ISHAGE dan pengukuran daya maju yang menggunakan penanda asid nukleus 7-amino actinomycin D.

Terdapat perkaitan statistik yang signifikan antara kumpulan kawalan wanita normal dan kumpulan wanita mGDM dari segi isi padu unit UCB dan jumlah serta daya maju TNC. Isi padu min UCB untuk wanita bukan-diabetes adalah 60.88 ± 18.16, manakala ia adalah 54.06 ± 19.40 bagi wanita mGDM ($p = 0.041$). Min TNC ialah 54.84 ± 26.79, 44.42 ± 24.13 dan daya maju TNC adalah 53.71 ± 25.52, 42.88 ± 24.12 ($p = 0.022$ dan 0.014) bagi wanita bukan-diabetes dan yang mengidap mGDM masing-masing. Walau bagaimanapun, analisis kiraan CD34+ dan viabilitinya antara dua kumpulan ini tidak menghasilkan perbezaan statistik yang signifikan. Sebaliknya, min-min untuk peratusan CD34+ di dalam mGDM (0.16 ± 0.19) adalah signifikan dari segi statistik berbanding dengan kumpulan kawalan (0.11 ± 0.10) dengan $p = 0.027$ yang mencerminkan bahawa mGDM menghasilkan % CD34+ yang lebih tinggi. Korelasi pekali untuk CD34+ menunjukkan perhubungan yang positif dan signifikan antara keberatan plasenta dan kiraan sel CD34+ ($r = 0.572$, $p < 0.001$) daya maju CD34+ ($r = 0.279$, $p = 0.027$), dan peratusan CD34+ ($r = 0.422$, $p = 0.001$). Isi padu UCB dan kiraan TNC serta viability adalah sangat signifikan taliannya dengan keberatan plasenta di mana nilai-nilai pekali korelasi adalah 0.438 ($p < 0.0010$), 0.3810 ($p = 0.002$), dan 0.382 ($p = 0.002$) masing-masing. Sebaliknya, di kalangan kumpulan kawalan, berat plasenta hanya berkorelasi kepada isi padu CB dan jumlah TNC dengan $r = 0.288$ ($p = 0.019$) dan 0.246 ($p = 0.047$) masing-masing. Kajian ini menunjukkan bahawa jantina juga tidak ada impak yang signifikan ke atas semua parameter UCB tidak kira sama ada ibu mengandung mengidap diabetes mellitus ringan atau kumpulan kawalan kerana ($p > 0.05$). Berat lahir bayi dalam kumpulan mGDM mempunyai korelasi positif dengan isi padu UCB, kiraan TNC dan viability, tetapi tidak parameter CD34+ (kiraan, viability dan peratusan) ($p > 0.05$). Apabila dibandingkan dengan neonat berberat lahir biasa (NBW) mempunyai isi padu darah, kiraan TNC dan daya maju yang lebih tinggi, berbanding neonat berberat badan lahir rendah (LBW) ($p = 0.007$, 0.010, 0.013). Di dalam kumpulan kawalan semua parameter UCB tidak terjejas sama ada neonat adalah NBW atau LBW ($p > 0.05$).

Dapat disimpulkan bahawa mGDM mempunyai kesan negatif ke atas isipadu UCB dan kiraan TNC serta daya maju jika diperolehi daripada darah tali umbilikus semasa kelahiran, tetapi tidak ke atas hasil HSC. Berat plasenta dan berat lahir bayi adalah faktor terpenting yang mempengaruhi bilangan HSC darah tali umbilicus. Penemuan ini akan memberi panduan kepada ibu-ibu yang mengidap GDM untuk menjalani kawalan pemakanan dan kepada penjaga kesihatan mereka untuk membuat keputusan yang tepat mengenai pengumpulan dan perbankan sel punca UCB.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

The members of the Supervisory Committee were as follows:

Maiza Tusimin, PhD
Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Norshariza Nordin, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Sabariah Md Noor, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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Name and Matric No: Sara. M.El. Ahmed GS4522

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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Signature: _____
Name of Chairman of Supervisory Committee: Dr. Maiza Tusimin

Signature: _____
Name of Member of Supervisory Committee: Dr. Norshariza Nordin

Signature: _____
Name of Member of Supervisory Committee: Dr. Sabariah Md Noor

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LIST OF ABBREVIATIONS

7- AAD	7- Amino Actinomycin D
ADS	Adult Stem Cells
BMI	Body Mass Index
BM	Bone Marrow
CS	Caesarean Section
C-Kit	Cell Kit
CD	Cluster of Differentiation
CB	Cord Blood
CFU	Colony Forming Unit
CFU-GM	Colony forming Units Granulocyte/ macrophages
CFU-GEMM	Colony forming Units Granulocyte/ Erythroid/Macrophage/Mega karyocyte
CMV	Cyto-Megalo Virus
DM	Diabetes Mellitus
ESCs	Embryonic Stem Cells
FBG	Fasting Blood glucose
FACS	Fluorescence Activated Cell Sorting
FITC	Fluorescein Isothiocyanate,
GDM	Gestational Diabetes Mellitus
GA	Gestational Age
G6PD	Glucose-6-Phosphate Dehydrogenase
GVHD	Graft versus Host Disease
HbA1c	haemoglobin A1c
HSCs	Hematopoietic Stem Cells

HPCs	Hematopoietic Progenitor Cells
HPP	Hours Postprandial
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HES	Hydroxyethyl Starch
ISHAGE	International Society of Haematotherapy and Graft Engineering
MSCs	Mesenchymal Stem Cells
mGDM	Mild Gestational Diabetes Mellitus
MGTT	Modified Glucose Tolerance Test
MNC	Mononucleotide Count
MLR	Multiple Linear Regression
NHLBI	National Heart Lung and Blood Institute
NC	Nucleated Cells
OGTT	Oral Glucose Tolerance Test
TTR	Time To Receipt
TNC	Total Nucleated Cells
T2DM	Type 2 Diabetes Mellitus
UCB	Umbilical Cord Blood
WHO	Word Human Organisation

CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Stem cells

Stem cells are defined as single cells that are clonal progenitors of both new identical stem cells and a determined set of differentiated progeny (Weissman et al., 2001). More explicitly, they are known as their capability to generating exact duplicates, their capability to dividing indefinitely and they are capable of differentiating into multiple cell lineages giving various cell types that carry out different functions (Melissa Little et al., 2006; Sung and Chao, 2013). Stem cells are classified into three main groups according to their genomic and biochemical markers: embryonic stem cells (ESCs), Mesenchymal stromal cells (MSCs) and hematopoietic stem cells (HSCs) (dela Peña et al., 2015).

Many sources are rich with stem cells that can be isolated from human tissues including preimplantation embryos (embryonic stem cells), foetal sources such as aborted foetuses and birth associated tissues(foetal stem cells), and from adult peripheral blood and tissues (adult stem cells) (Peña et al., 2015). Adult Stem Cells (ADS) or somatic stem cells are immature cell groups characterised by its capability of tissue repair, preserving tissue homeostasis and redress in a niche-specific controlled microenvironment (Heissig et al., 2005). While, ADS have been shown in many tissues (including neural, muscular, hepatic and cardiovascular tissues), hematopoietic stem cells still the most characterised ADS population with clinical evidence. ADS have been emerged in many therapeutic trials compared to (ESC) cells. There are no existing clinical treatments based on ESC, but using animal models have shown in fact only a few modest published successes (McCulloch, 2003).

1.1.2 Cord blood banking & Haematopoietic Stem Cells

Over the last three decades, UCB has been clinically investigated as an alternative source of hematopoietic tissue for allogeneic transplantation of patients lacking a human leukocyte antigen (HLA)-matched marrow donor (Pranke and Canabarro, 2009). And during this period, the field of UCB banking and transplantation has frequently expanded, with more than 600,000 UCB units cryopreserved worldwide and over 30,000 UCB transplants having been performed.

The use of human umbilical cord blood (hUCB) as an important source of hematopoietic stem cells in transplantation has increased considerably since 1997. The use of UCB stem cells for transplantation has several advantages over HSCs gained

from bone marrow or peripheral blood; one of the most significant benefits is the stem cells of UCB have an immature T cell immunity, and therefore a much less strict HLA match is required for transplant. That contributes to the lower incidence of acute and chronic graft versus host disease (GVHD) occurs in recipients (Shahrokhi et al., 2012). Other advantages are the easy availability, the simplicity of umbilical cord blood collection, in addition to the lack of risk for both mother and new-born (Barini et al., 2011)

HSCs are multipotent cells that give generation to a finite number of cell types namely, blood and blood-related lineages. They are extracted from both the adult bone marrow and the umbilical cord (UC). Morphologically, haematopoietic stem cells resemble lymphocytes in shape that is rounded and medium-sized mononuclear cells with a low cytoplasm-to-nucleus ratio, basophilic cytoplasm with no granules, and with eminent nucleoli. Since HSCs cannot be isolated as a pure population and it is not possible to identify them in a light microscope, thus that description is based on the morphological characteristics of a heterogeneous population, of which haematopoietic stem cells are a part of the composition (Hordyjewska et al.,2015).

1.1.3 Assessment of UCB quality

International Standards for the collection, processing, testing, banking, and selection of cord blood were established by the NetCord-Foundation for the Accreditation of Cellular Therapy (FACT) in the year 2000 (Butler and Menitove, 2011). These standards aimed to promote quality medical practices, laboratory processes, and banking procedures so that there is consistent production of high quality umbilical cord blood units available for transplantation (NetCord-FACT, 2006). The parameters commonly used to evaluate the quality of umbilical cord blood and its suitability to predict transplant outcomes are total UCB volume, total nucleated cells (TNC's), and CD34+ cells concentration. A requiring TNC content from 6 to 10×10^8 for storage (Solves et al., 2007) and a minimal volume between 40 and 60 mL (Van Haute et al., 2005). While a dose of 2×10^5 $CD34^+$ cells per kilogram of recipient's weight before cryopreservation seems to be more suitable for the selection of CB units for storage. Notable, the CD34+ cell content has been shown to influence engraftment and survival after unrelated UCB transplantation, better predicting the hematopoietic potential of a CB unit than nucleated cell content (Wagner et al., 2002).

There are many factors that have the potential to impact the quantity and quality of stem cells collected from the umbilical cord vein. Pregnancy is naturally associated with a metabolic interaction between the mother and the foetus over a certain duration of time. The maternal metabolic status is closely related to the intrauterine environment of the foetus. Foetal programming is the theory that nutrition state of mother and medical risks during pregnancy such as gestational diabetes mellitus (GDM), preeclampsia, and obesity are commonly reflected in the intrauterine life, later life well-being and metabolism of the child (Lakshmy, 2013). Since foetal growth is primarily dependent on the functional safety of the placenta, the placenta is

physiologically efficient to support proportional foetal growth, organ development, and differentiation, as well as to adapt to the mother's nutritional and metabolic states (Thornburg et al., 2010). In addition, the metabolic disorders and other diseases during pregnancy have been reported to have an impact on cord blood yield in term of UCB volume, TNCs, MSCs and HSCs.

1.1.4 Gestational Diabetes Mellitus

1.1.4.1 Definition and prevalence

Gestational diabetes mellitus (GDM) is any degree of carbohydrate intolerance of variable severity. It is the most popular medical complication of pregnancy with the first recognition of hyperglycaemia during pregnancy(Clinical Practice Guidelines Development Group, 2015). GDM is characterized by both insulin resistance and impaired insulin secretion as observed in T2DM and may share the same genetic susceptibility (Khatijah et al., 2011). Approximately 200,000 GDM cases are recorded per year in the United States (Diabetes, 2013), whereas the prevalence in Malaysia has ranged 12.7-24.9% in a period of 1993 to 2003 (Idris et al., 2009).

Diabetic pregnancy associated with a significantly higher risk of pre-eclampsia, caesarean section, intrauterine foetal death, neonatal hypoglycaemia and hyperbilirubinemia for women with GDM as compared with normal glucose tolerance women (Kampan et al., 2013). GDM is a potent risk factor for the affected women to develop type 2 diabetes later in their lives. Published studies show that 35-60% of women who have a history of GDM exposed to getting type 2 diabetes within ten years (Idris et al., 2009). In addition, the child of GDM mother has a higher risk of obesity and getting T2DM in younger age as a result of the exposure of maternal hyperglycaemia since in utero (Khatijah et al., 2011).

1.1.4.2 Diagnosis for GDM

The Ministry of Health Malaysia recommends that screening for GDM should involve all pregnant women who are between weeks 24 to 28 of gestation or women at high risk of developing GDM at booking. GDM is diagnosed in the presence of any one of these results:

- FPG ≥5.1 mmol/L
- 2-hour post-prandial (2-HPP) ≥7.8 mmol/L

And overt DM is diagnosed in the presence of any one or more of these results

- FPG ≥7.0 mmol/L
- 2-HPP ≥11.1 mmol/L
- RPG ≥11.1 mmol/L with symptoms. (Clinical Practice Guidelines Development Group, 2015)

Mild gestational diabetes (GDM) is a common complication of pregnancy, affecting up to 9% of pregnant women. Treatment of mild GDM is known to reduce adverse perinatal outcomes such as macrosomia and associated birth injuries, such as shoulder dystocia, bone fractures and nerve palsies (Pirc et al., 2007). Mild GDM, diagnosed according to the WHO definitions (75-g OGTT 2-h plasma glucose >7.8–11.0 mmol/l [140–199 mg/dl]) and a fasting glucose <7. 0 mmol/l) (Natasha et al., 2012).

1.1.4.3 Effects of GDM on the foetus and UCB

GDM has an adverse effect on both mother and foetus. Early foetal complications include macrosomia, neonatal hypoglycaemia, perinatal mortality, congenital abnormalities, hyperbilirubinemia, polycythaemia, hypocalcaemia, and respiratory distress syndrome. While later through child's life are prone to have obesity, deformities and diabetes (Leddy et al., 2008). Diabetes during pregnancy may cause some defects in embryonic development due to variations in glucose levels (Singh, et al., 2013). In addition, it affects the quality of UCB stem cells by stimulating oxidative stress production, ageing and mitochondrial dysfunctions. The oxidative stress impacts the proliferation and mobilisation of cord blood stem cells regarding frequent hyperglycaemic situations led to stem cells early ageing and inhibited their renewal power. Therefore severely undermines stem cell-based therapies (Khan et al., 2011). Nevertheless, at present, there is no study linking the yield of UCB and a mild degree of GDM.

1.2 Study's problem statement

Successful engraftment is highly dependent on the quality and quantity of stem cells and nucleated cells in cord blood. Storage of umbilical cord blood is a very costly procedure and it will be very useful if factors that influence cell count and viability could be identified to aid in the decision to process and store cord blood collections for the ultimate aim of successful engraftment. Many variables that may improve the quality of UCB are still under study and research, and these factors can lead to lowering the cost and time consumed in processing and storage of unsuitable UCB. In contrast to the over-commercialisation of UCB Banking, a limited data in regards to the influence of medical disorder on its quality had created a burden to perinatal care providers, parental satisfaction, and health care costs. Hence, the knowledge acquired from this study with regards to UCB collection in the mGDM patient will aid the

health provider in providing adequate and appropriate counselling before the patient embarks on UCB banking. In addition, the research outcome can be utilized as evidence in the improvement of the National Guideline on Cord Blood Procurement and Banking.

1.3 Objectives:

- Main Objective: To determine the effects of mild gestational diabetes mellitus (mGDM) on the yield of UCB haematopoietic stem cells collected at delivery.
- Specific Objectives:

1. Describe the demographic data of the participants for both groups (healthy and mGDM).
2. To compare the number of UCB total nucleated cell count (TNC) and $CD34^+$ cell count among mGDM group with healthy normal group.
3. To analyse the association of neonatal factors (e.g. Apgar score, birth weight, gender, gestational age and placental weight) with the quality of the UCB hematopoietic stem cell in mGDM patients and compare with healthy women.
4. To analyse the association of maternal factors (e.g. maternal age, ethnicity, gravidity, parity, maternal BMI, duration of labour, and estimated blood loss at delivery) with the quality of the UCB hematopoietic stem cell in mGDM patients and compare with healthy women.

1.4 Research Hypothesis:

The mild degree of GDM can impact the quality and quantity of UCB total nucleated cells and hematopoietic stem cells compared to women with normal pregnancy.

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BIODATA OF STUDENT

Sara M. El. Ahmed was born on the 20 December 1981 in Tripoli, Libya. obtained a Bachelor of medicine and surgery (MBBS) from the Faculty of medicine, Zawia medical University in 2007. After graduation, she worked as Lecturer Assistant in Pathology Department, Faculty of medicine, Zawia medical University from 2008 to 2012.

She registered as Master student in the field of Haematology at Faculty of Medicine and Health Science (FMHS), Universiti Putra Malaysia (UPM) in February 2015.

During her postgraduate study, she participated in academic and scientific workshops, such as journal publishing, flow cytometry, and analysis of qualitative data organized by UPM.

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