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Ultrasound Intervention in Maternal Hyperparathyroidism Associated Hypercalcemia over Foetal Biochemical and Skeletal Mineralization Status

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ABSTRACT

Intrauterine bone mineralization requires large amount of calcium and phosphate inured to sustain the normal and adequate bone mineralization and growth in developing foetal. Inadequate skeletal mineralization increases the susceptibility of foetal to contract bone metabolic bone disease in postnatal life. Subsequent research has discovered a close relationship between low bone mass in neonatal with the development of osteoporosis. Intrauterine environment and maternal calcium status has direct effect over the skeletal mineralization. Parathyroid disorder associated with hypercalcemia during pregnancy reduced the normal foetal growth, skeletal mineralization and serum calcium concentration. In addition, human and animal study show benefits from local hyperthermia induced by ultrasound exposure. The purpose of this study is to evaluate the effects of ultrasound intervention during pregnancy in maternal hypercalcemia condition in relation to progeny outcome. Ten-month-old primiparous New Zealand White (NZW) does were assigned into three different groups; control (C), healthy NZW does and free from ultrasound insonation; hyperparathyroidism (HPT), positive control groups having hypercalcemia condition established through administering phosphate intravenously for 5 months and free from parathyroid ultrasound insonation; treatment (T), experimental groups having hypercalcemia condition and receiving parathyroid ultrasound insonation during pregnancy as intervention to hyperparathyroidism. In the treatment group, rabbits were exposed for 30, 60 and 90 minutes of parathyroid ultrasound exposure on 1st, 2nd and 3rd gestational stage accordingly. Subsequently they were subjected to other tests specifically serum calcium (SCa), tissue nonspecific alkaline phosphatase (TNALP), intact parathyroid hormone (iPTH), and femoral bone volume fraction (BV.TV). Maternal hypercalcemia during pregnancy produced small to gestational age progeny with significant reduction in progeny SCa, TNALP, iPTH and femoral BV.TV. Meanwhile, ultrasound exposure given during the middle 2nd gestational stage resulted in significant increase in progeny mean average SCa, TNALP, iPTH and femoral BV.TV compared to HPT group.

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INTRODUCTION

Foetal bone mineralization is a process of bone mineral accretion in the bone mass characterized by longitudinal bone growth which records changes in skeletal size and shape[1]. The most prominent bone changes occur during foetal and neonatal life [2]. Intrauterine skeletal mineralization requires adequate placenta calcium transfer to ensure normal growth and mineralization of the skeleton[1]. Numerous research report that intrauterine foetal health correlate with the adult disease susceptibility [3] for example the risk of osteoporosis in later life which may be the direct influence of the abnormal intrauterine environmental during foetal life [4]. Foetal skeletal growth and mineralization requires sufficient mineral to be delivered from the placenta to develop and mineralize normally. However intrauterine environment has direct effect over the skeletal mineralization [5]. Maternal parathyroid disease associated with disturbances in maternal calcium metabolism will result in calcium metabolic stress to the foetal thus altering the intrauterine longitudinal growth and resulting in metabolic bone disease or disorders of calcium homeostasis in neonates .

Animal study reported that maternal hypercalcemia gave birth to small to gestational age (SGA) offspring associated with low bone mass due to insufficient mineral supply to the foetal [6]. In the course of pregnancy,

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maternal hypercalcemia is inclined to expose the unborn foetal to intrauterine hypercalcemia suppressing the development of foetal parathyroid glands [7] thus producing immature neonatal parathyroid tissue [8]. Calcium, the most abundant mineral in the human body is essential for many biological path way and therefore a vital mineral for newborn infant [9]. Parathyroid hormone (PTH) plays a decisive role in maintaining calcium homeostasis. Disturbance in parathyroid hormone results in abnormal serum calcium concentration.

Parathyroid disorder during pregnancy can cause serious problems to the mother as well as the unborn baby if left untreated. Previous report stated that moderate or severe maternal HPT develop babies with severe calcium deficiency or permanent hypoparathyroidisms in worse cases [10] associated with other complications including intrauterine growth retardation, low birth weight, preterm delivery, neonatal hypocalcaemia, tetany and still-birth[11, 12]. Furthermore, human and animal study show benefits from local hyperthermia induced by ultrasound exposure. Human study discovered that ultrasound heating decreases PTH levels and the sizes of parathyroid tumours [13, 14], while animal study show reduction in tumour volume after ultrasound intervention as a treatment [15]. The present study will compare the outcome of ultrasound insonation as intervention in maternal HPT associated hypercalcemia condition in relation to foetal intrauterine skeletal mineralization, serum calcium concentration, intact parathyroid hormone level and tissue nonspecific alkaline phosphatase.

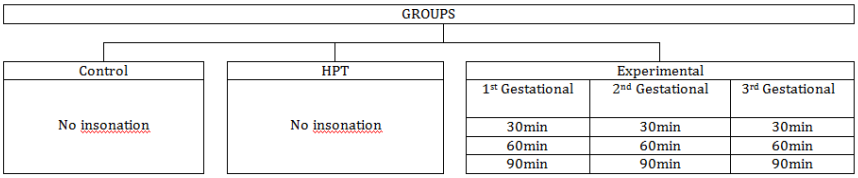

Fig. 1: Chart of ultrasound insonation according to groups.

Methodology:

The research protocol was approved by University's Committee on Animal Research & Ethics and all experimentswere carried out in Laboratory Animal Facility and Management, Faculty Pharmacies, UiTM.Ten-month-old primiparous New Zealand White (NZW) does were assigned in 3 major groups (Fig.1) based on their serum calcium concentrations; control (C), healthy NZW does and free from ultrasound insonation; hyperparathyroidism (HPT), positive control groups established through administering phosphate intravenously for 5 months [16] and free from parathyroid ultrasound insonation; treatment (T), having hypercalcemia condition establishedthrough administering phosphate intravenously for 5 months[16] and receiving parathyroid ultrasound insonation during pregnancy as intervention to hyperparathyroidism. Treatment groups were then further divided into 3 different groups which are 1st gestation, 2nd gestation and 3rd gestation groups based on the time of ultrasound insonation given during pregnancy. In the treatment group, each rabbit received ultrasound insonation once for 30 minutes, 60 minutes and 90 minutes in the middle of the gestational stage accordingly. Following ultrasound exposure intervention, each doe's blood sample was taken for serum calcium evaluation.

All does were mated with normal and healthy NZW bucks. The average gestational term for rabbit ranged between 30-33 days [17] and consists of three stages. Following birth, the progeny were euthanized using diethyl ether and blood was withdrawn via cardiac puncture. After 1 hour, the blood was centrifuged at 5000 rpm for 10 minutes and the serum was separated prior to storage at temperature of -80 ºC. The serum (SCa) and tissue nonspecific alkaline phosphatase (TNALP)were analysed atthe Faculty of Veterinary Medicine Universiti Putra Malaysia. The PTH levels were determined by immunoradiometricassay (IRMA). Femoral bone were dissected and placed in 10% buffered formalin. The volume fraction BV.TV were calculated using SkyScan 1176 high resolution micro-computed tomography (CT)

Results from the experimental groups were compared with the control and HPT groups to evaluate the effects of ultrasound intervention during pregnancy in hypercalcemia condition. Data were analysed using ANOVA one-way analysis of variance followed by post hoc using Scheffe's test. Analysis was done using Statistical Pack-age for Social Sciences software (SPSS version 21).

RESULTS AND DISCUSSION

Alteration in maternal serum calcium can possibly lead to placental insufficiency resulting in preeclampsia (PE)[18]. Epidemiological and clinical studies proposed that alteration in maternal calcium mineral metabolism play an important role in the pathogenesis of preeclampsia, disturbing the normal placenta nutrient transfer and altering the normal growth of foetal[18].Maternal parathyroid disorder associated with abnormal calcium homeostasis resulted in adverse effects to foetal BV.TV, SCa and iPTH. On the other hand, TNALPshow no significant difference between groups.

Table 1: Reference values for control and hyperparathyroidism progeny.

	CONTROL ($\bar{x}$)	SEM	HPT ($\bar{x}$)	SEM
Maternal Ca (mmol/l)	3.370	0.019	4.610	0.019
SCa (mmol/l)	3.432	0.093	2.849	0.022
TNAL (U/L)	107	5.319	99.50	5.536
iPTH (pg/mL)	7.208	0.222	5.702	0.350
BV.TV (%)	48.485	0.742	38.824	1.317
a) $\bar{x}$, mean; SEM, standard error; SCa, serum calcium level; ALP, tissue nonspecific alkaline phophatase level; iPTH, intact parathyroid hormone level; BV/TV, bone volume fraction				

Maternal hypercalcemia produced litters with significant low in SCa($P<0.05$) compared to normal calcium level condition.Untreated maternal hyperparathyroidism associated hypercalcemia predisposed the foetal to intrauterine hypercalcemia supressing the foetal parathyroid gland development and activity producing immature parathyroid gland in foetal[8]. During intrauterine life, foetal is consistently and significantly hypercalcemic[19, 20] compared to the mother in order to achieve normal mineralization of the foetal skeleton and aided the foetal survival caused by abrupt disconnection of placenta calcium infusion after birth[8]. Human and animal studies confirmed the decline of serum calcium concentration after birth[19, 21]. Thus a lower intrauterine foetal SCa may predispose to even lower concentration after birth, thereby increasing the risk of tetany and death[22]. Furthermore, mature parathyroid tissue unable to adapt with spontaneous abrupt cease of calciumafter cord ligation resulted in the early onset of foetal hypocalcemia. Following ultrasound treatment in middle of 2ndgestation with 30, 60 and 90 minutes and 3rd gestationwith 90 minute duration, the foetal SCa concentration were significantly increased compared to hypocalcemia foetal in maternal HPT. Local heating induced by parathyroid ultrasound exposure reduced the maternal serum calcium concentration to near normal level. Since there exista strong relationship between maternal serum calcium levels and neonatal calcium homeostasis[23], treating the mother will subsequently rectify the foetal serum calcium to near normal level.

Parathyroid hormone (PTH) exercises a decisive role in maintaining calcium homeostasis. In normal gestation, foetal parathyroid hormoneswere consistently lower compared to the mother because the foetal calcium were majorly controlled by the placenta. After birth parathyroid gland plays a pivotal role in regulating the calcium homeostasis and synthesises parathyroid hormone continuously in a pulsatile fashion in respond to decrease in serum calcium concentration.However, abnormal intrauterine hypercalcemia suppressed foetal parathyroid development producing immature parathyroid gland[8]incompetent to adapt and sufficiently secrete the parathyroid hormone due to the abruptcalcium changes during the postnatal period. iPTH were significantly increased in the treatment groups from 2nd gestation stage and 1st gestation stage with 60 minutes ultrasound exposure compared to maternal HPT. Parathyroid ultrasound exposure helps to reduce the severity of intrauterine hypercalcemia over parathyroid gland development by bringing the maternal serum calcium to near normal level thus reducing the severity of intrauterine hyperparathyroidism.

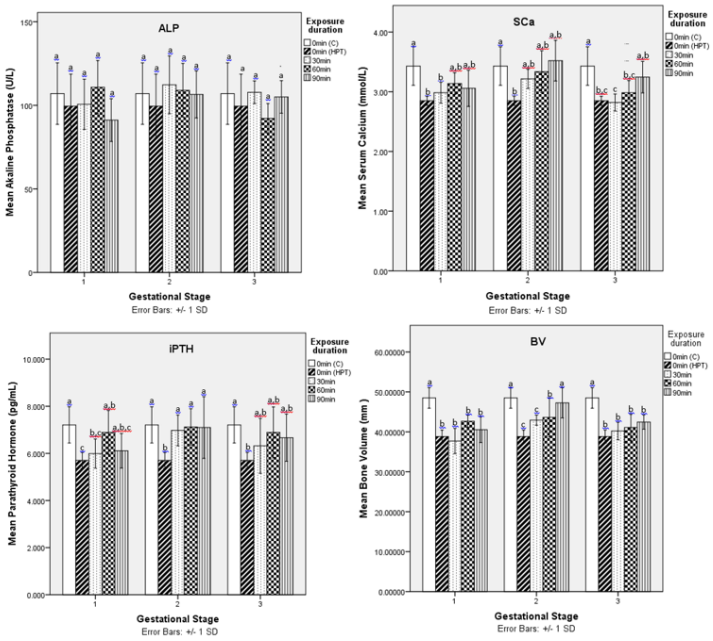

Fig. 2: Foetal SCa, iPTH and bone volume in 2nd gestation following ultrasound sow a significant improvement compare to foetal from maternal hypercalcemia.

Skeletal mineralization is known as a sensitive indicator to evaluate possible disruption of foetal intrauterine growth and often used to examine the maternal condition during pregnancy that might restrict the normal development of the offspring[24].Normal foetal and neonatal calcium homeostasis is dependent upon an adequate supply of calcium from maternal sources. Disturbance in maternal calcium homeostasis can cause metabolic bone disease or disorders of calcium homeostasis in neonates[24]. Statistically significant reduction in littersBV.TV were detected in maternal hypercalcemia. Meanwhile, 2nd gestation stage with 60 and 90 minute and 3rd gestation with 60 minutes exposure show significant increase in bone volume fraction compared to maternal hypercalcemia.Alteration in maternal calcium metabolism during pregnancy is a possible lead to foetal growth restriction. The ultrasound insonation during 2ndgestational stage initiate the maternal SCa and iPTH to near normal level thus improving the uteroplacental calcium transfer to foetal for skeletal mineralization and calcium deposition of organic bone matrix. Reduction of BV.TV show a reduction in bone mass per volume of tissue which may directly affect the bone quality.The propensity of bone fracture is determined by the strength of the bone[25]. BV.TVis an indicator to determine the bone strength as the Young's modulus E increase as the bone volume fraction increase[25].

No statistically significant change is detected in tissue nonspecific alkaline phosphatase (TNALP) level in HPT and treatment groups compared to Control. Even so, the mean average of ALP in progeny from HPT mom show a reduction compared to normal condition. Previous animal study of TNALP deficient mice has showncalcium content inhomogeneity of matrix mineralization[26].Mineral defect occur at the macroscopical level[27-29], microscopical and the nanostructural levels of the bone cortex[26]. TNALP is believed to play an important role in mineralization process. Subsequent to the ultrasound insonation at 2nd gestation, the mean average of TNALP registered an increase compared to maternal HPT.

Conclusion:

Hypercalcemia during pregnancy disturbed the intrauterine environment condition and reduced the foetal normal intrauterine bone growth and development. This ex-vivo study demonstrates the potential benefits of ultrasound local heating effects that might be of assistancein controlling PTH level in maternal hypercalcemia condition while foetus is still in the womb thus reducing the life –threatening post-natal complication in early days of foetal life.

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