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Detection of Aflatoxin B1 among Early and Middle Childhood Iraqi Patients

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Abstract:

The study was conducted for the detection of Aflatoxin B1 (AFB1) in the serum and urine of 42 early and middle childhood patients (26 male and 16 female) with renal function disease, liver function disease, in addition to atrophy in the growth and other symptoms depending on the information within consent obtained from each patient, in addition to 8 children, apparently healthy, as the control. The technique of HPLC was used for the detection of AFB1 from all samples. The results showed that out of 42 patient children, 19 (45.2%) gave positive detection of AFB1 in the serum among all age groups patients with a mean of 0.88 ng/ml and a range of (0.12-3.04) ng/ml. This was compared with the control that did not detect any level. On the other hand, AFB1 was not detected in any of urine samples in both of the sexes. Positive results of serum AFB1 were recorded in males more than females sample which reached 12(46.1%) and 7(43.7%) respectively with a mean/ range reached to (1.08 /0.12-2.91 and 0.82/0.12-1.30)ng/ml respectively, compared with 8 control samples that did not detect any value of aflatoxins.

Key words: Aflatoxin B1, Childhood, HPLC, Mycotoxins, Serum AFB1, Urine AFB1

Introduction:

Aflatoxins (AFs) are toxic group of fungal secondary metabolite naturally produced by, *Aspergillus flavus* and *A. parasiticus*, rarely by *Aspergillus nomius*, it is produced at temperature of 12- 40 C° and at humidity ranging between 3-18% (1) (2). AFs in nature have six forms B1, B2, G1, G2, M1 and M2. Group B contains cyclopentane ring the group G contains lactone ring, B and G refer to the first letter of green and blue fluorescent colors produced under UV light on thin layer chromatography plates (3). The subscript numbers 1 and 2 indicate major and minor compounds (4). AFs were discovered when more than 100,000 young Turkeys died in England overall the course of few months, due to an apparently new disease known as (Turkey-x disease). Aflatoxin B1 (AFB1) is the most commonly encountered and considered the most toxic, carcinogenic and hepatotoxic for animal and human. It was classified as group 1 carcinogenic by the International Agency for Research on Cancer due to its strong toxicity (5). AFB1 toxicity in animal/poultry may be expressed by changes in biochemical and hematological parameters' concentrations of blood serum and anaemia (6).

Additionally, the reduction of general immune function, hepatotoxic, haemorrhage and teratogenesis, in addition residue of AFB1 from animals will lead to carcinogenesis and mutagenesis that can appear in edible animal products for human consumption(7). AFB1 in the livestock animals and human effect on the metabolism. When the exposed to AFB1, metabolic activation of AFB1 predominantly in the hepatocytes by cytochrome 450 (CYP450) enzymes, toxic AFB1-8, 9-epoxide is formed which covalently binds to nucleotides and proteins. CYP2A13 predominantly in human respiratory tract enzyme, has been shown to be responsible for bioconversion of AFB1 into AFs b19-epoxide (8). AFB1 and its metabolites are excreted through urinary routes (9, 10). AFB1 was detected in the local paddy and polished rice in Iraq, the concentration of AFB1 in local paddy and polished was (0.3-0) ppb respectively (11). Also, another study in Iraq detected some fungal genus was the production of MTs such as *Aspergillus* and *Penicillium* in the imported American rice grains and local corn grains (12, 13) and found the same fungal genus in wheat grains in Iraq (14).

The aim of this study is to detect AFB1 in the serum and urine samples among early and middle Iraqi childhood.

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Materials and Methods:

A total of 50 children (early and middle) in both sexes were included in this study. 42 patients (26 male and 16 female) with liver function disease, renal function disease, in addition to atrophy in the growth and other symptoms depending on the information taken from the consent obtained from each patient.

The samples were taken from Children Welfare Teaching Hospital in medicine city of Baghdad during the period from June 2018 to September 2018. 8 samples from healthy children (4 for each sex) were taken as control from one of the primary schools in Baghdad.

Collection of blood samples

Ten ml of venous blood were collected from each child under sterile condition using disposable syringe. The blood samples were placed in a plain tube and let stand for 20 min. at room temperature to clot. The serum was separated from clot by centrifugation at 3000 r.p.m for 10 min. The obtained serum was divided into four parts in the Eppendorf tube to avoid repeated freezing and thawing cycle, then stored at -20 C° till analysis.

Collection of urine samples

Morning urine samples were taken from each child who was informed a day prior to the day of samples collected using a urine collection bag. Approximately 40 ml of urine was collected from each child, then immediately transferred to a urine cup and put in the centrifuge at 3000 r.p.m. Before blood and urine sampling, each child was asked some questions according to the questionnaire sheet (age, weight, length, and diet) prepared previously.

HPLC Condition

The examination was conducted in the laboratories of the Ministry of Science and Technology /Department of Water and Environment using the HPLC technique for the detection and evaluation of fungal toxins AFB1 in serum and urine samples, according to (15).

The reversed-phase HPLC(Cuknm, Germany model) on an Agilent 1100 system consisting of a diode-array UV detector at 362 nm was connected in series with a fluorescence detector (366 nm excitation and 436 nm emission). The HPLC column used was a C18 5 µm (150 × 4.6 mm) (Waters Ltd., Watford, United Kingdom). Chromatographic separation was obtained by a 5–25% ethanol linear gradient in water generated over a 25 min period followed by isocratic elution with 25% ethanol in water, all at a flow rate of 1 ml/min.

The mobile phase was buffered with 5 mM triethylammonium formate (pH 3.0) and the column temperature was maintained at 35°C. The eluted peaks were integrated and AFB1 was quantitated with the standard curve. Authentic AFB1 was eluted at 15.5 min. The detection limit of for the technique was 5 pg .

AFB1 Standard Curve

About 0.5 mg of AFB1 was taken and placed in 100 ml volumetric flask and the volume was completed to the mark where the concentration became (5 ppb). Using the dilution law ($C_1V_1=C_2V_2$), several concentrations were prepared and injected into the HPLC to draw a calibration curve.

The standard substances of AFB1 were injected in HPLC technique devices in concentration 0.12 ppb to diagnose the retention time(Fig. 1).

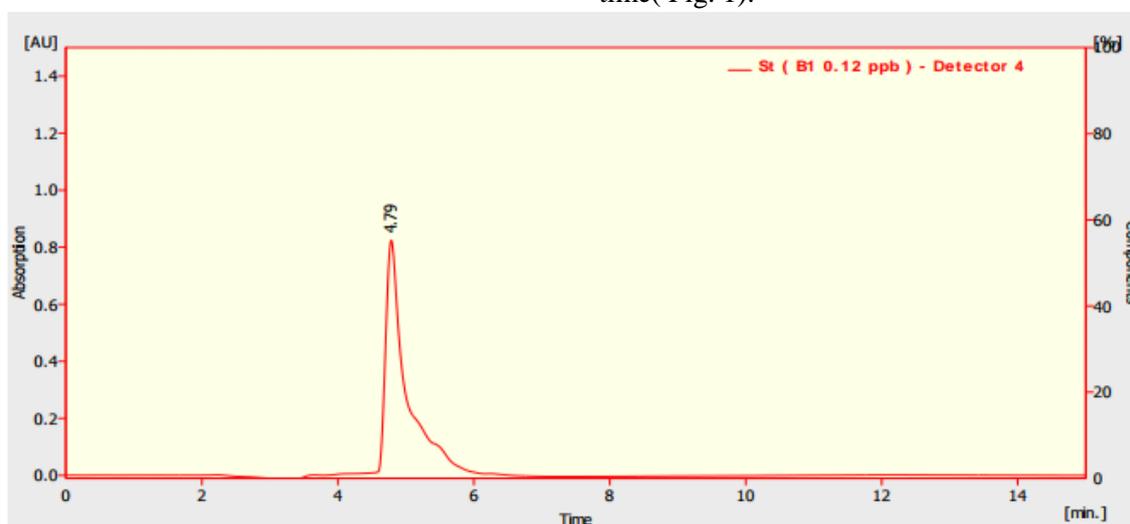


Figure 1. Standard curve of AFB1

Results and Discussion:

The current study is one of the few studies in the world and in Iraq to detect MTs AFB1 in the

body fluids (serum and urine). It is among one of the most important age bracket of all segments of society that is early and middle stage of childhood.

Many studies in the detection of MTs from different food sources have been reported

Detection of AFB1 in the serum

Table 1 shows that out of 42 children patients, 19 (45.2%) gave positive detection of AFB1 in the serum by HPLC technique among all age patient groups from (1-15) year, with a mean 0.88 ng/ml and a range (0.12-3.04) ng/ml, compared with 8 healthy children as control that did not detect any value. The highest mean of AFB1 (1.34 ng/ml) was recorded in the first group (1-5) years, at n= 6/10 (60 %) ranged from (0.12-3.04) ng/ml. That is followed by the second group, (6-10) years with a mean 1.05 ng/ml at n=6/20 (30%) and ranged from (0.12-2.97) ng/ml, while the third group (11-15) years recorded the lowest mean of AFB1 (0.12 ng/ml) at n=7/12 (58%) with ranged from (0.12-0.13) ng/ml.

There were highly significant differences ($P < 0.01$) between these groups, in addition, to control.

Table 1. Distribution of AFB1 in the serum by HPLC technique according to age groups of the study samples

Age group (year)	No. sample	Positive No. (%)	Mean \pm SE (Range) ng/ml
1-5	10	6 (60)	1.34 \pm 0.09 a (0.12- 3.04)
6-10	20	6 (30)	1.05 \pm 0.07 a (0.12-2.97)
11- 15	12	7 (58)	0.12 \pm 0.02 b (0.12-0.13)
Chi-Square (χ^2) Patients	---	8.92 **	LSD = 0.50 ** 0.88 \pm 0.07 a (0.12-3.04)
Healthy(Control)	8	0(0)	0.00 \pm 0.00 b (0)
Chi-Square (χ^2)	---	11.75 **	LSD = 0.35 **

The different letters in the same column refer to different significant values. ** ($P < 0.01$)

The current results disagree with a previous study by (16) in which the HPLC technique was the least sensitive method to detect AFTs in serum of human although there were few studies for AFB1 detection in serum of human by HPLC technique. However, Hatem *et al.*, (17) conducted a study to investigate the presence of MTs in serum blood and urine by thin- layer chromatography (TLC) method on 60 Egyptian infants with protein- energy malnutrition (30 with kwashiorkor and 30 with marasmus) compared with 10 healthy infants as control. They recorded that AFB1 was found in (80 and 46.7) %, respectively, with a mean: (32.38 and 13.62):(4-69 and 10-18) ng/100ml respectively, in addition to higher significant ($P < 0.01$) in

kwashiorkor patients and no AFB1 was detected in the control.

Detection of AFB1 in the urine

Although AFB1 was detected in 19/42 (45.42%) in serum samples as shown in the previous Table, there were no detection in any of urine samples in both of the sexes by the HPLC technique.

The current results coincided with Ayelign *et al.*, (18) where AFB1 was not detected in all of the urine samples in both of the sexes. Also the present results matched those of (19). Residues of AFTs B1 in addition to B2, G1, G2 and aflatoxinol were not detected in any urine sample among 60 volunteers at ages from 14 to 55 years old, when evaluating the short-term human exposure to AFTs in Brazil by a liquid chromatography coupled to mass spectrometry (UPLC-MS/MS) method.

However, in the current study AFB1 was not detected in any urine samples. But another study by (15) detected AFB1 in the urine of young children. The urine samples from the young children were examined for a total of five aflatoxins; AFB1, AFB2, AFG1, AFG2 and AFM1. One or more aflatoxins were detected in 34/200 (17%) urine samples collected which regions.

These are due to age differences, limits of detection and analytical methods performances. In Egypt, the prevalence of urinary AFB1 among infants with kwashiorkor and marasmus at a mean: range (8.29 and 6.92) (1-15 and 5-9) ng/100ml respectively was also reported, in addition to higher value in kwashiorkor than marasmus at 80% than 46.7%, respectively and no AFTB1 were detected in the control (17).

Distribution of AFB1 in the serum by HPLC technique among the study samples according to the sex

Table 2 shows that out of 42 patients, including 26 male and 16 female gave positive results of AFB1 in male more than female reaching to 12(46.1%) and 7(43.7%) respectively with a mean/ range reached to (1.08 /0.12-2.91 and 0.82/0.12-1.30)ng/ml respectively, compared with 8 control (4 of each sex) that did not give any value.

There were highly significant differences ($P < 0.01$) between these groups in addition to the control.

The highest mean/ range of AFB1 (1.66/0.12-3.05 and 0.71/0.12-1.30) ng/ml respectively, was recorded in the first group (1-5) years, at value n=4/7 (57.1%) and n= 2/3 (66.6%) respectively. It was followed by the second group (6-10) years with a mean (1.48 and 0.19) ng/ml respectively, while the third group (11-15) years recorded the lowest mean of AFB1 (0.12 and 0.13) ng/ml respectively.

Table 2. Distribution of AFB1 in the serum by HPLC technique among the study samples according to the sex

Age group (years)	No. sample	Male			Female		
		Male	Positive No. (%)	Average (range)ng/ml	Female	Positive No. (%)	Mean ± SE (Range) ng/ml
1-5	10	7	4(57.1)	1.66(0.12-3.05)	3	2(66.6)	0.71 (0.12-1.30)
6-10	20	13	4(30.1)	1.48(0.03-2.90)	7	2(28.5)	0.19 (0.12-0.26)
11-15	12	6	4(66.6)	0.12 (0.12-0.12)	6	3(50.0)	0.13 (0.12-0.13)
Chi-Square(χ^2)	---	---	9.478**	---	---	9.601**	---
Patients	42	26	12(46.1)	1.08 (0.12-2.91)	16	7(43.7)	0.82(0.12-1.30)
Control	8	4	0(0)	0(0)	4	0(0)	0(0)
Chi-Square(χ^2)	---	---	10.02 **	---	---	---	---

** (P<0.01).

There was a lack in the studies to compare the serum and urinary AFB1 concentration according to the sex. The increased of AFB1 in male more than female in the current results, may be attributed to the capability of male children to eat more light meals than females due to their movement, and these meals might contain contaminants with MTs, including AFTs. AFT exposure has been associated with growth faltering (20) and immune suppression in young children (21).

Conclusion:

The current study represents one of the few studies in the world and Arab countries for detecting AFB1 from serum and urine, and indicates frequent exposure to these toxins among childhood in Iraqi patients. A positive result of AFB1 in serum was occurred by the use of HPLC technique in all groups of children. Male children are more sensitive to methods of serum with AFB1 then female children

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have

been given the permission for re-publication attached with the manuscript.

- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

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الكشف عن سموم الأفلاتوكسين AFB1 بين مرضى عراقيين في مرحلة الطفولة المبكرة والمتوسطة

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الخلاصة:

أجريت الدراسة للكشف عن الأفلاتوكسين AFB1 في مصل وبول 42 مريض في مرحلة الطفولة المبكرة والمتوسطة (26 من الذكور و 16 من الإناث) يعانون مرض وظائف الكلى، وأمراض وظائف الكبد ، فضلا عن ضمور في النمو وأعراض أخرى اعتمادا على معلومات تم الحصول عليها من كل مريض. و 8 اطفال اصحاء ظاهريا كمجموعة سيطرة. تم استخدام تقنية HPLC للكشف عن AFB1 في جميع العينات. أظهرت النتائج أنه من بين 42 مريضا في مرحلة الطفولة ، 19 (45.2%) منها اعطت نتيجة ايجابية في وجود AFB1 في المصل من بين جميع الفئات العمرية للمرضى بمعدل 0.88 نانوغرام / مل ومدى (0.12-3.04) نانوغرام / مل ، بالمقارنة مع مجموعه السيطرة التي لم تكتشف أي مستوى من الأفلاتوكسين. بينما لم يتم اكتشاف عن الأفلاتوكسين في أي من عينات البول في كلا الجنسين. تم تسجيل النتائج الإيجابية للأفلاتوكسين AFB1 في عينات المصل للذكور أكثر من الإناث وصلت إلى 12 (46.1%) و 7 (43.7%) على التوالي مع متوسط / مدى وصل إلى (1.08/ 0.12- 2.91 و 0.82 / 0.12- 1.30) نانوغرام / مل على التوالي، مقارنة مع مجموعة السيطرة (8) والتي لم تكتشف عن وجود أي قيمة للأفلاتوكسين.

الكلمات المفتاحية: الأفلاتوكسين بي 1 ، طفولة، تقنية السائل ذات الضغط العالي، سموم فطرية، افلاتوكسين المصل، افلاتوكسين الادرار .