ORIGINAL ARTICLE

IMPACT OF PRENATAL ADMINISTRATION OF MELAMINE ON FOETAL GROWTH IN RATS

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Background: Data on the potential effects of maternal exposure to melamine is scarce. We aimed to evaluate the impact of melamine administration on pregnancy outcome and foetal growth in rats. **Methods:** Positively-mated female Sprague-Dawley rats (n=24) were treated from day 6 to day 20 of gestation with vehicle (control), melamine 300 mg/kg/day (group-1) or melamine 450 mg/kg/day (group 2). On day 21, the numbers of foetal resorptions and dead foetuses were recorded. Thereafter, pups were examined for external anomalies, and various growth parameters were measured. **Results:** A remarkable increase in the number of resorptions was observed in group-2 compared to the other two groups. A significant increase in foetal weight and placental weight was seen in group-2 compared to control. Head length and placental diameter were low in group-1 compared to control. The ratio between crown-rump length and head length was significantly greater in group 2 compared to control indicating asymmetrical intrauterine growth restriction. The only influence observed in group 1 compared to control was a decrease in placental diameter. No gross foetal malformations or changes in umbilical cord length, crown-rump length or biparietal diameter were observed in both melamine-treated groups. **Conclusions**: Maternal exposure to melamine during pregnancy increased the incidence of resorption and resulted in asymmetrical intrauterine growth restriction.

Keywords: Melamine; Maternal Exposure; Foetal Growth; Rat

Citation: Tayem Y, Veeramuthu S, Rashid A, Sequeira R, Fadel R. Impact of prenatal administration of melamine on foetal growth in rats. J Ayub Med Coll Abbottabad 2019;31(4):502–5.

INTRODUCTION

Melamine attracted global attention following the illegal marketing of melamine-adulterated pet food which led to chronic toxicity in pets in several countries around the world in 2004 and 2007.[1] A few years later, another melamine poisoning outbreak affected thousands of Chinese infants and children who were fed contaminated milk formula which resulted in serious morbidity and mortality.[2] As a result of these incidences, which took place in September 2008, more than 47,000 children were hospitalized, and four died.[3] Examination of deceased and ill babies revealed that many of them suffered from renal stones while others developed acute renal failure as a result of tubular injury and obstructive nephropathy.[4,5] This devastating melamine poisoning in humans was considered a global threat to health worldwide.

Melamine is a nitrogen-rich compound which is used for manufacturing durable plastic products, non-stick utensil coating and glue. Some dairy producers deliberately add melamine to food products, such as formula milk, in order to increase their apparent protein content and its monetary value.[6] Many animal studies have investigated the toxic effects of melamine on different body systems. Interestingly, acute exposure to low doses of melamine has not been shown to be toxic in animals, because greater than 90% of the ingested dose is eliminated from the body within 24 h.[7] However, subacute and chronic exposure to this compound have been shown to cause several toxic effects including haematuria, proteinuria, oliguria, renal failure, hepatic impairment, neurotoxicity, and transitional cell carcinoma in ureter and urinary bladder.[8–11] Wong *et al* investigated the mechanisms of melamine-induced renal injury and demonstrated that maternal exposure to melamine resulted in oxidative stress which caused endothelial dysfunction in the rat mothers. This endothelial injury induced renal vasoconstriction and diminished vasodilation. Interestingly, the investigators revealed that offspring were at high risk for developing vascular changes too.[12]

The effects of maternal exposure to melamine on the development of the foetus have not been adequately investigated before. Therefore, by using a rat model of melamine administration during pregnancy, we aimed to investigate the potential effect of melamine on pregnancy outcomes such as resorption, foetal death, external anomalies, and intrauterine growth.

MATERIAL AND METHODS

All animal studies were conducted in compliance with the specifications outlined in the Arabian

Gulf University (AGU) Guide for the Care and Use of Animals and in accordance with National Institutes of Health guide for the care and use of Laboratory animals. This work was approved by the Institutional Research and Ethics Committee (Approval number 17-PI-12/2013). Nulliparous, virgin female Sprague-Dawley rats (n=24) weighing 180–220 grams were kept in separate, spacious and wide-mesh cages, in a pathogen-free animal facility. Before starting the experiments, the rats were acclimated for one week in the animal house under ambient temperature of 25 ºC, 12-hour light-dark cycles, and *ad libitum* access to standard rodent chow and purified water. Following acclimatization, one fertile male rat was placed in each cage along with two female rats for mating. Pregnancy was tested every morning by microscopic examination of vaginal lavage smears. The female rats were confirmed to be pregnant when spermatozoa were observed in the smear and this was designated as day one gestation.

Starting from day 6 until day 20 of gestation, pregnant rats were treated once daily between 9–10 am via oral gavage. Rats were randomly allocated into three experimental groups: Control (n=8) received the vehicle 1% carboxymethyllcelulose (CMC) in water, group 1 (n=8) and group 2 (n=8) were administered melamines (Alfa-Aesar, Germany) at doses of 300 or 450 mg/kg/day, respectively.

Between 9 and 10 am in the morning of day 21 of gestation, the rats were euthanized by ether inhalation and laparotomy was carried out. During this procedure, foetuses were collected from the uterine horns, numbers of resorptions and dead foetuses were recorded and alive pups were euthanized by ether. Following euthanizia, the pups were examined for gross external malformations. The following foetal growth parameters were measured: foetal weight (FW), crown-rump length (CRL), head length (HL), biparietal diameter (BPD), placental weight (PW), placental diameter (PD), and umbilical cord length (UCL). The weight parameters were determined by using a sensitive balance (Mettler PE 360, Ohio, USA) while the dimensional measures were determined by using digital Vernier scale (Cen-Tech, Virginia, USA).

Collected data was analysed by using the SPSS-23. The differences between the means of growth parameters among the experimental groups, were assessed by using the independent sample *t*-test whereas correlation between these parameters was determined by using Pearson`s correlation coefficient. Statistical significance was set at *p*-value less than 0.05.

RESULTS

The total number of implants in all the three experimental groups reached 241. The amount of foetal loss and resorptions was determined in this study (Table-1). One foetus was found dead in both control (1.1%) and group 1 (1.3%). However, no foetal death was observed in group 2 (0%). Regarding the number of resorptions, we observed three in control (3.4%) and three in group 1 (3.8%). Nevertheless, the number of resorptions significantly escalated in group 2 and reached 15 (19.7%). Correlation analysis revealed a significant increase in the number of resorptions in group 2 compared to control ($p<0.01$) and group 1 ($p<0.01$). However, no significant difference in the number of resorptions was observed in group 1 compared to control ($p>0.05$).

Regarding the data on growth parameters, our findings revealed a significant increase in FW and PW in group 2 compared to control ($p<0.001$) (Table-2). Both HL and PD were also found to decline in group 1 compared to control ($p<0.05$). However, the other growth parameters, namely CRL, BPD and UCL, were not affected in group 2 compared to control. On the other hand, all growth parameters were not found to be significantly different in group 1 compared to control, except a significant decrease in placental diameter ($p<0.05$).

The ratio between CRL and HL was significantly higher in group 2 in comparison to control ($p=0.039$) indicating asymmetrical intrauterine growth restriction (IUGR). However, the same ratio was not significantly different in group 1 compared to control. The correlation coefficient between CRL, HL and BPD in the three experimental groups was also calculated (Table-3): CRL was positively correlated with HL and BPD in groups 1 and 2.

Table-1: Effects of maternal exposure to melamine on embryo-lethality and resorption in rats

	Control (n=8)	Group 1 (n=8)	Group 2 (n=8)
Total implants	87	78	76
Resorption	3 (3.4%)	3 (3.8%)	15 (19.7%)*
Dead foetus	1 (1.1%)	1 (1.3%)	0 (0%)
Alive foetus	83 (95.5%)	74 (94.9%)	61 (80.3%)

Chi-Square: *$p<0.01$ compared to control and group 1. Group 1: melamine 300 mg/kg/day, group 2: melamine 450 mg/kg/day.

Table-2: Effects of melamine exposure during pregnancy on the growth parameters in rat foetuses

Growth parameters	Groups		
	Control±SD	Group 1±SD	Group 2±SD
Foetal weight (FW); g	3.1347±0.35388	3.2549±0.48761	3.4328±0.53858**
Placental weight (PW); g	0.6097±0.1027	0.6207±0.8595	0.6702±0.10056**
Placental diameter (PD); cm	1.3988±0.10714	1.3560±0.12219*	1.3438±0.17984*
Head length (HL); cm	1.3494±0.8886	1.3541±0.10094	1.3094±0.14983*
Biparietal diameter (BPD); cm	0.8626±0.06718	0.8599±0.06227	0.8567±0.06305
Crown rump length (CRL); cm	3.1434±0.21253	3.0932±0.23486	3.1468±0.14901
Umbilical cord length (UCL); cm	2.1963±0.33973	2.0919±0.35798	2.1250±0.35367
CRL/HL ratio	2.3396±0.19722	2.2899±0.16503	2.4074±0.19127*

Independent sample t-test compared to control: *p< 0.05, **p< 0.001. Group 1: melamine 300 mg/kg/day, group 2: melamine 450 mg/kg/day.

Table-3: Correlation between CRL and the other growth parameters in the control and treated groups

Groups	HL	BPD	PW	PD	FW	UCL
CRL control	0.247*	0.299**	0.248*	0.042	-0.098	0.116
CRL group 1	0.599**	0.356**	0.101	0.209	0.571**	0.283**
CRL group 2	0.298*	0.546**	0.123	0.006	0.867*	-0.23

Pearson`s correlation coefficient: *p< 0.05, **p<0.01. Group 1: melamine 300 mg/kg/day, group 2: melamine 450 mg/kg/day.

DISCUSSION

Little is known about the impact of maternal exposure to melamine on pregnancy outcome and foetal development. In this study, we used a rat-model to investigate the effects of melamine administration on pregnancy outcomes including occurrence of malformations and intrauterine foetal growth. Although no external anomalies were observed under our settings, a dose-dependent increase in asymmetrical IUGR and increased risk of resorption were reported.

Our findings showed no occurrence of external anomalies in all melamine-treated groups, but we found a statistically significant rise in the number of resorptions in group-2 but not in group 1. Previous studies have reported that melamine can cross the placenta and reach the foetus in a dose-dependent manner. Jingbin and colleagues reported that, in a dose-dependent manner, melamine crossed the placental barrier and reached the foetuses.[13] By using an ex-vivo model of placental perfusion, El-Nezami and co-authors confirmed melamine's ability to cross the placental barrier. The researchers showed that the trans-placental transfer of melamine is dose-dependent and tends to increase as pregnancy progresses.[14] The ability of melamine to cross the placental barrier in a dose-dependent manner may explain the significant rise in the number of resorptions reported in group 2.

In line with our findings, by using a similar rat model of prenatal melamine administration, Wang *et al* reported an increase in foetal loss and perinatal death but no congenital malformations.[15] Our data clearly demonstrated that at high dose, maternal exposure to melamine could increase the incidence of resorption in rats.

Our findings on foetal growth parameters showed a remarkable increase in foetal and placental weight in group 2. Previous studies have shown that melamine leads to kidney damage as a result of renal calculi formation. [16–18] Gao *et al* followed children who were exposed to melamine-tainted milk in China for 18 months. More than 90% of them passed a stone at the end of the observation period, and melamine exposure resulted in an increase in microalbuminuria. We speculate that the observed increase in foetal and placental weight in our study might be related to foetal oedema as result of nephrotoxicity and proteinuria. Our data on foetal weight appeared to be in contrast with the findings of Wang *et al*, who reported a decrease in the weight of rat foetuses following melamine exposure during gestation.[15]

The ratio between CRL and HL significantly increased in group 2 compared to control. However, there was a positive correlation between CRL, HL and BPD suggesting that all three parameters followed a similar trend, albeit in different proportions. These findings indicate possible asymmetrical IUGR in foetuses born to rats who were exposed to melamine at a dose of 450 mg/kg. To our knowledge, this is the first study that reported melamine-induced IUGR in rat foetuses.

CONCLUSION

Maternal exposure to melamine during pregnancy was not associated with external anomalies but it increased risk of resorption and resulted in asymmetrical intrauterine growth restriction in pups.

ACKNOWLEDGMENT

This study was funded by the Arabian Gulf University (grant number 17-PI-12/2013), Manama, Bahrain.

AUTHORS' CONTRIBUTION

YT: Was the main investigator. He significantly contributed to experimental design, animal treatment, data collection, analysis, interpretation and manuscript preparation. SV: Significantly contributed to animal treatment, data collection, analysis and interpretation. AR: Significantly contributed to animal treatment, data collection, analysis and interpretation. RS: Significantly contributed to experimental design, data analysis, interpretation and manuscript preparation. RF: Significantly contributed to experimental design, animal treatment, data collection, analysis, interpretation and manuscript preparation.

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Submitted: 5 November, 2018	*Revised: --*	*Accepted: 16 June, 2019*

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