1The Egyptian Journal of Hospital Medicine (October 2021) Vol. 85 (1), Page 2725-2729

Evaluation of Ochratoxins in Lactating Mothers and its Transfer to Their Exclusively Breast-Fed Infants through Breast milk

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ABSTRACT

Background: Ochratoxin A (OTA) is a well-known widely-spread mycotoxin all over the world that constitutes a real human threat. Its presence in human milk has previously been reported in different countries.

Objective: This study aimed to detect the presence of OTA in both mothers' milk, sera, and infants' sera and compare the results with a previous study done in Egypt.

Patients and Methods: Forty-eight healthy breast-lactating mothers and their infants who were exclusively breast-fed for at least 4 months were included. All of them were subjected to a thorough laboratory evaluation including determination of OTA concentration by (ELISA) Enzyme-Linked Immunosorbent Assay. **Results:** Fifteen mothers (31.3%) and their infants had been contaminated with OTA. The analysis showed that all infants of affected mothers had OTA in their sera. **Conclusion:** Multivariate logistic regression analysis showed that there was a significant correlation between OTA levels in mothers' sera, milk, and their infants' sera.

Keywords: Human milk, Kidney functions, Lactation, Ochratoxin A- urinary NGAl.

INTRODUCTION

Ochratoxin A (OTA) is a well-known widely-spread mycotoxin all over the world[(1)]. Ochratoxins (A, B, and C) are secondary metabolites of Penicillium and Aspergillus microfungi of which the most hazardous type is Ochratoxin A that causes harmful effects in humans and animals[(2)]. OTA was first found in the Balkan region; however, it can be detected practically in all territories, it is accumulated in animal feed and human food due to the favorable weather conditions and microclimate, and/or to improper storage of food components[(2)].

Ochratoxin A occurs in wheat, fruits, oilseeds, and animal feed resulting in its presence in milk, meat, and even in eggs[(1)]. Therefore, many drinks (e.g., wine, beer, coffee, tea, milk, etc.) **Bellver Soto, *et al.*[(3)] and Flores-Flores *et al.*[(4)])**, as well as common meals (bakery, meat, and dairy products) **El Khoury *et al.*[(5)]** contain more or fewer amounts of OTA. Furthermore, recent studies also highlighted its presence in herbal medicines **Shim *et al.*[(6)]** food coloring agents, spices **Ostry *et al.*[(7)],** and even in bottled water **Mat *et al.*[(8)]**. The wide occurrence of OTA and its high thermal stability makes the eradication of OTA from the food chain very difficult.

Excretion:

1. Renal Excretion: In vivo studies verify that the toxin can be reabsorbed from practically any part of the nephron both by active transport and by passive diffusion in a pH-dependent fashion[(9)].

Excretion of OTA is primarily done through tubular secretion. The tubular reabsorption of the toxin might be considered to be partially responsible for the intracellular accumulation of OTA[(1)].

2. Fecal Excretion and Entero-Hepatic Circulation.

3. Excretion through Breast Milk: There is a direct relationship between the ingestion of OTA and its concentration in the milk[(10)]. In a human study, it was observed that the highest OTA level was found in breast milk during the first few days after delivery[(11)]. Its presence may be associated with the chronic tubulo-interstitial kidney disease called Balkan Endemic Nephropathy (BEN) [(12)].

BEN is a chronic progressive disease with a period of 6–10 years leading to irreversible renal failure. Due to its high heat stability, complete removal of OTA from food is practically impossible although several approaches exist for reducing OTA contamination[(13)]. After the absorption of OTA from the gastrointestinal tract, it binds primarily to albumin with high affinity, which results in its very long half-life (from a few days to one month, depending on species).

Although glomerular filtration of OTA is strongly limited due to its albumin binding, the small filtrated and secreted fraction is partially reabsorbed, which might help the accumulation of the toxin in the kidney tubule cells. OTA toxicity is strongly correlated with the occurrence of BEN **Castegnaro *et al.*[(9)]**; however, its mechanism of action is very complex **Hadjeba-Medjdoub *et al.*[(14)]**. It is thought to be carcinogenic, teratogenic, hepatotoxic, neurotoxic, and immunotoxic, based on in vitro and on animal studies[(5)].

IARC (International Agency for Research on Cancer) categorizes OTA as a member of the 2B subgroup which means that, based on animal studies, OTA is a potential human carcinogen[(15)]. The NCI/NTP (National Cancer Institute/National Toxicological

Received: 8 /5 /2021
Accepted: 4 /7 /2021

Program) renders OTA to be the most potent renal carcinogen in rodents ever studied[2].

The incidence of upper urinary tract tumors in endemic regions of Bulgaria is 90-fold higher compared to that of non-endemic regions[2].

In addition to BEN, some studies emphasize the role of OTA in the development of Tunisian Nephropathy **Hassen *et al.*[16]** gastric and esophageal tumors in some regions of China **Cui *et al.*[17]** as well as testicular cancer[18].

This study aimed to investigate the presence of chratoxin A in the serum and milk of lactating mothers and its affection on the renal function of their exclusive breastfed infants.

SUBJECTS AND METHODS

This cross-sectional study has been performed at the outpatient clinic of pediatrics of Zagazig University Hospital during the period from January 2020 to November 2020.

The study was done on 48 lactating mothers and their infants with the following inclusion and exclusion criteria.

Inclusion criteria:

1-Infant age <6 months
2- Infants with exclusive breast feeding.
3- Apparently healthy mothers and infants.
4-Both sex was involved.

Exclusion criteria:

1-Refusal to participate in the study
2- Infants who were not exclusively breast fed
3-Those diagnosed with renal diseases.
4-Infant age >6 months

All subjects included in our study were subjected to a thorough evaluation including clinical assessment and laboratory investigations with special emphasis on liver function tests and kidney function tests (serum urea and creatinine). Both mothers' and infants' kidneys were examined by ultrasound. Then Serum ochratoxin level for mothers and infants and milk ochratoxin level for mothers were done in addition to Urinary NGAL for infants.

Ethical Considerations:

An approval of the study was obtained from Zagazig University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Specimen collection and storage:

Serum and milk: Three ml of venous blood by vein puncture were collected under complete aseptic condition from every subject then put in a sterile, clean separator gel tube for serum isolation and left to clot. Centrifugation was done for 20-min at the speed of 2000-3000 r.p.m. and the supernatant was removed and kept in the refrigerator at (-4^{o}C) till analysis. If precipitation appeared, the sample is centrifuged again. The same was done for milk samples.

Measurement of Ochratoxin A:

Ochratoxin A was measured in serum samples and milk samples by ELISA. Kit was provided from SunRed biotechnology company (China) Catalogue No. 201-13-00895 named Ochratoxin A (OTA) ELISA Kit.

Test principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Ochratoxin Ain serum samples. Ochratoxin Ais added to monoclonal antibody Enzyme well which is pre-coated with Ochratoxin A monoclonal antibody, incubation; then Ochratoxin A antibodies labeled with biotin, and combined with Streptavidin-HRP are added to form an immune complex; then incubation and washing again to remove the uncombined enzyme are done. Then Chromogen Solution A, B is added, the color of the liquid changes into blue, and at the effect of acid, the color finally became yellow. The chroma of color and the concentration of the plant substance Ochratoxin A of the sample were positively correlated.

Statistical Analysis

Data collected throughout history, basic clinical examination, laboratory investigations, and outcome measures coded, entered, and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis.

According to the type of data qualitative were represented as number and percentage, quantitative data were represented by mean±SD, Differences between quantitative independent groups by t-test, correlation by Pearson's correlation. P-value was set at <0.05 for significant results &<0.001 for a highly significant result.

RESULTS

The clinical demographic characteristics and laboratory parameters of both mothers and their infants at the study entry are summarized in Table 1. All infants of mothers who were proven to have OTA in their serum and milk, have OTA in their serum.

Table (1): Demographic and laboratory characteristics of mothers and their infants. All quantitative variables are presented as mean ± SD and qualitative variables as the total sum of cases .

Mother age (years)	**Mean± SD**	25.37±3.58	
	Median (Range)	25.0 (20-32)	
Baby age (month)	**Mean± SD**	3.97±1.34	
	Median (Range)	4.0 (2-6)	
		N	**%**
Residence	**Urban**	21	43.8
	Rural	27	56.3
	Total	48	100.0
Baby Sex	**Male**	22	45.8
	Female	26	54.2
	Total	48	100.0
mothers	**ALT**	**Mean± SD**	32.11±.09
	AST	**Mean± SD**	35.70±3.47
	S. urea	**Mean± SD**	32.88±3.98
	S. Cr	**Mean± SD**	0.80±0.13
Babies	**ALT**	**Mean± SD**	22.13±1.92
	AST	**Mean± SD**	30.10±2.48
	S. urea	**Mean± SD**	15.79±2.17
	S. Cr	**Mean± SD**	0.38±0.04
S. Ochratoxin mothers		**Mean± SD**	13.71±5.58
		Median (Range)	10.84 (-VE-31)
Milk Ochratoxin mothers		**Mean± SD**	2.45±0.93
		Median (Range)	1.43 (-VE-6)
S. Ochratoxin baby		**Mean± SD**	10.07±4.23
		Median (Range)	7.55 (-VE-26)

Table (2): Correlations between Ochratoxins level between baby and mothers

		S Ochratoxins baby
Milk Ochratoxins	**r**	0.889**
	P	0.001
S Ochratoxins	**r**	0.864**
	P	0.001

There is a significant positive correlation between mothers' and babies' levels of ochratoxins.

Table (3): Comparison between Urban and Rural regarding Ochratoxins in mothers and infants.

	Urban	**Rural**	**t**	**P**
S Ochratoxins	4.91±1.74	18.30±6.21	4.321	0.001**
Milk Ochratoxins	0.52±0.19	3.46±1.36	4.440	0.001**
S Ochratoxins baby	3.70±1.28	13.39±4.68	3.452	0.002*

Table (4): High Ochratoxins percentage according to cutoffs distribution

		N	**%**
S. Ochratoxins mother	**<15.5**	33	68.8
	>15.5	15	31.3
Milk Ochratoxins	**<2.24**	33	68.8
	>2.24	15	31.3
Baby Ochratoxins	**<12.7**	36	75.0
	>12.7	12	25.0
	Total	48	100.0

31.3% of serum Ochratoxins in mothers were high and the same percentage in milk and babies 25.0% were high regarding cutoff.

DISCUSSION

Various fungi are widespread contaminants of Egyptian cereals especially Ochratoxin A. The positive Egyptian food samples for OTA belonged to white maize, wheat, wheat bran, beans, rice germ, rice germ cake, broilers feed egg production feed, and milk production feed[19]. The contamination of foods with Ochratoxins is a significant problem for the adverse effects on humans, animals, and crops that result in illnesses and economic losses. It has been extensively found in food items like grains, bread, nuts, spices, coffee, beer wine, grapes, and with high levels in animal feedstuff [19]**.** This may explain the high contamination rate among our studied individuals.

The levels of ochratoxin among rural groups were found to be higher than those among urban groups as in table number (3). This is in agreement with **Hassan *et al.*[20].** It was reported that a higher Ochratoxin exposure was evident in rural areas, on the assumption that they produced and stored their cereals in unhygienic conditions[21]**.** One important factor is due to the climate difference between the studied zones and also the deficient procedures of conservation of cereals and other food items[19]**.**

The presence of OTA in human milk samples from different countries has been previously reported. OTA was found in 38 (33%) of 115 human milk samples in different regions in Norway, causing a daily intake of OTA from human milk exceeding the suggested tolerable dose[22]**.**

Also, it was reported that infants in Sierra Leone are exposed to OTA at levels that, in some cases, far exceed those permissible in animal feed in developed countries[10]**.** Another study determined the concentration of OTA in the breast milk of donor mothers in Italy. Out of 111 samples, 22 were contaminated in a range of 0.1–12 g/kg[23]**.** In Hassan et al study in Egypt in 2006, the milk level of OTA was 1.89±0.98. In our study, the levels of ochratoxin in the milk of the studied lactating mothers were 2.45±0.93 ng/ml.

Our observation detected that Ochratoxin is secreted in the milk of lactating mothers which depend on their levels in the serum of mothers and transferred to their exclusive breast fed infants in agreement with **Hassan *et al.*[20]** and **Muñoz *et al.*[13]** who claimed the transfer of ochratoxin from affected mothers to their infants through their breast milk.

Using the cutoff level of urinary NGAL above which renal impairment might occur (140 ng/ml) as reported by **Aghel *et al.* [24]**, the affected percentage of mothers was **(31.3%)** and babies percentage was **25%** of total studied babies as shown in table number (4).

No published data is supporting our observation. In comparison with the previous study done by **Hassan *et al.* [20]** in Egypt, Our study revealed a lower rate for mothers' milk contamination with OTA **(31.3%)** than did the previous reports, and so infants are exposed to a lower contamination risk than before.

This means much more improvement in the methods of storage of different seeds including wheat. This improvement is due to the role of our country and the increased awareness of public health. Despite the public gap between our country and other countries so much more effort is recommended in the ways of prevention of Ochratoxin-A spread and exposure.

In conclusion, OTA is secreted in mothers' milk and may produce hazardous effects on their infants so much more effort is needed and much more studies to detect its effect on babies.

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