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DETECTION OF OCHRATOXIN IN EXCLUSIVELY BREAST FED INFANTS AND THEIR MOTHERS AND ITS EFFECT ON THEM USING URINARY NGAL

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ABSTRACT

Background: Ochratoxin A (OTA) is one of the most important mycotoxins with nephrotoxic, carcinogenic, teratogenic, genotoxic, and immunotoxic properties. Thus, we carried out this study to determine the concentration of ochratoxin A in human breast milkin different countries. Thisstudy aimed to detect the presence of OTA in both mother's milk and infant's serausingOTA then evaluating its effect on the renal function of infants using urinary neutrophil gelatinase-associated lipocalin (NGAL). **Patients and methods:** A cross sectional study includedfourty 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 and NGAL concentration byEnzyme-Linked Immunosorbent Assay (ELISA).According to standards' concentration and the corresponding absorbance, we calculated out the standard curve linear regression equation.**Results:**Urine NGAL was distributed as 169.71±124.33 with minimum 20 and maximum 411 and high NGAL and affected baby were 25%.Urine NGAL was significantly positive correlated with baby serum Ochratoxins. Urine NGAL was significantly positive correlated with mother milk Ochratoxins level. Urine NGAL was significantly positive correlated withmother serumOchratoxins level**.** About 31.3% of serum Ochratoxins in mothers were high and the same percentage in milk and in babies 25.0% were high regarding cutoff**.**Significant AUC and cutoff as >15.5, >2.24 and >12.7 respectively among mothers S Ochratoxins, Milk Ochratoxins and S Ochratoxins baby with sensitivity 78.0%, 75.0% and 76.5% respectively and specificity 74.0%, 73.3% and 82.0% respectively. **Conclusion:** Concentration of NGAL in infants may aid in early detection of renal impairment. Despite advances in diagnostics and treatment, renal impairment is still a major medical problem with high mortality. Therefore, novel biomarkers for early detection of renal affection should be used.

Keywords: Ochratoxin A; Urinary NGAl;AKI; mother milk

INTRODUCTION

The presence of OTA in human milk was previously reported from several countries.ochratoxin A (OTA) levels in food have shown the presence of local pockets of higher concentration in some countries, especially Tunisia Canada, Bulgaria, Australia, Germany and Egypt **(1)**.

A potential biomarkers that are measurable in urine or plasma of patients with acute kidney injury AKI have been including neutrophil gelatinase-associated lipocalin (NGAL)**(2)**.NGAL is filtered by the glomerulus and is reabsorbed by the proximal tubules by a megalin-dependent pathway **(3)**. A decrease in tubular reabsorption after AKI may lead to a further increase in urine NGAL concentration. NGAL elevation is detectable as early as 3 h after tubular injury and peaks approximately 6 to 12 hr after injury, depending on the severity of the injury **(4)**.

NGAL provides antiapoptotic effects and enhances proliferation of renal tubular cells, which establishes its potential pathways in kidney protection during AKI. Although NGAL is represented in some human tissues, it is one of the most upregulated transcripts in the kidney early after ischemic, septic, or toxic AKI in animal models and human neonates, children, and adults, implying its role as an early marker of structural renal tubular damage**(5)**. There is also substantial evidence that increased urine NGAL can differentiate intrinsic renal damage from hemodynamic alterations due to volume depletion**(6)**.

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The aim of this study to detect the presence of OTA in both mother's milk and infant's serausing OTA then evaluating its effect on the renal function of infants using urinary neutrophil gelatinase-associated lipocalin (NGAL)..

PATIENTS AND METHODS:

This cross sectional study has been performed at outpatient clinic of pediatrics of Zagazig University Hospital during the period.The study was done on 48 lactating mothers and their infants.Ethical permission for the study was obtained from the parents who were fully informed about all study procedures and their consents were obtained prior to the children enrollment in the study. Our study was approved by the ethical committee of the faculty of Medicine, Zagazig University Hospitals.

Inclusion criteria:

Infants with exclusive breast feeding in age<6 months .Apparently healthy mothers and infants. Both sex were involved.

Exclusion criteria:

Refusal to participate in the study, infants who were not exclusively breast fedthose diagnosed with renal diseases and age >6 months

All subjects were subjected full history, clinical assesment and laboratory investigations with special emphasis on kidney function tests.Both mothers and infants kidneys were examined by ultrasound. Serum ochratoxin level for mothers and infants and milk ochratoxin level for mothers were done in addition to Urinary NGAL for infants.

Investigations:

Serum and milk Samples: 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 done for 20-min at the speed of 2000-3000 r.p.m. andsupernatant removed and kept in refrigerator at (-4^{o})C till analysis. If precipitation appeared, sample is centrifugedagain. The same ws done for milk samples.

Urine samples: three ml of urine were collected using a sterile container, Centrifugation done for 20-min at the speed of 2000 -3000 r.p.m. and supernatant removed and kept in refrigerator at (-4^{o})C till analysis. If precipitation appeared, sample is centrifuged again.

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.The chroma of color and the concentration of the plant substance Ochratoxin A of sample were positively correlated.

Measurement of human NGAL:

Human NGALwas measured in urine samples by ELISA. Kit was provided from SunRed biotechnology company (China) Catalogue No. 201-12-1720 named human (NGAL)ELISA Kit.Thechroma of color and the concentration of the plant substance NGAL of 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 20.0software for analysis. 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 high significant result.A receiver operating characteristic (ROC), or simply ROC curve performed .

Results

The present study showed urine NGAL was distributed as 169.71±124.33 with minimum 20 and maximum 411 and high NGAL and affected baby were 25%(**Table 1**).Urine NGAL was significantly positive correlated with baby serum Ochratoxins(**Figure 1**). Urine NGAL was significantly positive correlated with mother milk Ochratoxins level (**Figure 2**). Urine NGAL was significantly positive correlated withmother serumOchratoxins level(**Figure 3).**

About 31.3% of serum Ochratoxins in mothers were high and the same percentage in milk and in babies 25.0% were high regarding cutoff(**Figure 4).**Significant AUC and cutoff as >15.5, >2.24 and

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>12.7 respectively among mothers S Ochratoxins, Milk Ochratoxins and S Ochratoxins baby with sensitivity 78.0%, 75.0% and 76.5% respectively and specificity 74.0%, 73.3% and 82.0% respectively**(Table 2)**.

Table (1): NGAL level distribution among babies

		Mean ±SD	
Urine NGAL		169.71±124.33	
		129.05 (20-411)	
		N	**%**
NGAL*	**Normal <140**	36	75.0
	High >140	12	25.0
	Total	48	100.0

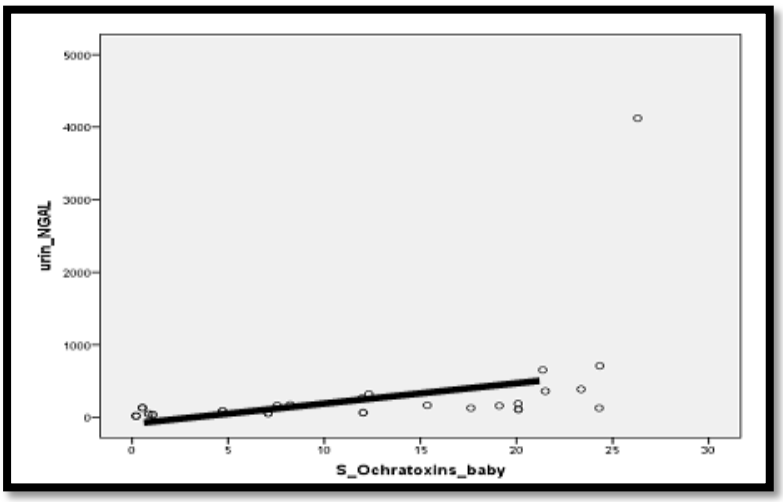

Figure (1):Correlation between urine NGAL and serum Ochratoxin of babies.

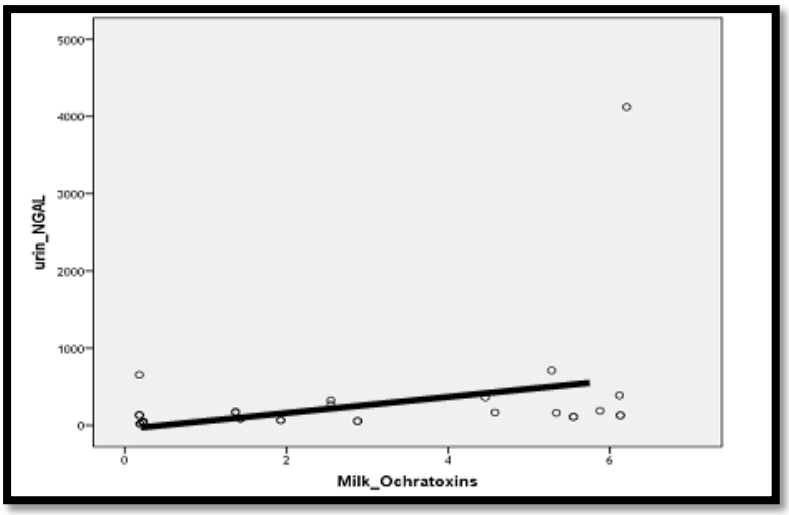

Figure (2):Correlation between babies urinary NGAL and milk Ochratoxin of mothers.

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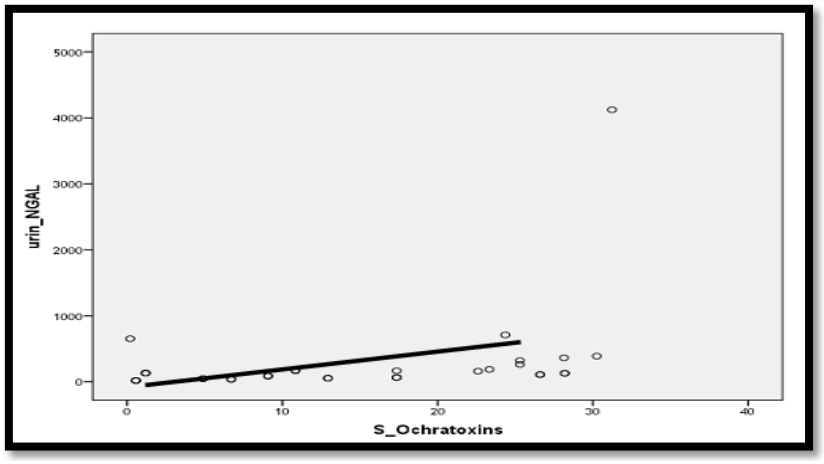

Figure (3): Correlation between babies urinary NGAL and serum Ochratoxin of mothers.

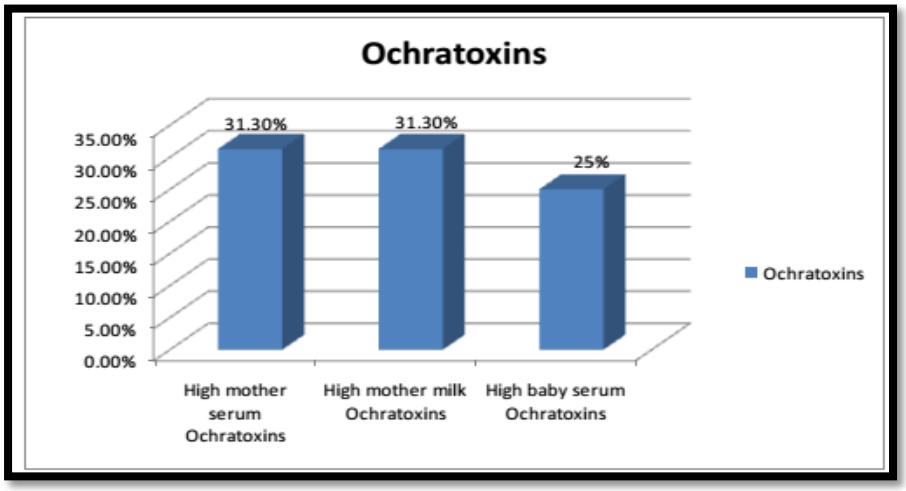

Figure (4): High Ochratoxins percentage according to cutoffs among serum and milk of mothers and serum of babies.

Table (2): Area under curve, cutoff and validity

Test Result Variable(s)	AUC	Cutoff	P	95% Confidence Interval		Sensitivity	Specificity
				Lower Bound	Upper Bound		
S Ochratoxins mother	0.761	>15.5	0.012*	0.580	0.942	78.0%	74.0%
Milk Ochratoxins	0.726	>2.24	0.030*	0.545	0.908	75.0%	73.3%
S Ochratoxins baby	0.873	>12.7	0.000*	0.759	0.987	76.5%	82.0%

Discussion

Ochratoxins (OTA) 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 **(7)**. Human exposed to OTA directly by the consumption of contaminated cereal food products and their derivatives (plant sources) as well as derived from meat (animal sources) consume the OTA contaminated feed **(8).**

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It was hypothesized that OTA-induced extracellular matrix deposition by proximal tubular cells may be of importance in generation of renal diseases in humans **(9).** Another finding suggested a link between OTA and the outcome of karyomegalic nephropathy **(10).**

NGAL is the most extensively investigated AKI biomarker. Many investigators have examined various patient populations and determined the performance of NGAL. Its performance has been evaluated in various settings **(11)**.

Our study was conducted on patients attending Pediatric outpatient Clinic of Zagazig University Hospitals from January 2020 to November 2020. Apparently healthy control children were taken from outpatient clinic. Full examination with detailed history was done and laboratory work was conducted in Clinical Pathology Department, ZagazigUniversity.This aim of the current study to detect the presence of OTA in both mother's milk and infant's serausing urinary NGAL.

In our study, the new marker (NGAL) was used for early detection of renal injury, the levels were high (169.71±124.33).Using the cutoff level of urinary NGAL above which renal impairment might occur(140 ng/ml) as reported by **Aghel et al.(12)**, the affected percentage of babies was 25% of total studied babies (level of urinary NGAL>140 ng/ml) as shown in table number (2).There is no published data supporting our observation.

Our study observed that there is a positive correlation between urinary NGAL and serum Ochratoxin of infants where the levels of OTA above the cutoff point were detected in the serum of mothers in 15 out of 48 mothers(31.3%). In addition, the high levels of OTA in breast milk were detected in 15 out of 48 samples(31.3%). While the high levels (above cutoff point) of OTA in the serum of babies were detected in 12 out of 48 samples (25%).This explains that there is accumulation of exposure to ochratoxins not only during the intra uterine period but also during the breast feading period.Ochratoxins levels in babies serum and mothers serum and milk were significantly higher among affected (high urinary NGAL) babies group than non affected babies (low urinary NGAL).

In comparison with the previous study done by **Hassan et al (13)**at 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.

In our study,significant AUC and cutoff as >15.5, >2.24 and >12.7 respectively among mothers S Ochratoxins, Milk Ochratoxins and S Ochratoxins baby with sensitivity 78.0%, 75.0% and 76.5% respectively and specificity 74.0%, 73.3% and 82.0% respectively.

In a study of **Ho et al. (14)** including 16 studies with a total of 2906 patients investigated urine NGAL as a biomarker for the prediction of AKI after cardiac surgery in adult patients. The composite area under the receiver operating characteristic curve (AUC) of urine NGAL was 0.72.

Also, **Chen et al.(15)**reported their findings of a prospective study investigating the potential use of urinary AGT in combination with 3 AKI biomarkers, including NGAL, IL-18, and KIM-1, in the prediction of AKI progression. patients with the highest values of urine AGT had a 10.8fold greater risk of AKI progression compared with those with the lowest urine AGT values. In addition, urine AGT outperformed the other 3 biomarkers, with an AUC of 0.78 for AKI progression and 0.85 for AKI progression with death.

Determination of appropriate cutoff values in different settings appears to be the next important step in the clinical validation of NGAL testing **(16)**.

The lower rate for mothers' milk contamination with OTA in our study compared with the previous study done at Egypt means much more improvement in the methods of storage of different seeds including the wheat. This improvement is due to the role of our country and the increased awareness of the puplic health. Inspite of 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.

Conclusion:

The concentration of NGAL in infants may aid in early detection of renal impairment .Our results suggest that Ochratoxin A may be involved in the pathogenesis of renal impairment. Despite advances in diagnostics and treatment, renal impairment is still a major medical problem with high mortality. Therefore, novel biomarkers for early detection of renal affection should be used.

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