The Universiti Sains Malaysia Pregnancy Cohort Study: Maternal-infant Adiposity Development until the First Year of Life

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ABSTRACT: Increasing rates of overweight and obesity have prompted the need to prevent the risk from early life. This idea was originated from the 'Developmental Origins of Adult Disease' hypothesis. Historical cohort studies had indicated that maternal nutritional status was associated with later obesity development. However, little attention has been paid on the specific roles of prenatal oxidative stress status and adipokines levels in affecting postpartum and infant obesity. The Universiti Sains Malaysia (USM) Pregnancy Cohort Study was thus established in year 2009 to measure the prenatal environment of 153 Malay women aged 19 to 40 years, and then followed-up these women and their delivered infants until the first year of life. Healthy women in the second trimester of pregnancy were recruited from two clinics in Kelantan, Malaysia. Maternal blood and breast milk samples were collected for analyzing oxidative stress and adipokines profiles, together with the maternal and infant anthropometric measurements, nicotine exposure, dietary intakes and physical activities. Preliminary findings that indicated the association of maternal environment based on the aspects of prenatal dietary intake and pregnancy symptoms with birth outcomes were presented. Overall, this paper seeks to provide a brief introduction to the USM Pregnancy Cohort Study, to summarize the study content, to consider the strengths and weaknesses of the study and to address the issue of challenges that was faced throughout the study. An overview of the study and preliminary findings are important to serve as a basic reference of the project implementation for future studies.

Keywords: Adipokines, birth cohort, pregnancy, obesity, oxidative stress.

Introduction

An escalating epidemic of obesity is affecting many countries and population groups. In Malaysia, obesity is on the rise. The Third National Health and Morbidity Survey (NHMS III 2006) reported that 14% of adult populations studied were obese, with higher prevalence for women (17.4%) than men (10%) (IPH, 2008). Among children, 5.4% were overweight, with slightly higher prevalence among boys (6.0%) than girls (4.7%) (IPH, 2008).

Issue on obesity in pregnancy is gaining wide spread attention throughout the world, not only because of adverse effect on obstetric outcomes (Jarvie and Ramsay, 2010), but its detrimental effects on subsequent child health (Cottrell and Ozanne, 2008). Children of obese women who are complicated with gestational diabetes showed higher fat tissue mass and become overweight or obese in later life (Gillman *et al*., 2003; Ehrenberg *et al*., 2004). This phenomenon is supported by the hypothesis of 'Developmental Origins of Adult Disease', which proposed that childhood and adult obesity are 'programmed' by early life (Langley-Evans, 2006). The concept of early life events on long-term health and disease outcomes has been discussed in past decades. However, the underlying mechanisms of 'programming' remain controversial. Aside from genetic determined factors, intrauterine environment have been suggested to exert profound and permanent effect on the programming pathway. Plausibly, oxidative stress (Luo *et al*., 2006) and adipokine alterations (Cottrell and Ozanne, 2008) may be the key factors or links that contribute to this disease programming effect.

Obesity and Oxidative Stress

Obesity is a chronic state of oxidative stress (Vincent *et al*., 2007). Pregnancy is a pro-inflammatory state associated with enhanced oxidative stress (Furness *et al*., 2011). Maternal obesity accompanied with increasing oxidative metabolism in pregnancy due to reduced uteroplacental perfusion, increased energy demand and oxygen requirement of mother and foetus, exaggerate free radicals formations (Quanungo and Mukherjea, 2000; Zavalza-Gomez, 2011). Exposure to pollutants or unhealthy lifestyle further increases the susceptibility of mother to oxidative insults (Luo *et al*., 2006; Furness *et al*., 2011). These may directly modify gene

expression or indirectly produce more free radicals, damaging biologic molecules such as lipid, protein and deoxyribonucleic acid (DNA) that bring adverse effects to the foetus via alteration in adipogenesis *in utero* or early postnatal life, and subsequently predispose to obesity in later life based on oxidative stress programming hypothesis (Luo *et al*., 2006). Antioxidant defence system is important to counterbalance the progressive rise in oxidant levels in order to protect both maternal-foetal units from such developmental programming of obesity (Arikan *et al*., 2001). However, the validity of this hypothesis still needs to be proven (**Figure 1**).

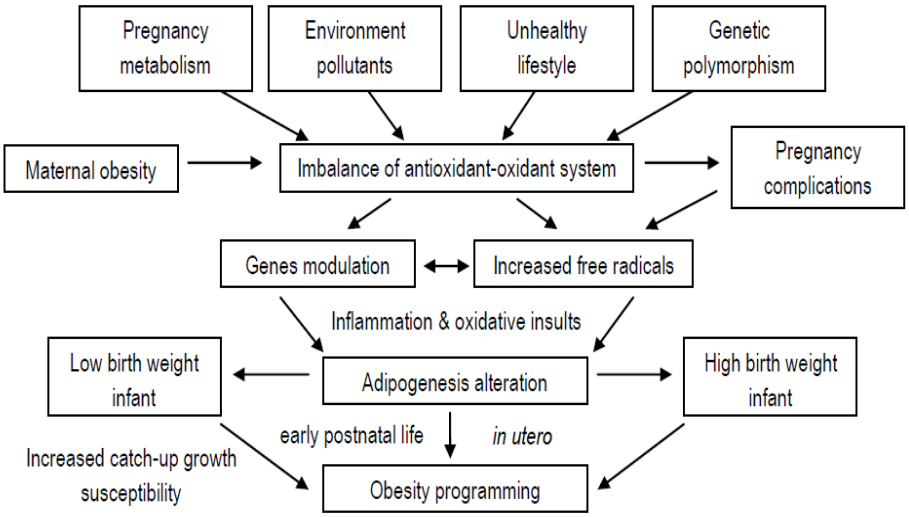

Figure 1: Proposed mechanisms of oxidative stress programming hypothesis. Endogenous and exogenous oxidative insults that occur during pregnancy impair antioxidant defence system and trigger oxidative injuries which may alter adipogenesis in critical periods, leading to obesity development in later life.

Obesity and Adipokines

Increased obesity risk is observed in individuals who were either experienced undernutrition (growth restriction) or overnutrition (postnatal overfeeding) in early life (Tabacchi *et al*., 2007). Animal models suggest that fundamental alterations in adipokine signalling components may

contribute to the development of insulin resistance, resulted in subsequent adult chronic diseases (Cottrell and Ozanne, 2008; Galic *et al*., 2010).

Obesity is characterized by an excess of adipose tissue deposition in body due to deregulation of energy metabolism. Other than functioning as energy store, adipose tissue is an active endocrine organ which involves in releasing multiple adipokines, including pro-inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and C-reactive protein (CRP), and anti-inflammatory adipokines such as adiponectin as well as other adipokines such as leptin and resistin (Galic *et al*., 2010; Fernandez-Sanchez *et al*., 2011). Expansion of adipose tissue in obesity induces alteration in adipokines secretion which may cause disruptions in hypothalamic energy balance systems and decreased insulin sensitivity (Cottrell and Ozanne, 2008; Galic *et al*., 2010). These may eventually lead to persistent pathophysiological changes and contribute to the development of obesity. Recently, majority of the researches have focused on adiponectin and leptin, which show beneficial role on insulin resistance and lipid metabolism. However, functional role of breast milk adiponectin on maternal and infancy obesity yet to be explored.

Study Aims

Oxidative insults and adipokine alterations may occur in either prenatal or early postnatal periods. Thus, recent interest has focused on the possible role of pre- and postnatal environment interactions in the pathogenesis of obesity. Predictions and intervention strategies aimed at this critical period provide hopes for the novel approaches to reduce the health burdens of obesity in future. The Universiti Sains Malaysia (USM) Pregnancy Cohort Study was therefore established (i) to determine the predictors of prenatal oxidative stress markers and adipokines levels; and (ii) to examine the influences of prenatal and postnatal nutritional status, physical activity, oxidative stress and adipokine profiles on postnatal maternal-infancy adiposity.

This paper summarized the study design and implementation of the Universiti Sains Malaysia (USM) Pregnancy Cohort Study in order to serve as a basic reference for future local birth cohort study. To our knowledge, this is the first pregnancy cohort study conducted in Malaysia.

Therefore, it is important to present an overview of the study implementation for any potential modification or improvement in future.

Methodology

Sample Recruitment

The USM Pregnancy Cohort Study was approved by the Human Research Ethics Committee of USM and Medical Research Ethics Committee of Ministry of Health, Malaysia. All pregnant women were identified from the Obstetrics and Gynaecology Clinic of Hospital Universiti Sains Malaysia (HUSM) and Health Clinic of Kubang Kerian based on purposive sampling technique. The subjects' inclusion criteria were: 1) Malaysian citizen and Malay ethnicity; 2) aged 19 to 40 years old; 3) with singleton pregnancy; 4) gestational age 24 weeks and less based on last menstrual period or early ultrasound scan; 5) plan to give birth in HUSM; 6) live within a distance of 50 km from HUSM. Women with pre-existing chronic diseases such as diabetes mellitus, hypertension or heart disease and with preterm delivery before 37 weeks of gestation were excluded. The eligible women were informed of the objectives and characteristics of the study, followed by showing a study flowchart. Written informed consent was obtained from those women who agreed to participate. Subject recruitment was started from May 2010 until June 2011.

Study Design

This was a cohort study where subjects were recruited and followed-up until 1 year of life of the offspring. The subjects were interviewed by the researchers about social circumstances, obstetric and smoking histories, diet and physical activities. Anthropometric measurements were taken in the clinic within the gestational age of ≤24 weeks and ≥32 weeks. A total of 10 ml fasting blood samples were collected by venipuncture in tubes containing EDTA, oxalate, lithium heparin and in plain tubes. Within 1 to 5 days of birth, maternal and infant anthropometric measurements were done; breast milk and hair samples were collected either in the postnatal ward or at home.

The offspring were followed-up at home at 2 months, 6 months and 1 year of age. **Figure 2** shows the flowchart of study design of the USM Pregnancy Cohort Study.

Data Collection

Maternal diet intakes were assessed using 24 h dietary recalls (DR) and validated semi-quantitative food frequency questionnaire (FFQ) (Loy *et al*., 2011). Maternal DRs were taken in the second and third trimesters of pregnancy, as well as during 2 months, 6 months and 12 months postpartum. The FFQ was administered at late gestation to cover habitual food intake in the past six months of pregnancy. The questionnaire was consisted of 10 food groups with a number of food categories listed under each food group. The frequency of intake was based on the options, which are 'per day', 'per week', 'per month' and 'never'. Nutritionist Pro ™ software (Axxya Systems LLC., USA) was used to analyze the nutrient intakes. Appetite changes and supplement intakes were recorded twice during the gestation. Infant feeding histories were asked in details at each home visit.

Maternal pregnancy symptoms throughout the gestations were assessed using validated pregnancy symptoms questionnaire (PSQ) (Marhazlina *et al.*, 2011). A total of 22 common pregnancy symptoms were interviewed in the late trimester (≥32 gestation weeks), according to frequency of occurrence and level of severity based on Likert scaling. Frequency of occurrence was defined as 'never', 'rarely' (once per week), 'sometimes' (three to five times per week), 'often' (once per day) and 'very often' (more than once per day). Level of severity was rated as 'none', 'mild', 'moderate', 'very severe' and 'extremely severe'.

Maternal anthropometric measurements such as body weight, height, mid-upper arm circumference, waist circumference and total body fat were assessed at each stage of the study. Maternal body fat was measured using a bioelectrical impedance analysis (BIA) device (Tanita SC-330ST, Tokyo, Japan). The current introduced to the women was 90 μA at 50 khz. The resistance to electric current is inversely related to the fat free mass. This assessment method was shown to be safe and suitable on pregnant and postpartum women (To and Wong, 2009; Fattah *et al.*, 2010).

Pregnant women (≤24 weeks of gestation) aged 19-40 years interviewed.

Anthropometric measurements and blood samples were taken.

2nd interview at ≥32 weeks of gestation.

Anthropometric measurements and blood samples were taken.

Maternal- infant anthropometry, breast milk and hair samples

were taken within 1 to 14 days after delivery.

Children followed up at 2, 6 and 12 months of age.

Figure 2: Study flowchart of the USM Pregnancy Cohort Study

Though an increase in total body water during pregnancy would lead to underestimation of body fat mass using BIA (To and Wong, 2009), the use of segmental BIA has potential advantages in pregnancy as the limb measurements of fat are independent of the evolving uterine content (Fattah *et al.*, 2009). Also, this assessment method has been suggested to be useful to provide information on series measurement in longitudinal study (To and Wong, 2009). When comparisons were made between the total body fat levels that assessed using BIA and skinfolds methods, high correlations were observed. During gestation period, total body fat mass assessed by BIA method was found to be correlated well with body fat mass that computed from two sites skinfold thicknesses (biceps and triceps) (Durnin and Womersley, 1974; van Raaij *et al.*,1988) among 100 women in the second trimester (r=0.96) and third trimester of pregnancy (r=0.98). During postpartum period, comparison of total body fat percentage was made between BIA method and four sites skinfold thicknesses (biceps, triceps, subscapular and suprailiac) (Durnin & Womersley, 1974) in 100 women after delivery (r=0.88), 2 months (r=0.95), 6 months (0.93) and 12 months (r=0.93) postpartum.

Infant weight, length, head circumference and umbilical abdominal circumference were measured at birth, 2, 6 and 12 months of age. Triceps and subscapular skinfold thicknesses were taken at 6 months and 12 months of age. Based on the chronological age and anthropometric measurements, infant growth was assessed by using the WHO Child Growth Standards 2006 to derive weight-for-age Z-scores, length-for-age Z-scores, weight-for-length Z-scores, BMI-for-age Z-scores, triceps skinfold-for-age Z-scores and subscapular skinfold-for-age Z-scores. These growth indices were computed using the WHO Anthro Software (Version 3.2.2). Infant nutritional status was defined according to the Z-scores (WHO, 2008). All anthropometric measurements were performed by one researcher to reduce inter-observer variation.

A minimum of 8-hour, 10 ml fasting venous blood samples were taken from the women in the second and third trimesters of pregnancy. The blood samples were kept at 4ºC until centrifuged. The fresh whole blood samples were analyzed for deoxyribonucleic acid (DNA) damage. The centrifuged (3500 rpm, 10 min, 4ºC) serum and plasma were distributed in aliquots to analyze for glucose and lipid profiles, malondialdehyde and protein carbonyl concentrations, total antioxidant capacity, superoxide dismutase, catalase and glutathione peroxidase levels,

adiponectin and leptin concentrations. Sample aliquots were stored at -80ºC until needed for analyses.

Plasma glucose and serum lipid profiles such as total cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-C) concentrations were determined using Randox commercial enzymatic kits (Randox, UK). Plasma malondialdehyde, protein carbonyl, superoxide dismutase, catalase and glutathione peroxidase levels were measured using Cayman commercial assay kits (Cayman Chemical, Ann Arbor, Michigan) according to manufacturer's instructions. The comet assay of Singh *et al.* (1988) and the Ferric Reducing Ability of Plasma (FRAP) assay of Benzie and Strain (1996) were used to measure DNA damage and total antioxidant capacity levels, respectively. Serum adiponectin concentration was determined using Human Adiponectin Immunoassay Kit (Antibody and Immunoassay Services Center, the University of Hong Kong), whereas serum leptin concentration was measured using Leptin Enzyme-Linked Immunosorbent Assay kit (Alpco, United States).

Between 1 to 5 days after delivery, about 20-30 strands of maternal hair were collected from the posterior vertex region of the scalp to assess nicotine concentration. The hair sample was aligned and taped on a white paper, labelled at the hair root and kept in envelop at room temperature until analysis (Pichini *et al.*, 2003; *Florescu et al.*, 2007).

Approximately 1 ml of breast milk sample was obtained from the women using mini electric breast pump (Medela, Illinois, USA) after delivery (within 1 to 14 days), 2 months, 6 months and 1 year postpartum. Samples were aliquoted and stored at -80ºC until analysis for adiponectin concentration. Breast milk adiponectin levels were determined using High Sensitive Human Adiponectin Immunoassay Kit for Analysis of Milk Sample (Antibody and Immunoassay Services Center, the University of Hong Kong).

Statistical evaluations were done using the PASW® Statistics 19 (SPSS, Inc., Chicago, IL, USA).

Preliminary Findings and Discussion

Prior to the USM Pregnancy Cohort Study, pilot studies had been carried out to validate the newly developed Food Frequency Questionnaire (FFQ) and Pregnancy Symptoms Questionnaire (PSQ). Further analyses had been done to examine the association between maternal dietary intakes with birth outcomes, and pregnancy symptoms with maternal nutritional status. Both of these aspects were examined as they are associated with maternal oxidative stress and adipokines levels which tend to exert an impact on later maternal-infant adiposity. Some of the findings were presented and summarized below.

By using multiple regression analysis, fruits and vegetables intakes were found to be associated positively with birth size. However, no significant association was found between any of the measured nutrients and birth size. The study indicated that intake of leafy vegetables was associated with head circumference ($p<0.05$), whereas intake of tuber vegetables was associated with birth length and head circumference ($p<0.05$). Fruits intake was associated with birth weight, birth length and head circumference ($p<0.05$) respectively (Loy *et al*., 2011). These findings suggested that increasing consumption of fruits and vegetables during pregnancy helped to promote better birth outcomes. The beneficial role might be attributed to the presence of unmeasured micronutrients and phytochemicals in the fruits and vegetables that warrant further investigation (Loy *et al*., 2011).

Factor analysis using Promax rotation method was used to determine the construct components of pregnancy symptoms, which were classified into three categories: general, constitutional and somatic pain with a total of 40.8% for the cumulative percentage (Marhazlina *et al*., 2011). From the scoring of each pregnancy symptom, the most frequent experienced symptoms throughout the gestation were fatigue (84.9%), nausea (75.9%) and backache (74.1%). Further correlation between symptoms and maternal nutritional status showed symptoms of fatigue and backache, which were grouped under the category of somatic pain, were found to be positively associated with maternal weight gain ($r=0.18$, $p<0.05$) (Marhazlina *et al*., 2010). Study suggested that the association between frequency of somatic pain and maternal weight gain may contribute to the

health and nutritional status of pregnant women and later postnatal health (Marhazlina *et al*., 2010).

Overall, these preliminary data showed promising results and positive direction of the birth cohort in building up local knowledge related to the 'Foetal Origins' hypothesis (Barker, 2004). Currently, the study is ongoing with data collection and analyses. To date, data collection for prenatal stage was complete and progressed to the postnatal stage.

Strengths and Weaknesses

To our knowledge, there is no previous information available regarding the longitudinal changes of antioxidant and adipokines levels in pregnancy among Malaysian pregnant women. Cohort study design is able to show causality of the offspring obesity with maternal factors. The main feature of this study is highlighting the changes of blood oxidative stress status and adipokines levels during gestation, which makes the study unique to represent etiologic determinants *in utero* environment. Although currently there is no concrete evidence that shows exposure to oxidative insults or adipokines alterations in pregnancy may lead to postpartum or childhood obesity, the findings of this study are therefore important to elucidate the potential role of oxidative stress and adipokines levels during pregnancy and regulation of adiposity development among child-bearing women and children. Given that the modifiable nature of oxidative stress and adipokines concentrations, a better understanding of these underlying pathways of obesity may be vital to fight against the obesity epidemic in maternal and child setting. In addition, the detailed longitudinal anthropometric measurements taken on both mothers and infants help in establishing local baseline data of maternal nutritional status and infant growth-up rate.

Study time frame limitation caused the loss of prenatal characteristics data. Women were not recruited before conception as shown in other studies such as Southampton Women's Survey (Inskip *et al*., 2006) and Pune Maternal Nutrition Study (MRC, 2011). Another disadvantage of the study is ethnic consideration. Approximately 95% of the Kelantan population is Malay which makes this study only restricts to one ethnicity. The complexity of the follow up study also limits the sampling method to be done in such a way to improve the generalizability of these study

findings. However, this study represents the Malay which is the largest ethnic group in Malaysia. Yet, it should be noted that dietary intake of Malay in Kelantan is different regionally, especially from those in Sabah and Sarawak. The confinement diet among this Malay community may also differ from others, which was not measured in this study. It is therefore recommended that a larger national study which involves multiethnic pregnant population in Malaysia should be conducted in future.

Difficulties and Challenges

As per other cohort studies, the main difficulty in the study is to retain the number of subject throughout the follow-up period. The USM Pregnancy Cohort Study was established to estimate the average risk of maternal-infant adiposity during the first year of postnatal life. Loss of to follow-up during the study period will prevent direct measurements of these average risks. In this study, the total loss of subjects is 18.2% until the first year of postnatal life. The major reasons for withdrawal are unreachable (loss contact), the decision of subjects to retract their original consent to participate or the moving of subject's whole family to a new place that is beyond the 50 km distance limit. Those women's partners who are not cooperative also lead to the loss of data on the paternal side.

Conclusion

The USM Pregnancy Cohort study is a prospective follow-up study that focuses on the effect of maternal environment on maternal-infancy adiposity development until the first year of life. The preliminary findings addressed the issue of early life and gestational experience in determining later phenotype of obesity. It contributes new knowledge on the establishment of local data related to the 'Foetal Origins' hypothesis and also serves as the national reference data for Malaysian children. Understanding the study design and project implementation will provide a good platform on challenges and issues for future in other ethnic groups in Malaysia.

Acknowledgement

We thank the research team, hospital staff and pregnant women for their cooperation and support. This study was supported by Universiti Sains Malaysia Research University Grant Scheme (1001/PPSK/811087) and Tanita Healthy Weight Community Trust (JAPAN) Grant (304/PPSK/6150119/T122). Loy SL was funded by USM Fellowship Scheme.

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