

Mycotoxins – Risk Factors for Human Health

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Abstract. Of the many types of fungi that produce mycotoxins, *Aspergillus* and *Penicillium* are the ones considered to be extremely harmful for human and animal health. The research followed the qualitative and quantitative analysis of OTA in 10 serum samples obtained from patients undergoing dialysis in a clinic in Bucharest. Mycotoxicological analysis was performed by immunoenzymatic method ELISA, using R-Biopharm RIDASCREEN Ochratoxin A 30/15 test kits. At the same time, at the examined patients, has also be conducted an investigation regarding nutrition. Was aimed obtaining information regarding the frequency of consumption of several types of food, some having a high risk of contamination with mycotoxins. Daily and weekly consumption of products with a high risk of contamination is high for cereals, spice mixtures of condiments, tea and coffee. Out of the analyzed serum samples, 60% of them were contaminated with OTA, with values ranging between 49.94 ppt and 128.00 ppt.

Keywords: ochratoxin A, human serum, types of food.

INTRODUCTION

Of the many types of fungi that produce mycotoxins, *Aspergillus* and *Penicillium* are the ones considered to be extremely harmful for human and animal health. Fungi are ubiquitous in the environment where they survive by increasing their food by paralising other living organisms by living saprophyte on plants or by paralising dead organisms. Besides primary metabolites (for ex. polysaccharide), the funguses also produce secondary metabolites-micotoxines, which are products developed to survive competition with other species of microorganisms, including other fungus.

The study of mycotoxins and especially their effects on human and animal health is still at the beginning, many of these substances are hazardous when inhaled, digested or come into contact with the skin.

Ochratoxin A (OTA) was first identified in 1965, as a metabolite of *Aspergillus ochraceus* [6] and *Penicillium viridicatum* species. Ochratoxin production is common in mouldy or hot feed and is favoured by the presence of trace elements, a temperature of 20-28 °C and a humidity of of 18-19% for wheat, in the case of *P. viridicatum* or 22% in corn. Therefore, these two foods that are found frequently in human food (bread, pasta, pastries, and porridge) are possible and consistent sources of mycotoxins. OTA has been identified by WHO as a potential carcinogen in humans while other research shows that may be responsible for a series of nephrosis and neoplasms in urinal system. At the same time, numerous studies have shown that Balkan Endemic

Nephropathy is a common disease found in a limited geographical area, represented by the following countries: Romania, Bulgaria, Yugoslavia, Croatia and Bosnia [2,3,4,5].

MATERIALS AND METHODS

The research followed the qualitative and quantitative analysis of OTA in 10 serum samples obtained from patients undergoing dialysis in a clinic in Bucharest. Mycotoxicological analysis was performed by immunoenzymatic method ELISA, using R-Biopharm RIDASCREEN Ochratoxin A 30/15 test kits (Diamedix company). In order to analyze blood mycotoxicological ochratoxin A the following work protocol was established (Photo 1): blood sample was processed to obtain serum; have been taken 2 ml serum and was added 2.5 ml HCl 1 N and 4 ml dichloromethane (DCM); was stirred for 5 minutes then centrifuged for 15 minutes at 3500 rpm; the aqueous top layer was removed and then were taken from filtration 2 ml over which were added 2 ml NaHCO₃ 0.13 M followed by homogenization for 5 minutes and centrifugation for 5 minutes; the superior layer was collected and left into another tube; over the filtrate was added 2 ml NaHCO₃ 0.13 M stirred for 5 minutes and then centrifuged for 5 minutes; the top layer of NaHCO₃ was taken and added over the previous layer of NaHCO₃; over the two layers were added 0.75 ml HCl and 2 ml DCM followed by a new stir and centrifugation; the top layer was removed and the DCM layer had evaporated at 60 degrees Celsius; then was added 1 ml NaHCO₃ 0.13 M; in the end had been taken in work 50 µl/well plates. Were analyzed 10 serum samples using the Dynex analyzer, belonging to GRAL Medical Clinic (Fig. 2).

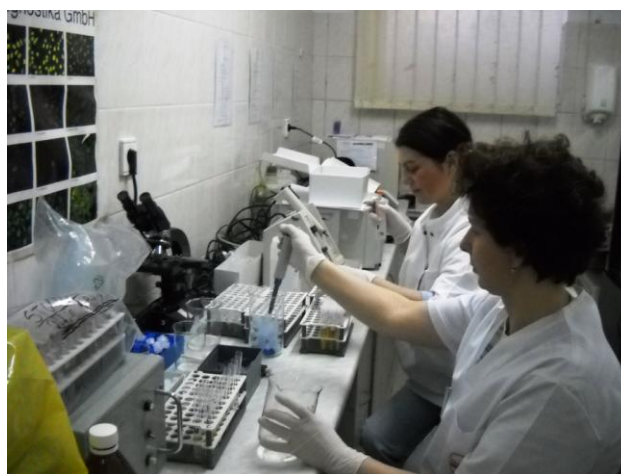


Fig 1. Preparation of samples for analysis



Fig. 2. Processing and analyzing samples with Dynex

At the same time, at the examined patients, has also be conducted an investigation regarding nutrition. Was aimed obtaining information regarding the frequency of consumption of several types of food, some having a high risk of contamination with mycotoxins. Were targeted five categories of food: grains (corn, flour, bread, breakfast cereals, biscuits, corn puffs), seeds, nuts (sunflower seeds, pumpkin seeds, peanuts, hazelnuts, walnuts, pistachios), spices (spice mixtures of condiments, mixed steak spice, paprika, cinnamon, vanilla), beverages (coffee, tea, beer, wine) and animal products (meat, milk and cheese).

For all the above food was monitored the frequency of consumption (daily, weekly, monthly, rarely or not at all) by the patients involved.

RESULTS AND DISCUSSIONS

The results of the investigation for the five nutritional food categories are listed below.

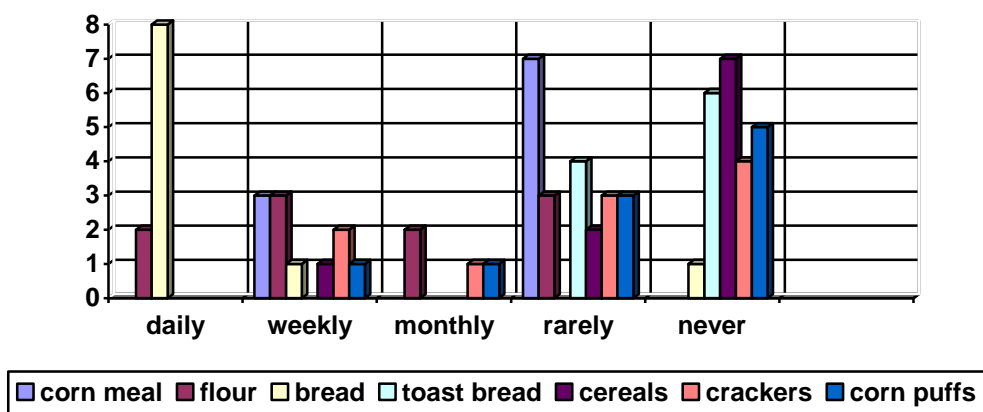


Fig. 3. Summary of the nutritional survey for the investigated group; Cereals.

The data and figure above shows that the highest frequency of daily consumption was in white bread and flour while more than half of evaluated patients had rarely or never consumed these products (the conclusion being justified because of their pathological conditions).

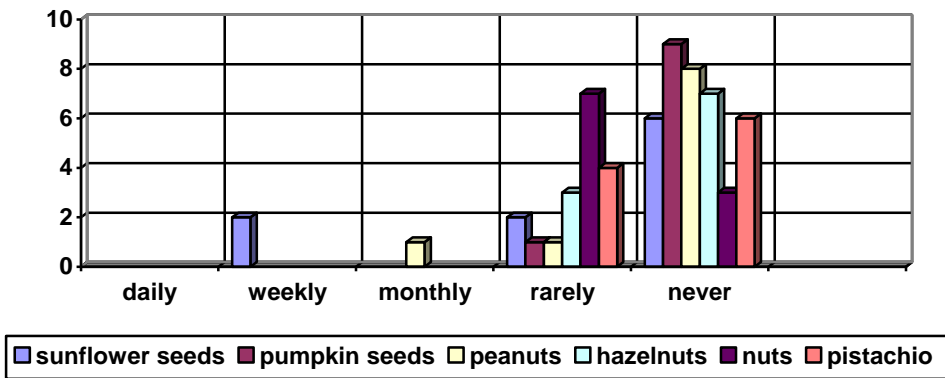


Fig. 4. Summary of the nutritional survey for the investigated group; Seeds, nuts

In terms of consumption of seeds and nuts (foods with a high risk of mycotoxin contamination) can be observed a low consumption frequency in most investigated patients. Of these, sunflower seeds are consumed weekly while nuts, walnuts and pistachios are rarely eaten.

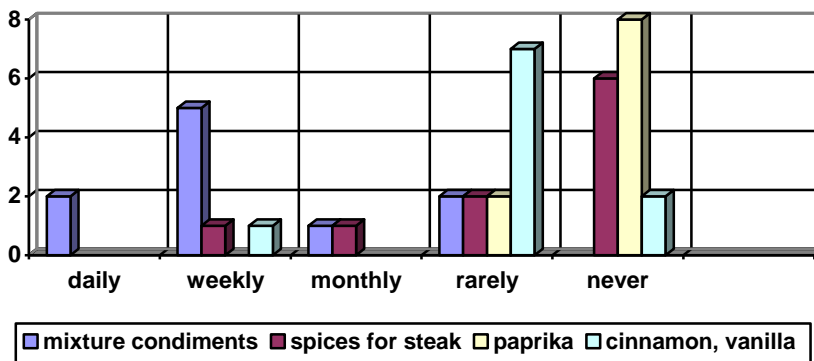


Fig. 5. Summary of the nutritional survey for the investigated group.

The data presented shows that although most patients rarely or never consume these foods, there are people who are eating daily spice mixtures of condiments, foods also with a high risk of contamination.

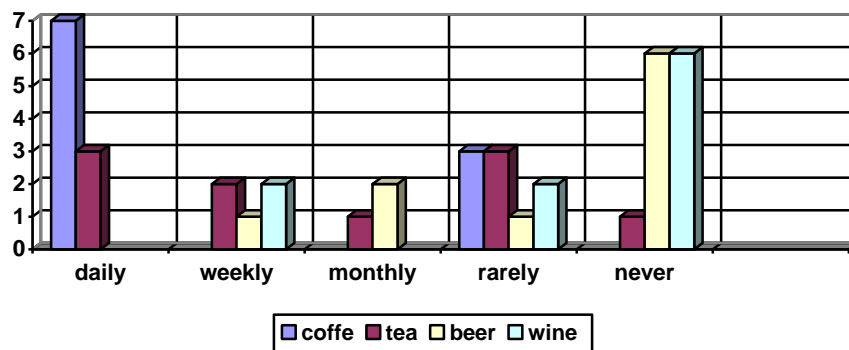


Fig. 6. Summary of the nutritional survey for the investigated group; Drinks.

Also in these categories can be found an increased daily consumption of coffee and tea at more than half of the investigated patients but, in addition to this there is a low or almost none consumption of beer or wine in the rest of the patients.

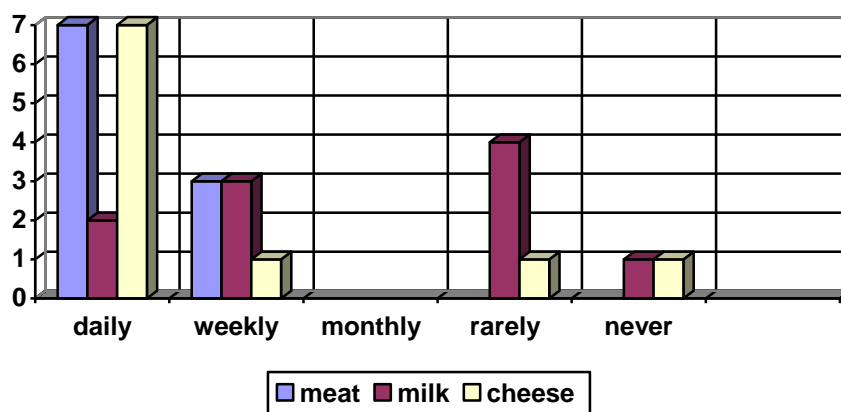


Fig. 7. Summary of the nutritional survey for the investigated group; Animal products.

These foods are consumed daily or weekly by most patients. The risk of contamination with mycotoxins is relatively low for this category given that these products are well controlled sanitary and veterinary, stored under refrigeration and sold relatively quickly from the manufacture day.

In the mycotoxicological analysis of serum samples regarding the presence and quantity of OTA, the results were positive in 6 samples (60%) having values ranging from 49.94 to 128.00 ppt (4 of them having values $</>$ LD).

In dialyzed patients with positive results and significantly increased by OTA in serum (61.77 ppt, 98.87 ppt and 128.00 ppt) was noticed the daily consumption of white bread and coffee, possible sources of OTA in food.

After the OTA enters the organism, the mycotoxin binds to the albumins at the plasma level and begins to metabolize relatively quickly, depending on the animal species. Among organs, OTA has the highest affinity for the liver and kidneys. An important aspect of OTA metabolism in the body is represented by the kidney reabsorption at the level of proximal tubule (2/3), at level of distal tubules and of the collecting channel (1/3). This phenomenon occurs due to the disruption of the pH's homeostasis in the cellular level of the nephrons wall which affects the acid-base transepithelial transport and causes urine acidification. Urine acidification favors OTA reabsorption leading to mycotoxin accumulation in the body by decreasing the rate of elimination [2,5].

CONCLUSIONS

- Daily and weekly consumption of products with a high risk of contamination is high for cereals, spice mixtures of condiments, tea and coffee.
- Out of the analyzed serum samples, 60% of them were contaminated with OTA, with values ranging between 49.94 ppt and 128.00 ppt.

ACKNOWLEDGEMENTS

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