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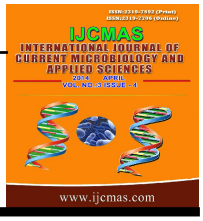

Original Research Article

Aflatoxins in the urine of children under five, economically vulnerable, and their potential involvement in developmental impairment by age

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A B S T R A C T

Evaluate the association between the existence or absence of Aflatoxins in urine of children under 5 years old who live in economically vulnerable settlements in the metropolitan area of Guadalajara with growth and development by age. We conducted a case-control study of 30 cases with developmental disabilities by age and 30 controls were analyzed. Sixty urine samples from children under 5 years were tested to determine the presence or absence of aflatoxins through Aflacheck and HPLC. The Results obtained suggest some intake of aflatoxigenic food. The cases with more consumption of oleaginous food, through Aflacheck, detected false positives. The Aflacheck test showed positivity towards B1 aflatoxin giving as a result false positives confirmed by HPLC due to the fact that none of the samples of the cases, neither of the controls, were positive towards the presence of B1 aflatoxin.

Keywords

Aflatoxins, aflatoxigenic food, cereal-oleaginous; AFB1 aflatoxin. HPLC

Introduction

In 1960, in England, the death of thousands of peacocks, which arose a week after the first symptoms and whose necropsy revealed hemorrhages and necrotic zones on their livers, led to the association between their deaths and the consumption of peanuts from Brazil which were contaminated by *Aspergillus flavus*. (Duarte and Villamil, 2006) Aflatoxins are secondary metabolites produced by the funguses *Aspergillus flavus, Aspergillus parasiticus*, as well as *Aspergillus nomius (Valdivia, et al, 2000; Lucas 2001; Santos, 2001)*. It is known that those three generate microtoxins capable of producing carcinogenic, mutagenic or teratogen

damage3-6. Such funguses have been detected on corn, peanuts and grains in general (Santos, 2001).

From the aflatoxins B1 (AFB1), B2 (AFB2), M (AFM), G1 (AFG1) y G2 (AFG2) (Ramis, 1998) the most important known is AFB1 regarding its toxigenic power. (Ramis, 1998; Valdivia, 2000,) It has been possible to detect the AFM as one of the secondary metabolites of AFB1 in blood and urine, (Jonsyn, et al, 1995)as well as on blood from the umbilical cord.(Jonsyn, 1995; Valdivia, 2000) And it is certain that those present have an association with liver cancer. (López and Avendaño, 2000).

The weight-height indicators byage, the realization of physical and intellectual development level of the child, allows a forecast of development including the cognitive level, the effect on their nutritional status, even are immersed as a result of diets poor in terms of food laws, therefore socially they could relate to the intake of an aflatoxigénic diet dependent on socio-economic indicators and represent risks for the intake of aflatoxigenic food, situation that could affect the general population and interfere with the development of children, without ignoring the impact on the rest of age groups with diverse effects.Mycotoxins are one of the public health problems who with greatest difficulty approach the legislature for to put limits on their presence in food.The potential harmful effects that these substances have in human health, even at low levels of exposure, only allow the adoption of preventive action and the reducing of pollution, but it does not complete its elimination.(Lucas, 2001)

Mycotoxins are fungal metabolites, the toxins produced by Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius are called aflatoxins,(Bourgeois, et al, 1994) which include aflatoxin B1 (AFB1), B2 (AFB2), M1 (AFM), G1 (AFG1) and G2 (AFG2). The most important is AFB1 due to their toxigenic degree. (Jonsyn, 1995; Valdivia, 2000) The chronic ingestion of these toxins havwe been associated with some of the chronic forms of malnutrition in children in growth process; as well as liver and kidney damage, carcinogenesis and immunosuppression, (Jonsyn, 1995; oJnathanet al, 2004) also the aflatoxins have been implicated in the pathogenesis of malnutrition such as wasting and stunting. (Gong et al, 2003 and 2004).

Aflatoxins have been detected in body fluids, including urine, and have been associated with malnutrition. (Turner et al 2009; Tchana, et al, 2010) In the study called Aflatoxins in body fluids and food of Nigerian children with protein-energy malnutrition byOnyemelukwe et al, 2012 say that they have detected all types of aflatoxins in urine samples of patients and controls with varying concentrations, and who in the controls have higher aflatoxin detection (90.9%), followed by kwashiorkor (84,6%), the marasmo (81,8%) and marasmo kwashiorkor (60%), in descending order, the differences observed in the detection rates in urine were not significant. ($p > 0.05$) (Tchana, et al, 2010;Onyemelukwe, et al, 2012).

The present research was being done with the finality to provide an answer to the following unknown: Does the urine of children under 5 living in economically vulnerable colonies have aflatoxin? Is the presence of Aflatoxin involved with the development and growth of these children because of their age?

For which the following variables were considered:

Variable Independent	Variable Intervening	Variable Dependent:
Aflatoxigenic diet and aflatoxins in urine.	Premature infants, children with diseases who affecting their growth and development, humid climates.	Growth-development and nutritional status.

Materials and Methods

We conducted a case-control study, for which we interviewed mothers in 30 cases with deficiencies in the developmental by their age and 30 controls, and 60 urine samples were analyzed to determine the presence or absence of aflatoxin. Regarding the determination of aflatoxins in urine, the analysis was performed with test strips AflaCheck and by HPCL in the same samples. A duplicate urine sample testing was usedwhich marked a standard concentration ranging between 2 and 10 ppb, this showed readings between 3,422 and 3,597 showing positivity of the sample. Statistical analysis was performed from building a database on D-BASE III plus and analysis in SPSS-15.

Results and Discussion

Both cases and controls have a similar food intake including the aflatoxigenic food. It was found that the group of cases reported an increase of the consumption of oil (Figure 1) such as walnuts, almonds and peanuts, this could make a difference in the final analysis, even depending on the quality of these foods, it represents an indicator of likely consumption and presence of aflatoxins, also it was found that in terms of intake of sweets and marzipan, cases have increased from the the intake. (Table 1).

The frequency of consumption could represent daily-daily intake, although in this study no significant difference was detected between the case and control groups. (Table 1 and table 2)

The test Afla Check showed positivity to aflatoxin B1 in urine samples and food resulting in false positives confirmed by HPLC, since none of the samples in cases and controls were positive to aflatoxin AFB1. While conducting the test, a urine sample was performed in a duplicate labeled with a standard of concentration ranging between 2 and 10 ppb. The readings for the standards were positive for the aflatoxin readings ranging from 3.422 to 3.597 ppb showing positivity of the sample to aflatoxin AFB1, situation who's not manifested in none of the problem samples for cases as for controls.(Table 3).

The differences detected in the eating pattern in cases and controls were not useful for the determination of aflatoxin AFB1 in urine with the development by age of the children.It Seemed that the issue is uncertain, however it, the results could be derived by the sample size, probably to a larger sample size, the probability of detecting children with aflatoxin B1 in urine would have been greater, and if detected, we could have performed the calculation of the OR, this would give greater certainty in the pursuit of this association, on the other hand the detection of false positives with a test based in reactive strips regarding to HPCL shows the importance of using this method in its determination, it denotes greater specificity and sensitivity respect to Afla Check.

The search of aflatoxin AFM1 as an indicator of the presence of aflatoxin AFB1 it confirms the exposure of the aflatoxin.

The same measurement of parameters were used to determine if there was a deficiency or not in the development, it could provide a higher probability of use with a greater reliability, dependent itself of the studied sample, even of the genetics issue implicated in each child detected as cases or as controls.

Results are important to highlight which makes the aflatoxin AFM1 an indicator of aflatoxin B1, it would be important check if there is aflatoxin AFM1 in children whose intake were described of dubious quality or suspicious of present aflatoxins, even if AFB1 cannot be detected in urine and has already been metabolized and bio-transformed into AFM1.

The frequency of consumption could represent daily intake, although in this study no significant difference were detected between the case and control groups. (Table 1 and table 2) According to aflatoxigenic intake, between junk food and corn tortilla intake, it showed a low quality $p > 0.05$, in comparison with the quality of the corn tortillas and peanuts, apparently causing other variables or even synergism to be necessary to increase the sample size to increasethe probability of being detected by HPLC aflatoxin AFB1 and AFM1.

Derived from the results it was determined that the molecular mechanism by which aflatoxins are produced cause a derivative teratogenic effect inhibition of cell division in metaphase level, the genome toxic effect depends on the metabolic activation of aflatoxin B1 when translocation causing thymine guanine.Epidemiological studies show translocation that causes mutations in the sequence 249 of the tumor suppressor gene p53, DNA 22 to 25 region, but the effect of food in humans aflatonxigenic is unknown, (Lunn , et al, 1997; Kirk, et al , 2000;Sudakin, 2003) for the same study the effect on children regarding the presence and effect of aflatoxins is an opportunity to investigate and seek alternatives to avoid exposure to such toxins.

Particularly in reference to aflatoxins exposition by means of the diet. Apparently this has not been studied in humans in a systematized way. (Ozturk, 1991; Sudakin, 2003) In addition, Evidences of their effect exist on animals, mutagenic and carcinogenic. (Lunn, et al , 1997) For instance, It is known that in acute out brakes that cause death, they can provoke embryonic death by inmunotoxicity of the embryo, as well as a diminishment in the production of eggs and in the size of those. In 1999, the FAO and WHO pointed out that food for human consumption should remain free of mycotoxins. Such substances in ruminants diminish their productivity and occasionally cause death. (González, 2013) In experiments carried out with female rabbits, it was detected that after the administration of 100 micrograms of aflatoxins by weighted Kg, pregnancy was obtained with a high degree of mortality in 47% of the cases. Some of the rabbits that stayed alive, aflatoxins and liver damage were determined; their birth rate was zero even (Galán and Rodríguez, 2003) when being administered with 50 micrograms by weight(Kg) for 10 days. As well as

Table.1 Structured interview to mothers of children under five years with and without developmental difficulties. **N** = 30 cases and 30 controls

	Sweets	Marzipan
Cases	100%	71.4%
Controls	90%	40%

60, Source: Direct

Table.2 Structured interview to mothers of children under five years with and without developmental difficulties. **N** = 30 cases and 30 controls

	Cobnut	Almond	Pistachio	Peanut
Cases	28.6	14.3	0	57.1
Controls	20	10	10	50

N = 60, Source:Direct.

Table.3 Average Determination of aflatoxin B1 in the children urine less than five years in standardized sample added with aflatoxin B1 respect to test samples in cases and controls

Standard sample	Standard sample
Average aflatoxin B1 detected.	Average aflatoxin B1 detected.
3.422	3.597
Problem sample HPLC	Problem sample HPLC
Promedio de aflatoxina B1detected.	**Promedio de aflatoxina B1 detected.**
0=Negativa	0=Negativa

N = 30 cases and 30 controls with your witness or standard sample and problem sample. **Source:** Analysis by HPLC of urine samples collected at ZMG vulnerable colony, Guadalajara Metropolitan Area.

testicular steroidogenesis in rats, it can also drastically affect trout (Turner, et al, 2007)in reference to its effect in humans, it is necesary to research the impact in development of boys byage, since there is evidence of the presence of aflatoxin AFB1 candy some companies and whether the daily intake is contaminated by aspergillus and its products intake based on sweets or peanut derivatives are a risk forutros health. Particularly on children. (Steven, 2004)

By AflaCheck test, it is possible to determine aflatoxins in urine and food, however, the test detects false positives, therefore it decreases their sensitivity and specificity discrediting its reliability.
Both cases and controls present similar food intake even respect to the aflatoxigenic food.

When looking for the determination of aflatoxin B1, it is suggested that we perform the aflatoxin determination by the HPLC method, even determined with more effectively the analysis in the search to detect aflatoxin M1, insomuch as this is a result of the metabolism of aflatoxin AFB1.

The results obtained in this research are denoted that it doesn´t allow the association between aflatoxin AFB1 and poor development for the age, insomuch as it does not detect aflatoxin AFB1 in the children´s urine with or without gaps in its development.

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