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The effects of exposure to endocrine-disrupting chemicals in intrauterine life on thyroid function tests during the neonatal period

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Ethics Committee Approval
The study protocol was approved by the Medical Ethics Research Committee at Erciyes University with a number of 2014/176.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Animal studies have shown that endocrine-disrupting chemicals can cause transient hypothyroidism. The aim of this study is to investigate the effects of exposure to endocrine disrupting chemicals (polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), bisphenol A (BPA) in plastics) in intrauterine life on thyroid function tests during the neonatal period.
Methods: In this observational cohort study, cord blood samples were obtained from all infants at birth to measure endocrine disruptors. Serum bisphenol A, di-(2-ethylhexyl) phthalate, and mono-ethylhexyl phthalate levels were measured by high performance liquid chromatography (HPLC). We excluded newborns whose mothers had thyroid function disorders.
Results: The male newborns' cord bisphenol A concentrations were significantly higher than those of female newborns (1.14 (0.26) ng/ml vs 0.85 (0.25) ng/ml, respectively; P=0.007). When we examined the correlation between the cord blood phthalate values and the maternal and newborn's thyroid function tests, a negative relationship between mono-ethylhexyl phthalate and newborn thyroid stimulating hormone was detected (r= -0.284, P=0.003).
Conclusion: A negative correlation was detected between cord blood mono-ethylhexyl phthalate levels and neonatal thyroid stimulating hormone levels suggesting that phthalate exposure may affect the thyroid function of babies in the prenatal period.

Keywords: Bisphenol A, di-(2-ethylhexyl) phthalate, Mono-ethylhexyl phthalate, Cord blood, Thyroid function

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Introduction

Endocrine disruptors affect the development and functions of the endocrine system. They are exogenous substances or their mixtures. These substances act on the production of hormone secretion, attachment, transport, activity, destruction, and excretion from the body. As they can be found in nature, they are also included in various industrial and synthetic products [1, 2].

Bisphenol-A (BPA) has been used as a synthetic estrogen in cattle and poultry since 1930 to achieve industrial gain. We are easily exposed to BPA due to the large numbers of plastic in various products, such as food containers, plastic bottles, canisters, thermal receipts, medical equipment, tableware, and water supply pipes [3]. Bisphenol-A may interfere with thyroid hormone (TH) action by interacting directly with the TH receptor. It was revealed that BPA acts a thyroid hormone receptor antagonist and suppresses the receptor's transcriptional activity, which is stimulated by the thyroid hormone (T3) [4].

Di-(2-ethylhexyl) phthalate (DEHP) is the most frequently used form of phthalate. The main area of use (95%) is PVC production. Di-(2-ethylhexyl) phthalate is considered an estrogen agonist and testosterone antagonist, but its mechanisms of toxicity are still not well understood [5].

Thyroid hormones (TH) play an essential role in pre- and postnatal growth and brain development in humans. Prenatal THs are essential for normal brain development. A number of studies have shown that variations in maternal T4 or TSH levels during gestation are associated with reduced cognitive abilities and increased risk of behavioral problems in childhood [6].

We evaluated whether BPA or phthalates (DEHP and MEHP) can affect thyroid functions and cause transient neonatal hypothyroidism. Experimental evidence supports this hypothesis. In an animal study, oral exposure to BPA resulted in a temporary decrease in free thyroxine (T4) in pregnant rats, but it had no effect on total thyroxine [7]. On the other hand, in another study, prenatal exposure to BPA was related to a temporary dose-related elevation in total T4 among both male and female pups (8) and increase in free T4 (at postnatal day 7) followed by a decrease (at postnatal day 21) among male pups only [7].

Few studies have examined the relationship between BPA and thyroid function and have yielded conflicting results. Meeker et al. [9] found no association among serum free T4, total T3 and TSH and BPA concentrations in urine samples collected from 167 men at an infertility clinic in Boston, Massachusetts.

There is very little information about the association between thyroid functions and DEHP and the results from previous studies are not consistent. This prompted us to undertake a study about this issue.

Materials and methods

Participants

After obtaining informed parental consent, we included 100 newborns to the study who were born with caesarian section or normal vaginal birth between May 2015-June 2015 in Erciyes University Maternity unit. Before delivery, thyroid function tests were obtained from mothers so that we could exclude newborns from the study whose mothers had thyroid function disorders.

The study protocol was approved by the Medical Ethics Research Committee at Erciyes University with the decision number 2014/176. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. We obtained informed consent from the parents.

Sample analysis

Thyroid function tests were performed in newborns at postnatal 3 to 7 days. Cord blood samples were obtained from all infants at birth to measure BPA, DEHP and MEHP levels.

In the delivery room, the cord was immediately clamped by the nurse from the mother's side and from the baby's side after birth, then 3 cc of cord blood was stored in a glass tube with a metal injector to avoid contact with plastic. Cord blood was centrifuged for 5 minutes at 4000 g, after which the plasma layer was transferred to plasma tubes. The plasma was stored at -80^{o}C. Serum levels of BPA, DEHP and MEHP were studied using HPLC method.

BPA measurement

Bisphenol-A measurements were made by using an Agillent 1100 series brand HPLC chromatographic system and C-18 column. (250mm X 4.6 mm) A fluorescence detector with a 227-nanometer wavelength extraction and 313-nanometer wavelength emission was used as a detector. Mobile phase A (70%) included acetonitrile and mobile phase B (30%) included water. The procedure was performed using a double pump. Chromatographic analysis was studied at 25 ° C, using a 1 ml/minute flow rate and a 20 ml injection volume.

Extraction of the serum samples was performed as the first step of the analysis. For this, 100 µl 0.01 mol/L ammonium acetate buffer and 4 ml n-hexane and a 70:30 mixture of a diethylether were added to 500 µl samples. Once the samples were centrifuged, the organic phase was evaporated under a stream of nitrogen. Subsequently, the sample was completed to 100 µl with HPLC compatible acetonitrile and analyzed.

Stock standard solutions of 0:50 mg/ml of BPA were prepared in methanol. The standard working solutions were obtained by using the stock standard solution diluted to varying concentrations of methanol again**.**

At the end of the study, the retention time was set at 3.7 min for BPA. According to the peak areas obtained from standard work, the linear calibration curve was calculated from the peak area of the sample. This way, we could calculate the BPA value.

Phthalate measurement

Di-(2-ethylhexyl) phthalate and MEHP concentration measurements were made by using an analytical ODS2 C-18 column (250 mm X 4.6 mm, Waters, Milford, MA) in the HPLC device (Hewlett Packard Agilent 1100 Series, Vienna, Austria). Separation was performed at room temperature. The mobile phase consisted of a 90:10 (v/v) mixture of acetonitrile and the 0.1% orthophosphoric acid. The mobile phase was prepared daily, and the flow rate was 1 ml/min. The peaks were detected by a UV detector at a 230 nm wavelength. Stock standard

solutions were prepared in acetonitrile containing 2000 ppm DEHP and MEHP. The standard working solutions of varying concentrations were prepared by diluting the stock solution with the mobile phase. Standard solutions at +4 degrees can remain stable for about 1 month. Extraction was carried out prior to analysis.

First, 400 ul 1 N NaOH and 100 uL of 50% H3PO4 and 600 µl of acetonitrile were added to the 200 µl sample. The supernatant was removed after each sample was centrifugated for 10 minutes at 3500 rpm and it was extracted with 600 µl of acetonitrile residue. After a second centrifugation under the same conditions, the supernatant was evaporated. In the last step, after the addition of the 400 µl mobile phase, 100 µl was injected into the chromatography system. The calibration curves were obtained according to the peak area of standards and sample concentrations were calculated.

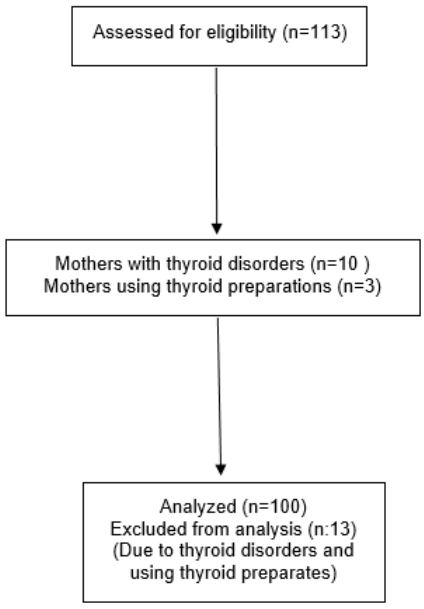
Statistical analysis

In G-power 3.1.9.2 program, power analysis was conducted at 0.25 effect size, 5% type 1 error, and 80% power. Accordingly, the number of samples required was calculated as 90. The study was planned with 100 patients considering 10% data loss. Data were evaluated in the IBM SPSS Statistics 22.0 statistical packages program (IBM Corp., Armonk, New York, USA). Normal distribution of the data was assessed by the Shapiro-Wilk normality test and Q-Q graphs. Descriptive statistic values were presented as mean (standard deviation) and median (25^{th}-75^{th}. percentile). Comparisons between groups were analyzed by t-test and Mann-Whitney U-test for independent samples. The relationship between the numerical variables were analyzed with Pearson and Spearman correlation analysis. The relationship between categorical variables was analyzed with the exact method of the chi-square test. $P<0.05$ was considered statistically significant.

Results

A total of 113 women were included in the study, but 13 had thyroid dysfunction or were using thyroid preparations (Figure 1). Finally, 100 newborns (52 males, 48 females) who were born in Erciyes University maternity unit between May 2015 and June 2015 were enrolled in the study.

Figure 1: Flow chart description of the trial



Infants born before 34 weeks were considered early premature, those born between 34-37 weeks were considered late premature and those born at 37 weeks or later were considered mature. The mean gestational age of newborns in the study was 37.38 (2.3) weeks (male newborns: 37.18 (2.4) weeks, female newborns: 37.53 (2.1) weeks); the mean body weight was 2907.2g (624.3 g) [male newborns: 2891.9 (690.6), female newborns: 2917.2 (557.9)]. The mean length was 48.6 (2.5) cm [male newborns: 48.4 (2.5) cm, female newborns: 48.9 (2.4cm)] (Table 1).

Table 1: Newborn anthropometric measurements and cord bisphenol, mono-ethylhexyl phthalate (MEHP) and Di-(2-ethylhexyl) phthalate (DEHP) values

	All n=100 (100%)	Male n=52 (52%)	Female n=48 (48%)	*P*-value
Gestational age (week) mean (SD)	37.38 (2.3)	37.53 (2.1)	37.18 (2.4)	0.174
Weight (kg) mean (SD)	2907.2 (624.3)	2891.9 (690.6)	2917.2 (557.9)	0.412
Height (cm) mean (SD)	48.6 (2.5)	48.4 (2.5)	48.9 (2.4)	0.321
BISPHENOL-A (ng/ml) mean (SD)	9.97 (2.93)	11.42 (2.60)[a]	8.52 (2.51)	0.007
MEHP (µg/ml) median (min-max)	0.20(0.03-0.78)	0.22 (0.14)	0.21 (0.16)	0.124
DEHP (µg/ml) median (min-max)	2.72(1.16-7.14)	2.72 (0.72)	3.04 (1.19)	0.137

DEHP: Di-(2-ethylhexyl) phthalate, MEHP: mono-ethylhexyl phthalate

The mean cord blood BPA levels of all newborns was 0.99 (0.29) ng/mL. The median DEHP value of all newborns was 2.72 µg/ml (1.16 to 7.14). The median MEHP value of all newborns was 0.20 µg/ml (0.03-0.78).

We found that the male newborns' cord BPA mean value was higher than that of female newborns (1.14 (0.26) ng/ml vs 0.85 (0.25) ng/ml, respectively) (P=0.007).

When the median MEHP value was compared by sex, we found that the male newborns' cord MEHP value was similar to that of female newborns (0.22 (0.14) µg/ml, 0.21 (0.16) µg/ml, respectively) (P=0.124).

The male newborns' cord DEHP mean value was slightly lower than that of female newborns (2.72 (0.72) µg/ml, 3.04 (1.19) µg/ml, respectively) (P=0.137).

There was no significant relationship between the cord blood BPA levels and the maternal and newborns' thyroid function tests; however, a negative relationship was found between MEHP and newborn TSH levels (r= -0.284 p=0.003) (Table 2). There was no significant relationship between MEHP values, maternal T3, newborn T3 and newborn T4 levels (P=0.214).

Table 2: Correlation coefficient between mono-ethylhexyl phthalate (MEHP) and newborn-mother thyroid function tests

MEHP Values (N:100)	MEHP (µg/ml)	Mother TSH (µIU/ml)	Mother fT3 (pg/ml)	Mother fT4 (ng/dl)	Newborn TSH (µIU/ml)	Newborn fT3 (pg/ml)	Newborn fT4 (ng/dl)
MEHP (µg/ml)	1						
Mother TSH (µIU/ml)	-0.23[a]	1					
Mother fT3 (pg/ml)	0.15	-0.10	1				
Mother fT4 (ng/dl)	-0.06	-0.14	0.09	1			
Newborn TSH (µIU/ml)	-0.28[b]	0.09	-0.07	0.11	1		
Newborn fT3 (pg/ml)	0.00	0.12	-0.08	-0.03	0.17	1	
Newborn fT4 (ng/dl)	0.00	0.16	-0.04	0.08	0.07	0.43[b]	1

[a] $P<0.05$, [b] $P<0.01$, MEHP: mono-ethylhexyl phthalate, TSH: Thyroid Stimulating Hormone, fT3: Free triiodothyronine, fT4: Free thyroxine

Discussion

The patient, who is informed about the need for an operation, faces a stressful situation. This anxiety reaches the maximum level especially in the preoperative preparation room. The main reason for this anxiety is pain and fear of not waking up after surgery [10-12]. Detailed information and premedication prior to the operation have an important role in the prevention of this preoperative incapability [9, 13]. This anxiety, pre-operative fasting, and fluid restriction significantly increase the level of stress. It induces various physiological, metabolic, and psychological responses to protect the body from this stress [3, 14]. The body's response is characterized by increased activation of catabolic and immunosuppressive hormones from the pituitary gland with activation of the sympathetic nervous system [15]. The aim of this study is to investigate the effects of stress and fluid restriction before anesthesia induction on patient clinic, endocrine responses, and a new peptide-made hormone, Nesfatin-1 level. The State-Continuity Anxiety Scale (STAI 1-STAI 2) is an easy-to-apply scale that can be answered by the individual as well as the patient's hemodynamic and biochemical data in the measurement of anxiety. Taşdemir et al. [16] showed that preoperative anxiety was significantly higher than postoperative anxiety. Domar et al. [17] found an average score of 45 preoperatively. In our study, according to the results of the statistical analysis for the STAI test, no significant differences were observed between the groups. The preoperative anxiety score in our study was 44 on average. This is similar to the results of other studies.

In our study, systolic and diastolic blood pressure values increased significantly just before induction in all groups. Although there were no statistically significant differences in the heart rate values between the groups, the intra-group evaluation increased significantly 1 hour before induction. This indicates that the anxiety caused by surgery increases as the time of operation approaches and premedication is not effective enough to prevent this.

The pituitary hormones secreted in response to stress and increased sympathetic activity cause the body to transition to a new state both hemodynamically and metabolically. Therefore, heart minute volume and tissue perfusion are increased, and body temperature rises. Blood glucose is increased with the increase of catabolic hormones such as cortisol, adrenaline, and insulin, in addition to glycolysis, gluconeogenesis, and lipolysis. The serum insulin levels in our study were similar in inter- and intra-group evaluations. Serum glucose levels of group 1A (fasted, fluid restricted and premedicated group) also showed a significant change compared to other groups. The high glucose levels of group 1A may be due to the anxiety caused by fluid restriction. Catecholamines have important physiological effects in response to stress. They activate glycogenolysis, gluconeogenesis, lipolysis and ketogenesis in the liver. This is because they lower insulin and increase glucagon [18-20]. They also increase blood pressure and heart rate [21]. There are many stimuli that lead to catecholamine release, such as hypovolemia, hypoglycemia, hypoxemia, pain and fear. Hypovolemia is best correlated with catecholamine release [22]. In our research, epinephrine and norepinephrine levels were higher in group 1A compared to the other groups. These results support our view that hunger and fluid restriction increase the stress level. It is known that surgical stimulation, anesthesia, psychic, and emotional stress increase cortisol release [23]. Cortisol potentiates the effects of epinephrine and glucagon, causing hyperglycemia. It also activates gluconeogenesis, proteolysis, and lipolysis. As a result of all these processes, blood glucose rises and tries to supply the vital organs with the necessary energy.

Nesfatin-1 is a recently described molecule. Studies show that besides the central nervous system, it is secreted from the pancreas, adipose tissue, and gastric mucosa [7, 24-26]. It has been shown in human milk [27]. Food and water intake has been shown to increase Nesfatin-1 levels [7, 24, 28]. Stengel et al. [29] found low levels of Nesfatin-1 in rats that were fasted for 24 hours. Tsuchiya et al. [30] stated that there is a negative correlation between Nesfatin-1 and BMI. The increase of Nesfatin-1 secretes glucose-stimulated insulin from pancreatic beta cells [31, 32]. Foo et al. [7] showed that Nesfatin-1 administration decreases blood glucose level of hyperglycemic rats (type 2 DM) depending on the dose and time. Nesfatin-1 is also effective in the regulation of emotional and behavioral states. In experimental animal models, Nesfatin-1 activation in the rat brain increased psychological stress [8]. In their study, Hofmann et al. [33] found that plasma Nesfatin-1 level was higher in the group of high anxiety compared to patients with low anxiety**.** There was a statistically significant correlation between plasma Nesfatin-1 level, total stress score and depression score. Günay et al. [34] reported that Nesfatin-1 level was low in their study on normal weight men with general anxiety. In our study, the nestfatin-1 levels of only group 2A was significantly higher than group 2B. In the intra-group evaluation, Nesfatin1 level in group 2A was high in the second period.

The level of Nesfatin1 in Group1A was significantly higher in the 3rd period than in the 2nd period. Also, group 2A had the lowest glucose levels in comparison to other groups. This can be regarded as an indicator of the antihyperglycemic effect of Nesfatin-1. Based on our results, anxiety score was compatible in the STAI 1 test, but no correlation was observed in the STAI-2 test. We think this may be related to glucose level.

The limitations of this study are as follows: The study was performed only in operations involving general anesthesia. Therefore, the data were more limited as it did not include patients with regional anesthesia. In addition, it should be kept in mind that anxiety analyses are affected by the sociodemographic statuses of the patients.

Conclusion

According to our findings, the highest reflection of stress in patients seems to coincide with the clinical and endocrine responses just before the induction period. Preoperative fluid replacement and premedication maintain hemodynamic stability and contribute positively to energy balance by increasing the level of Nesfatin-1. If the pathophysiological mechanisms are clarified, we think that Nesfatin-1 can be used in the treatment of diseases affecting energy metabolisms such as diabetes and obesity, and in reducing perioperative complications.

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