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RELATIONSHIP AND TREND OF CHANGES ON PARATHYROID HORMONE AND CREATININE LEVEL OF PRENATAL ULTRASOUND INDUCED EXPERIMENTAL SUBJECTS

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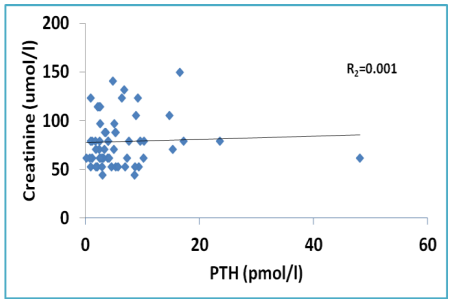
Graphical abstract



Abstract

The aim of this study is to experimentally investigate the effects of prenatal ultrasound exposure on parathyroid hormone and creatinine levels of young rabbit and its trend of changes, and the relationship between parathyroid hormone and creatinine levels of the experimental subjects. A total number of 14 rabbits (*Oryctolagus cuniculus*) served as the model, where seven pregnant rabbits (treated group) were scanned using ultrasound in the middle of pregnancy for 60 minutes, while another seven pregnant rabbits served as negative control group. After delivery term, the newborns resulted from treatment scheme served as the subject of this study. Blood samples were withdrawn between the ages of 1 month old to five months old and sent to the lab for parathyroid and creatinine level test. The parathyroid hormone (P=0.019) were significantly reduced and no significant difference in creatinine levels (P=0.485). There were significantly reduced PTH in 3 and 5 months old rabbits (P=0.046 and P=0.045, respectively and significantly reduced creatinine level in 2 months old rabbit (P=0.019). Correspondingly, there is no significant linear relationship between parathyroid hormone and creatinine levels (P = 0.835). For trend of changes test, A further long-term study should be taken into consideration to test whether prenatal ultrasound exposure might cause the opposite results in the next generation in young age rabbit.

Keywords: Ultrasound effect, parathyroid, creatinine, young rabbit

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1.0 INTRODUCTION

The practice of ultrasound diagnostic in obstetrics and gynaecology has achieved widespread acceptance for over the past four decades. The benefits of this technology are welcomed by both general community and health care providers. This technology has been used routinely from early to the end of pregnancy periods. Ultrasound is a non-invasive diagnostic medical imaging technique that uses high frequency sound waves in order to produce images of the internal part of the body. Ultrasound scan is considered to be a safe, non-invasive, accurate and cost-effective tool for foetal investigation [1].

Despite of its safety consideration, when ultrasound wave interacts with tissue, interactions will take place; absorption, reflection and refraction interaction were occurred [2]. These interactions of ultrasound caused the heating effect, known as ultrasound thermal effect. The thermal effect is created when the wave increases the kinetic energy of the soft tissue, and the ultrasound kinetic energy itself will then be converted into heat when absorbed by tissue.

Over many years of its usage, lack of information in the guidelines for the safe use of ultrasound is reported[3,4]. Based on the guidelines, it is recommended that the ultrasound usage are safe within a maximum of 1.5°C temperature rise from the physiological normal body temperature; 37°C [5]. Correspondingly, the foetal body temperature reported to be about 1°C higher than maternal temperatures [6]. With this, the estimated foetal body temperature was/is about 38°C. And reported by Zaiki et. al., that the temperature rises to 1.4°C at 60

minutes of ultrasound exposure insonated on pregnant rabbit [7]. Also reported that a study on diagnostic ultrasound on newborns are able to cause a temperature rise to 1.3°C [8]. Thus, we believe that the estimated foetal body temperature during the prenatal ultrasound exposure is about 39.3°C.

It is reported that a number of physiological effects might occur if a body tissue is heated at 40-45 °C. This includes the increase of the extensibility of soft tissue, the decrease of the viscosity of fluid elements, decrease of pain perception by slowing nerve conduction velocity, increase of metabolic rate, as well as increase of blood flow needed in the reduction of swelling and in stimulating the immune system.

However, it is unclear how much heat is needed to constitute a risk, and it is believed that the magnitude of the risk is depend on how long the exposure lasts, and the development stage of the foetus. Based on conservative estimation, the temperature towards the foetus should not rise more than 0.5°C above its normal temperature in order to avoid any effects [8]. Also few studies have reported that hyperthermia (overheating of the body) is recognized as a teratogen in mammalian laboratory animals and also suspected as a teratogen for humans [9].

While hormone concentration of maternal and foetus was also reported to be very sensitive to environment and heat [10,11]. Mete, et al. [12], discovered that heat exposure-induced stress may affect the endocrine system (in term of delayed reduction in serum corticosterone levels) which may then be associated with pre-pubertal male rats' behavioural deficiency. Most recently, Dom, et al., [13] found a significantly low PTH level in newborn rabbits with prenatal ultrasound effect, the same kind of results also reported that high ultrasound intensities are able to significantly reduce the parathyroid hormone levels in hyperparathyroidism patients [14,15]. Referring to parathyroid glands development, reported that the earliest available measurements in preterm infants are from 19 weeks of gestation (second trimester) and have shown suppressed PTH compared to the maternal value [16]. And reported that an absent of parathyroid glands in foetal resulted in a lower foetal blood calcium, and thus cause the active transport of calcium across placenta was found to be reduced [17]. These datas indicate that the parathyroid glands have a critical role in maintaining the foetal blood calcium and the active transport of calcium across the placenta. These findings also indicate that ultrasound intensities which able to disturb the parathyroid glands in secreting the PTH (in clinical and experimental subjects) also subsequently believed to cause a lower foetal blood calcium.

Low PTH level known to cause hypocalcaemia which showed by high serum creatinine concentration and characterised by the symptoms of thirst and polyuria in a patient having long standing sensorineural hearing loss (SNHL) [18]. Further studies on the patient reported a small kidney, presented with large right-sided cysts in the renal pelvis. Many more researches show the association between hypoparathyroidism and renal abnormalities [9-12]. Reported from previous studies that there can be a numerous renal anomalies, including renal dysplsia, hypoplasia, aplasia and also vesico-ureteral reflux (VUR) [8, 11-13].

As a summary, ultrasound which is known to reduce PTH towards the foetus is believed to be associated with the change levels of renal function, which in this paper we focused on creatinine levels as a reflection of renal function. Therefore, the objective of this study is to investigate the effects of prenatal ultrasound exposure on the parathyroid hormone and creatinine levels in young rabbits.

2.0 EXPERIMENTAL

The study was conducted in the Department of Medical Imaging, University Teknology MARA, Malaysia. A total number of 14 pregnant New Zealand White Rabbits (NZWR) were used as the model in this study. They were then divided into two groups (control and treated). Control group received no ultrasound exposure at all, while the treated group received ultrasound exposure for 60 minutes at the middle of pregnancy. The experiments were approved by the University Teknology MARA Ethics Committee on Experimental Animals.

2.1 Ultrasound Exposure

All studies were performed using a standard obstetric ultrasound scanning protocol. The ultrasound system used was a Philips HD3 (Philips Electronics E.V., Germany). This system was installed with all parameters that usually used for diagnostic ultrasound and the parameters were kept constant during the research. The ultrasound acoustic output parameters are as follow; exposure duration=60 minutes; frequency = 7.09 MHz; spatial peak temporal average intensity (ISPTA) = 49.4 W/cm2; power = 56.0 W; thermal index (TI) = 0.2; mechanical index (MI) = 1.0.

The ultrasound exposure on the treated group were insonated for 60 minutes based on the recommended maximum exposure time for embryo by the British Medical Ultrasound Society [23]. Meanwhile, the periods of the exposure which were set during the middle of second gestational stage were based on a research of determining the gestational effects of maternal hyperthermia due to febrile illnesses, which the researcher suggested that the second-trimester exposures to febrile maternal illnesses may trigger haemorrhages in vital foetal structures, causing vascular disruption and loss of fetal structures [24], also that the earliest available measurements of PTH levels in preterm infants are from 19 weeks of gestation [17].

Prior to the ultrasound exposure, the fur on the lower part of the abdominal area was removed to facilitate the transducer application. During the scanning, the transducer was applied to the whole womb area until all the foetuses can be seen on the screen, to ensure

that the sound wave were directed to the foetus as a whole, as well as the heating effect. The rabbits were moved back to the cage upon completion of the ultrasound exposure.

2.1 Laboratory Test

At 1 to 5 months old, blood were withdrawn from each kitten through auricular vein and labelled accordingly in EDTA tube (for PTH test) and no additive tubes (for creatinine test). The samples were then sent to a commercial laboratory (B.P Lab Clinical Sdn. Bhd., Glenmarie, Shah Alam, Selangor, Malaysia) for PTH and creatinine levels test.

3.0 RESULTS AND DISCUSSION

3.1 Results

For comparison between control and treated group, due to the distribution patterns of the sample followed a non-Gaussian distribution, the medians and interquartile ranges (IQR) of PTH levels were reported. While for creatinine levels, the mean and standard deviation (SD) are presented due to its normally distributed samples. Next, regression was done to look for the relationship between the PTH (independent) and creatinine (dependent). For the next test, in order to look for the trend of change of the PTH and creatinine level throughout rabbits' young age, the Mann-Whitney test was done to look for the differences between control and treated groups.

When comparing the median (IQR) PTH level of the control and the treated groups for all 65 subjects, a statistically significant difference was noted (7.06 pmol/l [7.41] and 3.13 pmol/l [3.00], respectively; Mann-Whitney test *P*=0.019), shown in Table 1. This indicates that, the prenatal ultrasound exposure has the possibility to cause significant differences in PTH level of young rabbit.

Table1 Mann-Whitney test of median PTH level

Variable	Control (n=34) Median (IQR)	Treated (n=31) Median (IQR)	Z statistic[a]	P value[a]
PTH (pmol/l)	7.06 (7.41)	3.13 (3.00)	-2.338	0.019

[a]Mann-Whitney test

Table 2 Independent t-test of mean creatinine level

Variables	Control (n=30) Mean (SD)	Treated (n=31) Mean (SD)	Mean diff. (95% CI)	t-stats (df)	*P* value[a]
Creatinine (umol/l)	80.96 (29.72)	76.36 (20.75)	4.60 (-8.50, 17.70)	0.73 (59)	0.485

[a]Independent t-test

Table 3 Relationship between creatinine and PTH levels

Variable	*B*	(95% CI)	F-stat (df)	*p*-value	R_2
PTH level	0.097	-0.830, 1.024	0.044 (1, 59)	0.835	0.001

SLR

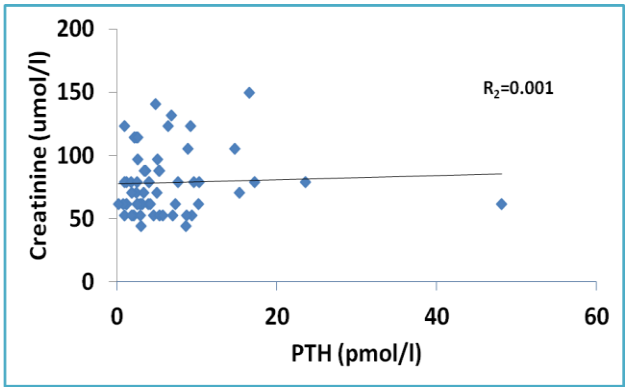

Figure 1 Therelationship between creatinine and PTH level

However, when comparing the mean (SD) creatinine level for the control and the treated groups for all 61 subjects, no statistically significant difference was noticed/observed (80.96 (29.72) vs 76.36 (20.75) umol/l, respectively; Independent t-test *P*=0.485) (Table 2). This indicates that the prenatal ultrasound exposure did not cause any differences in creatinine levels of young rabbit.

Next,the regression showed that there is no significant linear relationship between PTH (independent) and creatinine (dependent) (r = 0.001; *P* = 0.835). This is shown in Table 3 and Figure 1. This indicates that, the significantly reduced parathyroid hormone in young rabbit due to prenatal ultrasound exposure did not cause any significant changes in creatinine levels.

For the next test (to identify the trend of changes of the PTH and creatinine level based on month old) the researcher found that there were significant differences between the control and the treated groups of PTH level for the three and five months age groups (*P*=0.046 and *P*=0.045, respectively), shown in Table 4. While there was no statistically significant

difference between the control and the treated groups for the one, two and four months age groups (P > 0.05). These data's suggest that due to prenatal ultrasound effect on the middle of gestational for 60 minutes able to significantly reduced the PTH levels on the three and five month age groups, but there were no significance changes of PTH levels of one, two and four months old rabbit.

Table 4 The differences between control and treated group for each month of PTH level

Variable	PTH (pmol/l)		
Month	Control (n) Median (IQR)	Treated (n) Median (IQR)	P value[a]
1	(10) 8.635 (7.69)	(6) 3.21 (13.66)	0.278
2	(6) 2.68 (7.46)	(6) 4.235 (4.64)	0.522
3	(6) 8.91 (14.58)	(7) 2.41 (3.91)	0.046*
4	(6) 4.115 (6.36)	(6) 3.77 (1.96)	0.631
5	(6) 8.14 (9.46)	(6) 2.47 (4.09)	0.045*

[a]Mann-Whitney test

*P <0.05 is significant

Table 5 Mann-Whitney test for the creatinine level between control and treated groups for each month

Variable	Creatinine (umol/l)		
Month	Control (n) Median (IQR)	Treated (n) Median (IQR)	P value[a]
1	(6) 52.8 (11)	(6) 57.2 (8.8)	0.116
2	(6) 61.6 (61.6)	(6) 52.8 (8.8)	0.019*
3	(6) 74.8 (17.6)	(7) 79.2 (8.8)	0.359
4	(6) 105.6 (50.6)	(6) 79.2 (8.8)	0.241
5	(6) 110 (35.2)	(6) 114.4 (22)	0.744

[a]Mann-Whitney test

*P <0.05 is significant

For creatinine, we found that there was a statistically significant difference between control and treated groups for the second month age group (*P*=0.019), shown in Table 5. There was no statistically significant difference between the control and the treated groups for the first, third, fourth and fifth month age groups (P > 0.05). These data suggest that due to prenatal ultrasound effect, there were no significance changes of creatinine levels from one to five months old rabbit, but a significant reduction in creatinine level is spotted on two month old rabbit.

3.2 Discussion

In the current practice, ultrasound is accepted widespread as a screening tool to assess the foetus progression. However, there is lack of evidence that the ultrasound exposure of the developing embryo or foetus is free of hazard [25].

Basically, this experimental study using ultrasound exposure to pregnant rabbits, and the young born rabbits served as the subjects. This experimental study resulted that the treated group young aged subject showed a significantly reduced parathyroid hormonal levels. This finding is based on the analysis of young age rabbits' PTH levels using Mann-Whitney test by analyzing the differences between control and treatedgroups.

In relation to the effect of PTH level on the treated subjects reported by Dom, et al. [13], it is surmised that the ultrasound exposure towards pregnant rabbits will cause lower PTH level towards young born rabbits, as reported based on the significant difference found between the control and the treated group. This is a reflection of hormonal interruption of young born rabbits towards prenatal ultrasound beam effects. The same results were obtained earlier when Kovatcheva and her colleagues [14] found that serum PTH levels decreased and normalised after a series of high-intensity focused ultrasound (HIFU) treatment on primary hyperparathyroidism in menopausal women. Consequently, the same result was obtained when the treatment was given to the secondary hyperparathyroidism in patients with chronic kidney disease [15].

In combination of this study with previous reports from Dom, et al. [13]and Kovatcheva , et al. [14, 15], we can deduce that usage of high and diagnostic level of ultrasound wave able to cause a significant reduction towards the clinical and experimental subjects. These results can now be included in the next guidelines of ultrasound usage by the appropriate organizations.

Low PTH level, also known as hypoparathyroidism is one of the infrequent endocrine-deficiency disease characterized by hypocalcaemia with inappropriate response of parathyroid glands, as well as elevated levels of phosphorus. A suppression of foetal parathyroid gland reported to cause 50% rate of tetany and a 25% rate of neonatal death [26] . It is possible that the suppression of the parathyroid gland of young born rabbits due to prenatal ultrasound will be lasting for months, as concluded by Kovacs and Kronenberg [17] where they believe that gland suppression may occasionally be prolonged for months. This warrants for further studies to look for a longer effects. This is however, whether the foetal parathyroid gland suppression is caused either by direct effect of ultrasound beam or maternal

hypercalcaemia secondary to ultrasound effects is still yet to be determined.

This experimental study showed that the ultrasound exposure caused no significant changes toward the creatinine levels in young rabbits. The results also showed no significant linear relationship between creatinine and PTH levels. These results suggest that the significant reduction of PTH levels in this study do not associates with creatinine levels. This is however not in line with studies reporting that there is association between low parathyroid hormonal levels with creatinine levels and renal disorders [9-12]. This is however, in this particular study, the subject is the young born rabbits which arise from a different setting of methodology, where this can be the reason of no association stated. In turn, this result reporting that the prenatal ultrasound exposure induces in rabbit models, did not cause any significant difference in creatinine levels of the young born rabbits.

However, for the test of trend of changes throughout the young age (1, 2, 3, 4 and 5 month old) in PTH and creatinine level, the researcher found a different kind of result. The findings showed that there were significant differences in PTH levels as the parameters were compared regarding to a certain month old. The significant results dropped in the three and five months old subjects. The present study found that there was no significant difference in PTH levels when a comparison was made for one month old, while [13] reported that there was a significant difference in the PTH levels of newborns New Zealand White rabbits. These two findings contradict to each other. Reported that, for the first 48 hours, PTH levels were recorded below the normal range due to its glands that have not mature yet, however, it would rise up to normal range, or else it would be assumed as abnormally below normal ranges [27]. With this, it shows that the significantly decreased in PTH levels on newborns found in Dom's study will raise to normal level as it reached one month old.

However, for the five months old PTH levels, there was a significant difference in PTH levels of the treated group compared to the control group. It is reported that there is a positive correlation between PTH levels and age [28], but in this study, it has been found that the statistical values up to five months old are varried. This shows that prenatal ultrasound that is given during middle of pregnancy is statistically proven to cause a variation in the trend of change of PTH levels of the young age New Zealand White Rabbits.

Therefore, it is believed that prenatal ultrasound causes the next rabbit generations to have disturbance of parathyroid hormone levels. Such disturbance causes many unwanted side effects, which affect their life forever. However, whether the foetal parathyroid gland suppression is caused either by direct effect of ultrasound beams or maternal hypercalcemia due to ultrasound effect is still yet to be determined.

Despite of no significant difference in creatinine level between the control and the treated groups, there is an opposite finding where there was a significantly lower in the creatinine level of 2 months old rabbits (the rest were no significant difference) between the control and the treated groups, This finding proves that there is a linear relationship between serum creatinine and PTH levels [29] as stated previously, whereby there was a significantly lower PTH levels in the treated group compared to the control group. This finding, to a current knowledge, is new with regard to the effect of prenatal ultrasound on creatinine level of young rabbits. During childhood, an increase in renal muscular mass will increase serum creatinine [30]. Considering this new fact, the researcher highly recommend for future research to consider histological studies on the cells and tissues of the renal anatomy to further investigate the microscopic anatomy of the renal following ultrasound exposure.

In some reports, in a good way, low creatinine level indicates of a good working renal. On the other hand, low creatinine level might also be associated with lower muscle mass, severe liver disease, and low protein diet or pregnancy. Reported earlier that liver disease (chronic) is related to significantly lower serum creatinine level as compared to that of the control group [31]. Back to the finding, it is recommend for upcoming research to look for a relationship between low creatinine level in childhood age with liver condition in youngster.

Furthermore, it was reported that lower serum creatinine level is now a risk factor for type 2 diabetes [32]. It was also reported that there was a 10-fold increase in the incidence of type 2 diabetes among youngsters between 1982 and 1994, in which identical findings were also reported elsewhere [33]. In this case, the researcher highly propose for upcoming research to look for the relationship between prenatal ultrasound, serum creatinine level and in the incidence of type 2 diabetes among youngsters.

Nevertheless, this study has some limitations. Namely, the subjects were rabbits, where we could not directly use the data to correlate to human being and this study only covers a small part of prenatal ultrasound effect. In order to make the study of prenatal ultrasound heating effects is more reliable, we must study the effects on human itself.

In conclusion, the findings of this *in vivo* experimental study suggest that young rabbits' PTH and creatinine were affected when prenatal ultrasound exposure have been given on middle of pregnancy for 60 minutes. This however, shows that there is no relationship between PTH and creatinine levels in this experimental study of prenatal ultrasound heating effects. This scientific information can be applied in the body of knowledge of ultrasound field for its safety and prudent use.

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