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RESEARCH ARTICLE

DETERMINING THE LEVEL OF PCB (POLYCHLORINATED BIPHENYL) IN THE MILK OF NURSING MOTHERS AND ITS RELATIONSHIP WITH THE MOTHER'S DIET

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ABSTRACT

Background: Human human milk may contain high levels of fat and toxins, which exist in our country based on many reports during the first postnatal week.
Methods: This study was conducted on 50 mothers who gave birth to a healthy infant at Vali-Asr teaching hospital, affiliated to Tehran University of Medical Sciences, Tehran, Iran, from 2014 to 2015. Within the first postnatal week, a 20 cc sample of mother's human milk was obtained and was sent for laboratory analysis to measure PCB levels via GC Mass method.
Results: Maternal diet had a significant role in the concentrations of PCBs in human milk; mothers who had higher daily intake of chicken had significantly higher concentrations of PCB 28 and PCB 180 in their milk ($p=0.003$, $p=0.031$, respectively). Mothers who had fish in their meals 3-4 times a week had significantly lower levels of PCB153 in their milk ($p=0.025$). The percentage of harmful PCB153 was also significantly lower in their milk ($p=0.006$). Mothers who had twice a week intake of cereals had significantly higher levels of PCB28 in their human milk ($p=0.018$).
Conclusions: Maternal diet have direct influence on human milk PCB concentrations. Therefore, it is necessary to inform mothers of the potential harms that might be caused by overusing some ingredients.
Objective: evaluating the concentration of PCBs (polychlorinated biphenyl) in the mother's milk

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INTRODUCTION

During the industrial age, increasing the presence of chemical substances, human beings have been exposed to toxins and various pollutants. So reduction or elimination of such substances has become one of the biggest concerns for human beings. These toxins could also enter the human milk which need special attention from perinatologists (Heeschen, 1987; Jensen, 1983). In the present study, human milk contamination with chlorinated hydrocarbons, especially PCB (Polychlorinated biphenyls) was evaluated. PCB which belongs to dioxins and polychlorinated groups has two benzene rings in its chemical structure. This combination has 209 isomers based on the number of chlorine atoms (Bjerk, 1972; Brevik, 1978) and the structure of benzene ring. As a result, PCBs have diverse stabilities and toxicities. These pollutants, not

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completely metabolized, can be found everywhere, but not to a great extend. They have a cumulative impact as well. They can contaminate food chain, can be stored in lipid organs via foods, especially fish, meat and cheese, and can be transferred during pregnancy and breast –feeding to fetus and later to infants (Grasman and Fox, 2001). Environmental accumulation of organochlorines affects the endocrine and the immune system, increases the risk of prostate cancer (Brevik and Bjerk, 1978), Brest cancer, endometriosis cryptorchidism and hypospadias, has a long term impact on evolution of intelligence and neurobehavioral system, and may cause delayed CNC growth. The lipophilic nature of these toxins cause human milk to be one of its main sources of storage. This becomes a more significant way of PCB transmission, compared to transmission through placenta (Lin *et al*., 2009). Human milk contains high level of fat, drugs, allergens, pollutants, and toxins, especially lipophilictoxins. It could be a potential source of contamination transmission from mother to infant. Breast feeding makes infants to be affected by pollutions 50 to 100 percent more than adults. In some limited studies conducted in Iran, the existence of PCBs was reported in Rivers of Azerbaijan and Mazandaran (Behrooz *et al*., 2009; Röhrig and Meisch, 2000). The current study was carried out to indicate the amount of toxins in human milk and its relationship to mothers' diet. So we can estimate the risk of presence of pollutants in human milk. Since similar studies had never been done in Iran, and the Iranians diet is different from that in other countries, the research could be a helpful indicator for further studies.

MATERIALS AND METHODS

In this cross-sectional study, 50 human milk samples–in the period of the first week postpartum- was collected from mothers who delivered a baby from March 2014 to February2015 in Valiasr hospital. Inclusion criteria were nursing mothers, healthy and aged between 25 to 45 years old, who had no smoking or drug addiction, or a job that directly exposed them to pollutants. None of these mothers had chronic diseases such as diabetes and high blood pressure, or systematic diseases. At first, a 20 cc breast milk sample was expressed by mother or nurses using a sterile technique. It was then poured into 4 clean glass containers and was stored in +4 ^{C} . Then it was transferred to the laboratory and was frozen at-20 degrees Celsius. Next, all frozen samples were sent to the laboratory of environmental analysis research center for analysis. All experiments were done with (GCMass) 19/20/21 method, and the numbers of PCB28/52/101/138/153/and 180isomers were measured (Röhrig and Meisch, 2000). The technique used to assess pollutants in human milk was SPME (Solid phase Micro Extraction). In this method, a very thin fiber is used, the surface of which is covered with a polymer of dimethyl siloxane. It is used to extract analysis from different matrices. In the current study, head space extraction method was used, and a gas chromatograph/mass spectrometer (GC – MS/MS) device was utilized for analysis. Here, the amount of protein (meat and chicken), dairies (milk), and cereals in mothers' diet was measured through the verified Food Frequency Questionnaire (FFQ), and its relationship with PCB was determined. Based on the amount of above-mentioned ingredients in the diet, there are 3 groups of low, medium, and high;

1) Low: the consumption is twice a week
2) Average: the consumption is 3-4 times a week
3) High: the consumption is 5-6 times a week

Similar to some previous studies, in this research, the average dietary index (average daily intake), ADI, (10)(12) was measured through the following formula, according to the world health organization:

ADI=PCB*BW*human milk (1)/Bw

The purpose of this research was explained to all participants, and consequently, all mothers signed a written consent. The information will remain confidential and only the results will be published for scientific reasons. Considering descriptive statistics, absolute and relative frequencies were used for qualitative variable, and mean and standard deviation for qualitative variables. Analytical statistics was performed based on the objectives and for the comparison between our classified groups, in order to investigate the relationship of several factors with PCB in human milk. These statistical analysis were done with Man Whitney and CHI sequence tests, with the significance level of 95%. P value less than 0.05 was considered as significant.

RESULTS

The concentration of PCB 28, PCB 52, PCB 101, PCB 138, PCB153, and PCB 180 was measured during the first week postpartum milk. There was no significant correlation between human milk PCB level and diary consumption by mothers in the first week. Also, no correlation was observed between the average daily intake of PCB by infants and their human milk consumption. However, the average consumption (ADI) was higher than normal level in all cases. There was also no statistically significant correlation between human milk PCB level or average daily intake of PCB by infants and mothers' meat consumption. The level of PCB 28 (53/2 ± 82/6 to 51/2 ± 07/5 - P = 0.031) and PCB 180 (689/0 ± 762/0 to 435/0 ± 227/0 - P = 0.003) was significantly higher in those who ate chickenthan in those who did not. Furthermore, the level of ADI 28 (379/0 ± 02/1 to 377/0 ± 760/0 - P = 0.031) and ADI 180 (103/0 ± 114/0 to 065/0 ± 034/0 - P = 0.003) in infants of mothers who ate meat was significantly higher than those whose mothers did not. However, there was not any correlation between the number of meals mothers consumed meat or chicken in the first week and the level of PCBs in their human milk or the average daily intake of PCBs by infants.

There wasn't any significant correlation between the number of meals mothers consumed fish in a week and the level of PCBs in their human milk or the average daily intake of PCBs by infants. However, there was a significant correlation between the average daily intake of PCB153 by infants and fish consumption by mothers, (P = 0.025) so that, for mothers who ate fish 3 to 4 times a week, the average daily intake of PCB153 was significantly lower than others. The level of PCB 28 in the human milk of mothers who ate less cereals was significantly higher than others (P = 0.018). No significant correlation was found between the level of PCBs in human milk and the amount of fat consumption.

Table 1.The mean score of PCBs level in human milk samples

Variable	Mean ± SD N=50 *(µg/lit)*
PCB28	**5.92± 2.41**
PCB52	**4.91± 2.74**
PCB101	**1.99± 1.036**
PCB138	**4.21± 14.75**
PCB153	**1.93± 1.038**
PCB180*(µg/lit)*	**1.11± 0.38**

Table 2. The relation between the average daily intake in infants and the of fish in mother's nutrition

Fish usage per week		ADI28	ADI52	ADI138	ADI153	ADI180	ADI101
2time		n=23	n=23	n=23	n=23	n=23	n=23
	M	.9230	.7867	.5995	.2588	.0587	.2458
	SD	.36888	.41006	2.42284	.16688	.08637	.19083
3-4 time							
		n=5	n=5	n=5	n=5	n=5	n=5
	M	.7395	.5430	.0000	.0000	.0000	.1485
5-6 time							
		n=1	n=1	n=1	n=1	n=1	n=1
	M	1.2810	1.1025	.2430	.4035	.1350	.3315
	SD						
No use							
		n=19	n=19	n=19	n=19	n=19	n=19
	M	.7908	.6358	.1213	.2263	.0673	.2577
	SD	.47436	.48577	.11127	.20169	.10089	.18504
total							
		n=48	n=48	n=48	n=48	n=48	n=48
	M	.8657	.7110	.3526	.2265	.0643	.2452
	SD	.40066	.43392	1.67725	.18483	.09041	.18451
	P-Value	.317	.390	.586	.025***	.699	.595

*M=mean, **SD=Standard Deviation

***There is a significant relationship between PCB level in infants and the usage of fish in mothers daily meal.

Table 3. The relation between the average daily intake in infants and the cereals of in mother's nutrition

Grain usage per week		ADI28	ADI52	ADI138	ADI153	ADI180	ADI101
2time		n=6	n=6	n=6	n=6	n=6	n=6
	M*	1.1817	1.0328	.1040	.2050	.1105	.3475
	SD**	.26549	.39528	.13863	.06418	.09217	.08340
3-4 time		n=17	n=17	n=17	n=17	n=17	n=17
	M	.7414	.5891	.7883	.2833	.0580	.2416
	SD	.44090	.35799	2.81401	.22681	.09108	.24261
5-6 time		n=28	n=28	n=28	n=28	n=28	n=28
	M	.8510	.7123	.1084	.1772	.0548	.2068
	SD	.35967	.45161	.12950	.16637	.08657	.15404
total		n=51	n=51	n=51	n=51	n=51	n=51
	M	.8534	.7089	.3345	.2158	.0624	.2349
	SD	.39538	.42924	1.62787	.18499	.08870	.18526
	P Value	.018***	.167	.192	.876	.229	.277

*M=mean,**SD=Standard Deviation

***There is a positive correlation between the usage of cereals in daily meal and receiving PCB.

Also, there was no significant correlation between the average daily intake of PCBs by infants and the amount of fat consumption in mothers' diet.

DISCUSSION

PCBs are pollutants that are transferred to the human body through food in many parts of the world. According to the studies, PCBs have many toxic effects on the growth of some human parts such as nervous system, immune system, endocrine system and reproductive system (Jacobson *et al.*, 1985). PCBs were mainly used as a covering layer on wood and textile industry. They are chlorinated chemical compounds, 209 isomers of which have been identified (Covaci *et al.*, 2002). The presence of PCB in the air, soil and milk has been reported in urban areas of South Africa (Jacobson *et al.*, 1990). The estimated dietary intake of PCBs for an average adult was about 0.03 µg/kg/day in 1978, which declined to <0.001 µg/kg/day in 1991 (Zhao *et al.*, 2007). The level of PCBs has been assessed by measuring PCBs level in blood, human milk, and adipose tissue (Dahmardeh Behrooz *et al.*, 2009; Devanathan *et al.*, 2009). In Japan, this level was evaluated in human milk, maternal blood and umbilical cord blood. The concentration of PCB was higher in mother's human milk than in their blood, and PCB in mother's blood was higher than that in umbilical cord blood (Brevik, 1978). In the present study, we examined the human milk of 50 mothers who had recently delivered their baby. Previous studies have shown that human milk in first 2-4 days after delivery is more suitable for measuring PCBs, compared to human milk in the following days (Crinnion, 2011; Devanathan *et al*., 2009). In the present study, we evaluated the mothers' human milk in the first week after birth, and the level of this pollutant in all samples was considered to be high. In another Iranian study on the concentration of PCBs in butter, It was found that the highest concentrations of PCBs belonged to the samples from Mazandaran province in the North and big cities such as Tehran, Isfahan and Arak. These cities are among the most industrialized and the most polluted areas of the country. The lowest level of PCB was found in Chanqaralu in Orumieh and Dashte Abi in Qazvin that both of them are remote villages in the country (Tsydenova *et al*., 2007).

In China, measuring the level of PCB in mothers' human milk and the food mothers consumed showed the presence of PCB in all mothers' human milk and all types of food such as rice and eggs (Subramanian *et al*., 2007). According to a survey performed on human milk samples in Tabriz in 1385, it was found that the level of PCBs was considerably higher than what previously was approved in Canada (Sun *et al*., 2010). Another study on the human milk samples of mothers in the southern coasts of Caspian Sea, which is an agricultural area, showed that daily intake (ADI) of most infants was higher than the proposed standards, which clearly caused concerns about children's health (Çok *et al*., 2009) In a research that was done in Iran, PCBs were present in all human milk samples under study, which was in accordance with the results of another study in India (Barghi *et al*., 2011; Grandjean *et al*., 1997). In our study, no significant correlation was found between the human milk PCB level and the amount of milk consumption or meat consumption in the first week after delivery. However, a research conducted on 50 samples of human milk in north of China showed considerably high level of PCB. This research investigated the mother's diet and found that human milk PCB level had a positive significant correlation with the amount of fish intake, and a negative significant correlation with the amount of meat and chicken consumption (Grandjean *et al*., 1998). Also in the present study, no correlation was observed between the amount of fish consumption or the amount of fat in the diet and the level of human milk PCBs, but according to a research that was carried out on the level of dioxins in human milk in different parts of Turkey, In areas where fish consumption was higher, the level of PCB was also higher (Sudaryanto *et al*., 2006).

In the present study, the levels of PCB 28 and PCB 180 in those who ate chicken were significantly higher than that in those who did not (P = 0.003 & P = 0.031). Although, there was not any significant correlation with the number of meals containing chicken in a week. Regarding the consumption of cereals, the PCB 28 level in human milk of mothers who ate less cereals was significantly higher than others (P = 0.018). The lipophilic properties of PCBs and its slow metabolism may cause these compounds to be accumulated in fatty tissues of fish, birds, mammals, etc. over time (Raab *et al*., 2008). In our research, the ADI index (the average daily human milk intake by infants) was evaluated for each of PCB isomers, and its relation with mother's diet was estimated. Based on the results, there was not any significant correlation between the average daily intake of PCB by infants with mother's consumption of milk and meat and the number of their meals. However, the average intake (ADI) in all cases was higher than the standard level.

In a study conducted in India, the difference in diet in different areas increased the PCB concentration in human milk. In this study, it was stated that the amount of daily intake was higher than the standard level proposed in Canada in 1999 (Çok *et al*., 2009). In our research, the average daily intake of PCB 28 and PCB 180 in infants of mothers who ate chicken, were significantly higher than mothers who did not (P = 0.003 & P = 0.031), but there was not any significant correlation with the number of meals containing chicken. A study conducted in the Faroe Islands found a specific correlation between the concentration of mercury in umbilical cord blood and neurological disorders in children. Mercury was transferred through whale meat present in the pregnant women's diet (Raab *et al*., 2008). Umbilical cord blood in fetal stage has also been used for measuring PCBs level. In those who did not have high fish intake in their diet, the concentration of PCBs was 0.09 to1.5 micrograms per liter. Evidence show that high consumption of fish from waters contaminated with PCBs increases the entrance of PCBs into the body (Dahmardeh Behrooz *et al*., 2009). Regarding fish consumption in the present study, there was a significant correlation between the average daily intake of PCB153 in infants and the number of meals containing fish mothers consumed, in a way that those who ate fish 3 to 4 times a week had lower average daily intake of PCB153 (P = 0.025) than others. Also in this study, there was a significant correlation between the average daily intake of PCB28 in infants and the number of meals containing cereals mothers consumed, in a way that those who consumed cereals twice a week, the average daily intake of PCB28 was higher (P = 0.018) than those who ate more.

Conclusion

It seems that almost in countries that conducted studies to evaluate the level of pollutants, different amounts of PCB were reported. In each country, based on their geographical conditions and nutrition, some specific measures should be taken to reduce it. This study could be a good start for further researches in this countries.

Suggestion and Implications

It is suggested to have a follow-up research after 18 and 24 months on the same samples. It is highly recommended to also measure the concentration of PCBs in mother's blood and the blood of fetus. Furthermore, it is suggested to perform the same research with a larger sample size.

It is recommended to implement a comprehensive program educating mothers to have a healthy nutrition, and considering the long half-life of these pollutant, it's better to start it from younger ages.

Conflict of interest

- Authors have no conflict of interest

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