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ORIGINAL ARTICLE

Assessment of Ochratoxin A among Healthy Individuals in Baghdad, Iraq

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ABSTRACT

Background*: Mycotoxins have a significant impact on population health worldwide. Ochratoxin is a common mycotoxin that can be detected in the serum of healthy people due to its prevalence in food. Researches on ochratoxin and its metabolites in biological fluids can help us better understand the toxin's biological impacts.* ***Objective****: The aim of this study was to investigate whether individuals in Baghdad, Iraq, were exposed to ochratoxin A.* ***Methodology****: Serum samples were obtained from 90 healthy persons (ages 14–40 years) and evaluated for ochratoxin A using the human Ochratoxin A ELISA Kit, an accurate, quantitative, and sensitive technique (limit of detection 0.34 ng/ml).* ***Results****: We detected Ochratoxin A in all serum samples, with a mean concentration of 10.447 ng/ml (ranging from 2.66 to 71.447 ng/ml). The most prevalent concentration of ochratoxin A was between 10.1 and 20 ng/ml. The correlation between OTA concentration level and age groups was significant at the 0.01 level. The study did not show a significant difference in the concentration of ochratoxin between males and females.* ***Conclusion****: This study provides useful information about human exposure to OTA in Baghdad. Further research in youngsters, elderly, and adult cohorts from various regions is recommended.*

INTRODUCTION

Two important genera of filamentous fungi, *Penicillium* and *Aspergillus* spp., can produce ochratoxin, a secondary toxic metabolite that is one of the most important mycotoxins and contaminates food worldwide[1]. This substance disrupts biological processes such as protein creation, lipid peroxidation, calcium metabolism, mitochondrial respiration, and sugar metabolism, leading to ochratoxicosis in humans and animals[2, 3]. OTA is comprehensively (more than 99.98% in humans) binding to albumin and other proteins in serum, which increases the half-life of elimination to more than 35 days[4]. Ochratoxin has been found to be teratogenic, immunotoxic, nephrotoxic, and hepatotoxic in numerous animal species[5]. The ochratoxin family includes over 20 metabolites, with ochratoxin A being the most prevalent and dangerous molecule [6]. The toxin's genotoxicity has long been disputed. Co-exposure to ochratoxin with fumonisin B1 led to enhanced genotoxic damage and elevated biomarkers linked to cell death and cytostasis[7].

The chlorine substituent is a prominent component of the OTA structure, and it appears to be significant for OTA toxicity. Other correlated mycotoxins produced at low levels by OTA-producing fungus are methyl esters of Ochratoxin A and B. Ochratoxin B (OTB) is the non-chlorinated form, whereas OTC is the ethyl ester of OTA.

Contaminated soils at harvest levels can lead to mycotoxin contamination in food grains, including pods and seeds. High temperatures and humidity can promote fungus growth and form mycotoxin during storage. Other contamination parameters involve pH, fungus isolate, and substrate form [8, 9].

Insect infestation and pod breakage during handling can lead to the colonization of toxic fungi like Aspergillus and the formation of toxic chemicals[10]. Mycotoxin detection using human biological monitoring (HBM) is crucial due to their occurrence in food, skin, and mold/spores[11]. OTA has been linked to Danubian endemic family nephropathy, which is common in areas with high OTA concentrations in grains and cereals[12]. Mycotoxins have a significant impact on population health worldwide. The current study aimed to determine OTA values in the Baghdad population and assess how age and gender affect these levels.

METHODOLOGY

Serum Samples

Nineteen blood samples from healthy individuals living in the Baghdad district were collected in disposable vials. Blood allows coagulating for 10 to 20 minutes and is then centrifuged at 2000–3000 RPM for 20 minutes. The serum was collected and kept at 2-8 °C to be utilized within 5 days. Serum samples are kept at -20°C if utilized within one month or -80°C for six months.

Ethics Statement:

The ethical committee of the College of Science Research accepted this study (CSEC/0424/0039) on April 22, 2024. All participants provided informed consent and volunteered to submit blood samples during recruitment. This research does not involve any clinical trials

Analytical Methods

The human Ochratoxin A ELISA Kit (Bioassay Technology Laboratory, China) was used to detect and quantify ochratoxin A in serum samples. The ELISA technique is frequently used for mycotoxin screening and has been validated for many biological matrices[13]. The sandwich kit accurately detects the human ochratoxin A (OTA) in plasma, serum, cell culture supernatant, tissue homogenates, ascites, and other biological specimens. To create duplicate standard points, the stock solution of the standard (64 ng/ml) diluted to 1:2 with the diluent of the standard to yield 32 ng/ml, 16 ng/ml, 8 ng/ml, and 4ng/ml. The samples and ELISA reagents are poured into wells and incubated at 37°C for one hour, and the plate is washed five times. Substrate solutions (A and B) combine and incubate at 37°C for 10 minutes. The stop solution is added, and the color changes. Within 10 minutes after applying the stop solution, a microplate reader was used to measure the optical density (OD) using 450 nm. The readings and concentrations of OTA in the samples were determined using the standard curve's line equation.

Statistical Analysis

SPSS (version 22) was used for statistical analyses. To compare between two groups Pearson's chi-square test was conducted while for more than two groups Kruskal-Wallis test was used.

RESULTS

Calculating the regression value after making a standardized concentration level to create a standard curve and then draw a fit curve between the aspects on the graph. The measurement of linearity is meant to assess a method's capacity to generate an exponential reaction from assessments of standard concentration values; this is required for quantifiable analyses. Linearity is accomplished by solving a line equation for various concentrations of standard ochratoxin A values. The findings of linear regression analysis demonstrate that the coefficient of determination (R^2) exceeds 0.9, as shown in (Fig. 1).

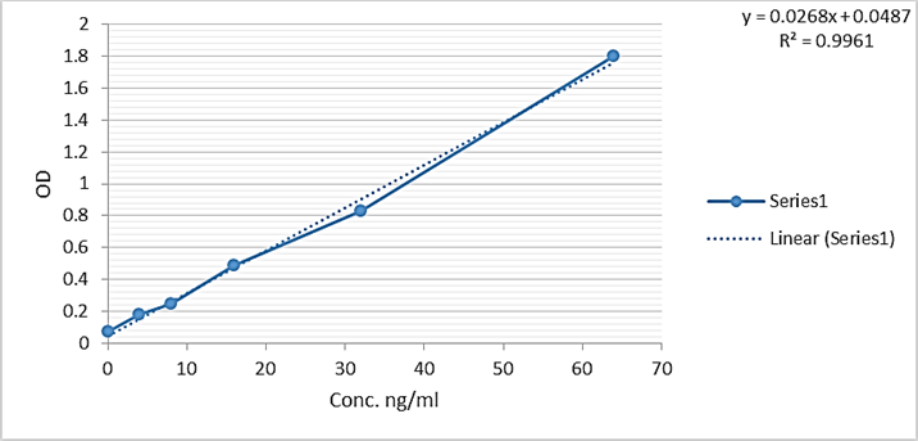

Fig. 1. The standard curve's line of OTA by ELISA

OTA was found in the serum of all participants and varied from 2.6 to 55.5 ng/ml after excluding the person with the peak value 71,914ng/ml, statistical analysis revealed that among the remaining 89 participants, the mean and median values were 17.9 and 15.75 ng/ml, respectively. The most prevalent concentration of OTA was between 10.1 - 20 ng/ml as shown in fig.2.

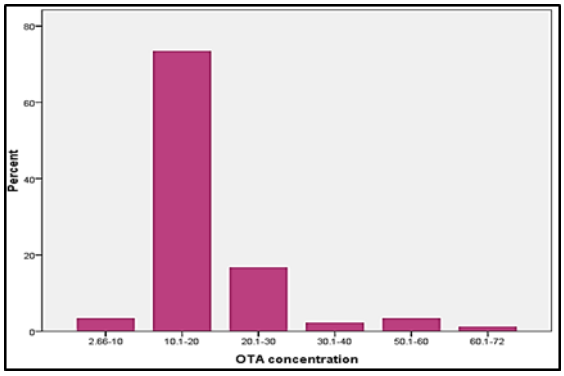

Fig. 2: The concentrations of OTA values among healthy individuals

Figure3 and 4 show the distribution of OTA values among age groups. The correlation between OTA concentration level and age groups was significant at the 0.01 level.

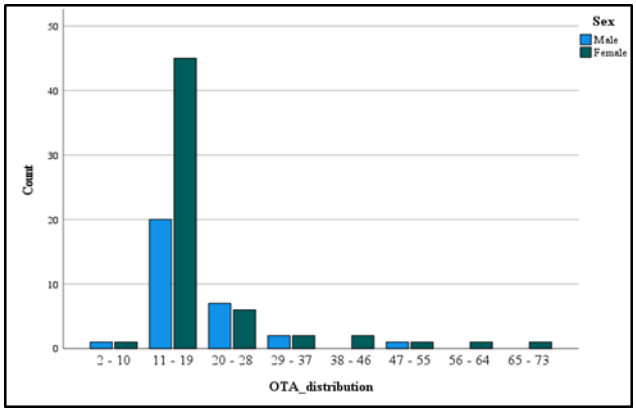

Fig. 3: The distribution of OTA values among age groups in relation to sex

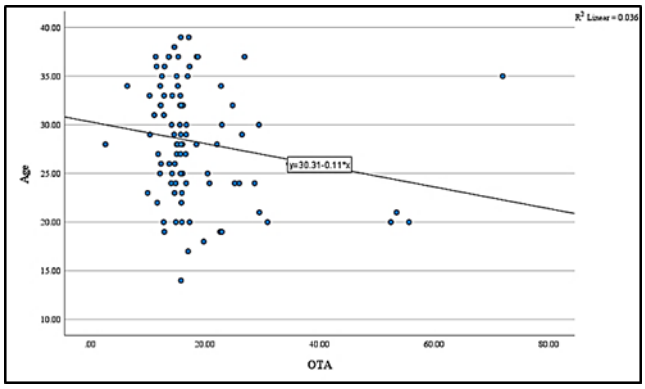

Fig. 4: Correlation between measurements of OTA serum levels in 90 subjects (Baghdad, Iraq). Linear regression function (R2 = 0.036)

DISCUSSION

ELISA is an immunochemical method constructed on a certain reaction between antigen and antibody with high sensitivity and specificity, employing enzymes as indicators. The main benefits of the ELISA technique are sensitivity, quick and easy implementation, relatively inexpensive, and efficiency. It is better to do a calibration test alongside the standard every time we do the test by calculating the regression value after making a standardized concentration level. The findings of linear regression analysis demonstrate that the coefficient of determination (R^2) exceeds 0.9, which is in line with other studies results[14, 15].

Age has little or no significant relationship with OTA levels in human blood, as it was mentioned in other studies[16]. Our study found OTA in all serum samples examined, indicating widespread contamination in the foods and beverages consumed by healthy persons in Baghdad. A global systematic review and meta-analysis conducted by Sharafi H. *et al.* [17] showed a widespread contamination of meat with ochratoxin in Iraq, with a rate of 77%, In addition to numerous other studies conducted on various food items consumed by individuals in Iraq [18, 19, 20].

OTA levels in human blood have been studied in multiple residents [21, 22, 23]. Consuming mycotoxin-contaminated foods can cause health effects such as diarrhea, reproductive abnormalities, cancer, growth impairment, and immunomodulation [24,25,26,27]. Ochratoxin A can have severe effects on human health, particularly in developing countries where social, agricultural, economic, and storage factors contribute to exposure[28, 29].

CONCLUSION

In summary, OTA is a common mycotoxin that can be detected in the serum of healthy people due to its prevalence in food. While studies have found detectable quantities in serum, the long-term health effects of low-level chronic exposure in otherwise healthy people are not well understood. Nephrotoxicity, carcinogenic risk, and immunological suppression are all major issues. Future research should look into the subclinical effects of low-dose exposure, long-term OTA buildup, and how genetic and environmental factors influence individual sensitivity to its harmful effects. Because of the potential health risks associated with OTA ingestion, researchers worldwide, as well as regulatory officials in many countries, have worked to measure OTA levels in foods. On the other hand, evaluating OTA levels in biological fluids provides an alternate method for determining the existence of this pollutant in foods and its risk to humans. Researches on OTA and its metabolites in biological fluids can help us better understand the toxin's biological impacts.

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Conflict of Interest
The authors have no financial interests or private relationships that could have prejudiced the work presented in this paper.

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