Neuroendocrinology Letters Volume 39 No. 4 2018
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Human mature milk zearalenone and deoxynivalenol levels in Turkey

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Submitted: 2018-06-19 Accepted: 2018-07-23 Published online: 2018-09-20

Key words: **Human milk; breastfeeding; mycotoxin; zearalenone; deoxynivalenol**

Neuroendocrinol Lett 2018; **39**(4):325–330 **PMID:** 30531702 NEL39041812

Abstract

INTRODUCTION: Zearalenone (ZEA) and deoxynivalenol (DON) are toxic fungal secondary metabolites, found mainly in contaminated food, that are associated with serious health problems. It is important to identify undesirable toxins and metabolites that may be present in human milk. The aim of this study was to evaluate human milk ZEA and DON levels, total daily intake of ZEA and DON; and their possible relationship with maternal dietary habits.

METHODS: We enrolled 90 lactating mothers who had 7- to 90-day-old babies. A dietary questionnaire was completed by each of the mothers. Human milk samples were obtained from 90 mothers, and human milk ZEA and DON levels were evaluated with the solid-phase direct enzyme immunoassay. The total daily intake (TDI) was calculated for the 63 exclusively breastfed infants.

RESULTS: ZEA was detected in all human milk samples; median was 173.8 ng/L (35.7–682 ng/L). The calculated median TDI for ZEA was 33.0 ng/kg body weight (bw) (10.4–120.5 ng/kg) among exclusively breast-fed infants, none of them had a TDI that was above the previously defined threshold levels. Human milk ZEA levels were associated with the maternal consumption of meat, fish, dry fig, dried apricot, flaked red spice and spice. The median DON levels was 3924 ng/L (400-14997 ng/L). The median TDI of DON was 750 ng/kg (240–2774 ng/kg) among exclusively breastfed infants and 36% out of them, the TDI for DON was above the previously defined threshold level. Human milk DON levels were associated with the maternal meat consumption.

CONCLUSIONS: Our findings are indicative of dietary exposure to mycotoxins during the pregnancy and lactation periods in nursing mothers. Further, the excessive TDI values for DON observed in 36% of the exclusively breastfed infants point to the need for further regulations and recommendations on the dietary habits of pregnant/nursing mothers in order to avoid exposure to potential mycotoxins.

INTRODUCTION

Breastfeeding is considered as a gold standard practice, and it is recommended that all infants be fed only breast milk for the first six months of life, after which solid foods can be added to their diet (Eidelman, 2012). There is no doubt that human milk is the ideal initial diet for infants from both the nutrition and immunity standpoints (Dietrich *et al.* 2013). Since human milk is a unique source of elements that are important for infants' nutrition and growth, breastfeeding is encouraged all over the world. However, nursing mothers may be exposed to different natural or artificial pollutants and consume foodstuffs contaminated with various amounts of toxins during the breastfeeding period (American Academy of Pediatrics Committee on Drugs, 2001). Children are uniquely vulnerable to environmental toxicants because of their greater relative exposure, less developed metabolism and higher rates of cell division, growth and development (American Academy of Pediatrics Committee on Drugs, 2001; Makri *et al.* 2004). Therefore, it is important to identify undesirable toxins and metabolites that may be present in human milk and get passed on to breastfeeding infants.

Some toxins (mainly mycotoxins) that are ingested by the mother through food pass through her bloodstream and to the baby through breast milk (Warth *et al.* 2016). Mycotoxins are ubiquitous secondary metabolites that are produced by filamentous fungi mainly belonging to the genera *Aspergillus, Penicillium and Fusarium* (Alshannaq and Yu, 2017). The Food and Agriculture Organization (FAO) has estimated that approximately 25% of food-derived mycotoxins are passed on through consumption of contaminated plant-derived foods or indirectly by consumption of animal-derived products (e.g., meat, eggs, and milk) (Alshannaq and Yu, 2017; Marin *et al.* 2013).

Zearalenone (ZEA) is mainly produced by *Fusarium graminearum* and *Fusarium semitectum*. It is described as an estrogenic mycotoxin that induces obvious estrogenic effects in human and animals, which is an important public health concern. ZEA is a common contaminant in cereal crops worldwide (Zinedine *et al.* 2007; Raiola *et al.*, 2015). Food contamination by ZEA is caused either by direct contamination of grains, fruits and their products or by "carry-over" of mycotoxins in animal tissues, milk and eggs after intake of contaminated foodstuff (Alshannaq and YU, 2017; Richar, 2007). Although ZEA compounds are ubiquitous, they are generally considered a potential danger for human health, particularly when they are absorbed in high amounts or over a long time of exposure (Zinedine *et al.* 2007; Raiola *et al.*, 2015). In children, however, the major effects of ZEA compounds are observed in the reproductive system, where they affect organ structure and function, leading to hyperestrogenism and premature thelarche and precocious puberty (Marin *et al.* 2013; Zinedine *et al.* 2007; Massart and Saggesse, 2010; Kowalska *et al.* 2016).

Deoxynivalenol (DON) is a toxic fungal secondary metabolite produced by molds of the Fusarium genus and is found in grains and cereal-based food and feed. It is known to cause a spectrum of diseases, both in humans and animals, such as nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, fever, anorexia, immunotoxicity, hematological disorders, impairment of maternal reproduction and fetal development (Alshannaq and YU, 2017; Raiola *et al.*, 2015; Sobrova *et al.*, 2010). DON is the most common mycotoxin that contaminates cereals in most countries with a mild climate. The International Agency for Research on Cancer has classified ZEA and DON under group 3 toxicants (not classifiable with regard to their carcinogenicity in humans) (Raiola *et al.* 2015), and previous data about the human milk ZEA and DON levels are limited (Wart *et al.* 2016; Massart *et al.* 2016; Rubert *et al.* 2014; Tonon *et al.* 2018; Degen *et al.* 2017; Mally *et al.* 2016). Therefore, the aim of this study was to evaluate human milk ZEA and DON levels, total daily ZEA and DON intake, and their possible relationship with maternal dietary habits.

MATERIAL AND METHOD

In this study, we investigated the levels of ZEA and DON in human milk samples taken from mothers with 7- to 90-day-old babies. Written informed consent was obtained from each of the participants. This study was approved by the Local Ethics Committee of Eskisehir Osmangazi University. This study was also supported by Eskisehir Osmangazi University Research Grant.

Mother's age, parity, delivery mode, and gestational age, and the baby's nutritional status were recorded. All participants completed a questionnaire about the consumption of certain foods. The dietary questionnaire was completed by each of the mothers; it contained questions about the diet and eating habits during later pregnancy and the postpartum period. The questionnaire also focused on foods that are more likely to be mycotoxins according to the European Commission Food Science and Techniques (Galvano *et al.* 2008). Based on the questionnaire results, participating mothers were classified according to their consumption frequency as "never," "once or twice a month," "once or twice a week," "3–5 times a week" or "everyday." This study is a part of the ongoing investigation with the "Mycotoxins, endocrine disruptors and antibiotic residues in human milk" dietary questionnaire, which includes different food groups: poultry, fish, milk products (milk, yoghurt and cheese), dried fruits (dried fig, dried apricot), honey, sea foods, flaked red spice and spice.

Samples of human milk (5 ml) were obtained from the mothers and stored at -20°C until analysis. Human

milk ZEA and DON levels were evaluated with the HELICA serum/milk assay (direct solid-phase enzyme immunoassay), which is designed for the quantitative measurement of human serum and milk.

Total daily intake (TDI) was calculated (among only exclusively breastfed infants) based on the concentration of human milk ZEA and DON levels, the daily intake of milk by infants, and the infants' body weight with the following equation: human milk ZEA or DON concentration (ng/L) × daily volume of estimated milk consumption (L)/body weight (kg). The daily intake of human milk is 150 ml per infant's body weight (kg).

All statistical analyses were performed with the SPSS for Windows package (Chicago, IL). Human milk ZEA and DON levels were not normally distributed and therefore shown as the median, minimum and maximum levels. The Mann-Whitney *U*-test and Kruskall-Wallis test were used for comparisons, and Spearman correlation test was used for assessing correlation. Analysis of variance was used to compare human milk ZEA and DON levels according to the maternal dietary and Games-Howell test was used to compare subgroups in cases where there was a statistically significant difference between the groups. $p < 0.05$ was considered to indicate significant differences.

RESULT

Ninety mothers aged between 17 and 41 years (mean age, 29.8 ± 6.16 years), who had 7- to 90-day-old newborn babies, were enrolled. With regard to the parity of the participants, 38.9% had one baby (n = 35), 34.4% had two children (n = 31), 6.7% had three children (n = 6), 10% had four children (n = 9) and 10% had 5 or more children (n = 9). Forty of the enrolled mothers had a history of normal delivery (44.4%), and 50 had a history of cesarean delivery (55.6%). Human milk samples were obtained from the mothers 7 to 90 days after delivery (mean, 35.4 ± 21.1 days). Mean gestational age was 37.7 ± 2.4 weeks. Of the babies, 63 were fed only breast milk, and the remaining 27 were breast milk with formula.

ZEA was detected in all the human milk samples, at a concentration ranging between 35.7 and 682 ng/L, and the median ZEA level was 173.8 ng/L (Table 1). In infants who were exclusively breastfed (n = 63), the calculated median TDI for ZEA was 33.0 ng/kg body weight (bw) (10.4–120.5 ng/kg bw) (Table 2). In none of the breastfed infants was the daily intake of ZEA above the previously defined threshold level of 0.5 μg/kg bw/day [18]. With regard to maternal dietary habits, human milk ZEA levels were associated with the consumption of meat, fish, dry fig, dried apricot, flaked red spice and spice (Table 3).

The DON concentration in the breast milk samples was found to vary between 400 and 14997 ng/L, with the median DON level being 3924 ng/L. In the infants who were exclusively breastfed (n = 63), the calculated median intake of DON was 750 (240–2774) ng/kg bw/day. In 36% of the breastfed infants (23/63), the daily intake of DON was higher than the previously reported threshold of 1 μg/kg bw [18] (Table 1-2). With regard to maternal dietary habits, human milk DON levels were associated with the consumption of meat only (Table 3).

Human milk ZEA levels were positively correlated with DON levels ($r = 0.622$, $p < 0.001$). However, human milk ZEA and DON levels were not correlated with gestational age or mother's age ($p > 0.05$). Moreover, the median human milk ZEA and DON levels were not significantly different between the cesarean delivery group and normal spontaneous delivery group ($p > 0.05$).

Tab. 1. Human milk zearelenone and deoxynivalenol levels

Human Milk	Median	95% CI	Minimum–Maximum
Zearelenone (ng/L)	173.8	176.1–236.5	35.7–682
Deoxynivalenol (ng/L)	3924	4192–5455	400–14997

Tab. 2. Total daily intake of zearelenone and deoxynivalenol among exclusively breastfed infants

	Median	Minimum–Maximum	% of breastfed infants >TDI thresholds
TDI for zearalenone (ng/kg bw)	33.0	10.4–120.5	0 (0/63)
TDI for deoxynivalenol (ng/kg bw)	750	240–2774	36 (23/63)

TDI = total daily intake, bw = body weight

DISCUSSION

In our study, ZEA was detected in all the human milk samples, at a concentration ranging between 35.7 and 682 ng/L (median ZEA level, 173.8 ng/L). Detection of ZEA in human milk has been reported in two previous studies, which indicated that infants are exposed to this estrogenic contaminant and its main metabolites (Massart *et al.*, 2016; Rubert *et al.* 2014). In Massart *et al.*'s (2016) study, human milk samples were collected within the first six weeks after delivery from 47 healthy primiparous women in Naples, Italy; they were found to contain mean ZEA levels of 1.13 ± 0.34 μg/L (0.26–1.78 μg/L), which were correlated with the weight of the mother before pregnancy and at the time of delivery (Massart *et al.*, 2016). The ZEA levels reported in our study were lower than those reported in the previous Italian study (while the method of these two studies are different) : the median levels were 173 ng/L and maximum level was 682 ng/L in our study, while the mean level was

Tab. 3. Maternal dietary habits and correlations with human milk zearelenone and deoxynivalenol levels.

Food	n (%)	ZEA	DON
CHICKEN			
Never	2 (2.2%)		
Once or twice a month	31 (34.4%)		
Once or twice a week	47 (52.2%)	0.114	0.376
Three to five times a week	8 (8.9%)		
Every day	2 (2.2%)		
FISH			
Never	16 (17.8%)		
Once or twice a month	32 (35.6%)		
Once or twice a week	42 (45.7%)	**0.033**	0.224
Three to five times a week	–		
Every day	–		
MEAT			
Never	6 (6.7%)		
Once or twice a month	35 (38.9%)		
Once or twice a week	38 (42.2%)	**0.014**	**0.015**
Three to five times a week	9 (10%)		
Every day	2 (2.2%)		
HONEY			
Never	32 (35.6%)		
Once or twice a month	11 (12.2%)		
Once or twice a week	8 (8.9%)	0.751	0.995
Three to five times a week	9 (10%)		
Every day	30 (33.3%)		
DRY FIG			
Never	20 (22.2%)		
Once or twice a month	17 (18.9%)		
Once or twice a week	17 (18.9%)	**0.018**	0.360
Three to five times a week	17 (18.9%)		
Every day	19 (21.1%)		
DRY APRICOT			
Never	22 (24.4%)		
Once or twice a month	16 (17.8%)		
Once or twice a week	16 (17.8%)	**0.022**	0.283
Three to five times a week	17 (18.9%)		
Every day	19 (21.1%)		
MILK			
Never	33 (36.7%)		
Once or twice a month	4 (4.4%)		
Once or twice a week	8 (8.9%)	0.224	0.194
Three to five times a week	13 (14.4%)		
Every day	32 (35.6%)		
FLAKED RED PEPPER			
Never	37 (41.1%)		
Once or twice a month	5 (5.5%)		
Once or twice a week	14 (15.6%)	**0.019**	0.390
Three to five times a week	4 (4.4%)		
Every day	30 (33.3%)		
SPICE			
Never	16 (17.8%)		
Once or twice a month	5 (5.5%)		
Once or twice a week	17 (18.9%)	**0.013**	0.744
Three to five times a week	5 (5.5%)		
Every day	47 (52.2%)		

1130 ng/L in Italy (Massart *et al.*, 2016). A provisional maximum TDI of 0.5 μg/kg bw has been established for ZEA by the Joint Committee of the FAO/World Health Organization (WHO) (Degen *et al.* 2017). In the study from Italy, the calculated TDI for ZEA was 0.2 μg/kg bw, which is slightly lower than the TDI of 0.5 μg/kg bw that has been set for adults. In our study, the TDI for ZEA was 0.03 μg/kg bw (range, 0.01–0.12 μg/kg), which is lower than the previously defined limit as well as the values reported in the study from Italy (Massart *et al.* 2016; Rubert *et al.* 2014; Tonon *et al.* 2018; Degen *et al.* 2017). In an Italian study, no correlation was found between human milk ZEA levels and maternal dietary habits (Massart *et al.*, 2016). In the present study, however, human milk ZEA levels were associated with maternal consumption of meat, fish, dry fig, dried apricot, flaked red spice and spice, but the levels were lower than the threshold levels reported. It is possible that neonates are more susceptible than adults to the estrogenic effects of ZEA, as a result of higher internal exposures due to metabolic and physiological immaturity (Warth *et al.* 2016; Zinedine *et al.*, 2007; Kowalska *et al.* 2016; Degen *et al.* 2017). Degen *et al.* (2017) calculated exposure for a single dose or continuous daily intake of some mycotoxins, including ZEA, and reported that it can exceed the age-adjusted TDI values for infants; therefore, the safety of exclusively breastfed infants should be considered when setting the TDI.

In this study, human milk DON levels were found to vary between 400 and 14997 ng/L, with the median DON levels in infants who were exclusively breastfed being 3924 ng/L; further, the calculated median TDI for DON was 726 ng/kg bw (range, 74–2774 ng/kg). In this study, the calculated median TDI for breastfed newborns was 0.75 μg/kg bw (range, 0.24–2.77 μg/kg), which is lower than the previously set TDI of 1 μg/kg bw

(Degen *et al.* 2017). However, in the present study, 36% of the breastfed infants had TDI >1 μg/kg bw. In contrast, Tonon *et al.* (2018) evaluated human milk DON levels (as well as the aflatoxin M1 and ochratoxin A levels) among 86 nursing mothers, but did not detect DON in any of the samples (even though the LC-MS/MS findings were indicative of DON in four samples). In addition, aflatoxin M1 and ochratoxin A were also not detected in the samples (Rubert *et al.* 2014). However, we previously found higher ochratoxin A levels in human milk samples in our study population than in other studies in Turkey (Warth *et al.* 2016; Dinleyici *et al.* 2018). The difference between our studies and other published ones might be related to geographical and diet-related exposure differences. A previous study that evaluated the urine DON levels among newborns reported that there was no significant difference in the mean urinary DON level across the different weaning categories; however, the authors concluded that most of the children with detectable levels of DON were either partially breastfed or fully weaned (EFSA J 2013). Chronic administration of DON in animals causes weight loss, anorexia, decreased nutritional efficiency, immune disorders such as immunosuppression and immunostimulation (depending of the dosage and exposure frequency), and increased susceptibility to facultative pathogens (Raiola *et al.* 2015; Sobrova *et al.* 2010). DON was found to be a frequent contaminant in a large number of samples of cereal grains such as wheat, maize, oats, barley, rye, and rice, and in some processed food products such as wheat flour, bread, breakfast cereals, noodles, baby and infant foods and cooked pancakes (EFSA J 2013).

In this study, human milk DON levels were positively correlated with maternal meat consumption. Mycotoxins can appear in the food chain because crops might be consumed by livestock. According to the metabolism of the individual, the ingested mycotoxins could accumulate in different organs or tissues, and eventually enter the food chain through meat, milk or eggs (Alshannaq and Yu, 2017; Marin *et al.* 2013; Raiola *et al.* 2015; Richard 2007). Thus, the DON contamination levels in meat products are far lower than those in cereal-based food and feeds, but they may still be cause for concern. In Turkey, DON levels have been evaluated in dairy cattle, beef cattle, and lamb–calf feeds with ELISA, and the DON levels in feed samples were found to be lower than the legal limits in Turkey (Kocasari *et al.* 2013). However, in this study, we did not analyze food consumed by the mothers including meat; therefore, it is difficult to directly correlate meat consumption with human milk DON levels based on the present findings. Further studies should be conducted in the future to understand this correlation better.

DON is probably the best known and most common contaminant in food and feed; it is found in more than 90% of food samples and is a potential marker for other mycotoxins. DON may also coexist with ZEA, which is produced by fungi of the same genus. In support of this, human milk DON levels were positively correlated with human milk ZEA levels in our study, despite the lower ZEA levels and higher DON levels observed.

We employed enzyme-linked immunosorbent assay (ELISA) for analysis, as it is the most commonly used assay for mycotoxin determination. Thin-layer chromatography is another effective method for assessing DON. ELISA provides rapid screening, and many kits are commercially available for the detection and quantification of all major mycotoxins including ZEA and DON. This technique provides is rapid, specific, and relatively easy to use for the analysis of mycotoxins in food. However, ELISA has certain disadvantages including potential cross-reactivity and dependence on a specific matrix (Alshannaq and Yu, 2017).

In conclusion, the presence and higher levels of measurable ZEA and DON in nursing Turkish mothers indicate that they are exposed to mycotoxins as a result of their dietary habits. Very little is known about the effects of ZEA and DON on childhood growth and development, but children are believed to be particularly vulnerable, since their rate of food consumption is higher than that in adults. Such data will improve risk communication and will be informative to policy makers. Such research activities will also help to provide support to and protect those who are more susceptible to the negative effects of mycotoxins and other contaminants. These toxins have long-term side effects; however, it is not recommended that mothers stop breastfeeding their infants, as the advantages of breastfeeding are clear. Instead, comprehensive programs should be developed to regularly investigate and control these toxins in both human and animal food chains so that the amount of toxins can be reduced and their side effects can be prevented. Further regulations/recommendations are needed with regard to the dietary habits of nursing mothers so as to reduce the risk of maternal exposure to mycotoxins. More studies are also needed to provide new dietary recommendations for women during the pregnancy and lactation period.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

FUNDING

This study has been funded by Eskisehir Osmangazi University Research Grant.

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