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STUDY THE CORRELATION BETWEEN AFLATOXIN B1 AND MALE INFERTILITY IN IRAQI PATIENTS

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ABASTRCT

The purpose of the investigation was to identify aflatoxin B1 (AFB1) in the blood serum and seminal fluid of 50 of patients from Iraqi male who suffering from infertility include (oligozoospermia, asthenozoospermia and teratozoospermia) with 16 fertile males as control depending on seminal fluid profile examination. The HPLC technique was used for AFB1 detection of the samples (serum and semen). The results showed that all the patient and control were gave positive detected of AFB1 50/50 (100%) and 16/16 (100%) respectively, in the serum at mean (81.08 ±3.95) ng/ml at range (114-60) and (8.31 ±0.63) ng/ml at range (5-12), respectively. Also detected AFB1 in the seminal fluid of patients at mean 39/50(78%) ng/ml at range (37-0), but not appear of AFB1 in any sample of control. There were highly significant differences (P≤0.01) between the mean level of AFB1 in the infertile male patients and control.

Key words: high performance liquid chromatography, infertility, oligozoospermia. mycotoxins.

دراسة العلاقة بين الأفلاتوكسين ب1 والعقم عند الذكور في المرضى العراقيين

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الخلاصة

هدفت الدراسة عن التحري على تشخيص سم الافلا ب 1 في مصل وسائل منوي لخمسون مريض من رجال عراقيين يعانون من عقم ، شمل (قلة الحيامن , وهن الحيامن , تشوهات الحيامن) مع 16 رجل غير عقيم كسيطرة، بالاعتماد على فحص صورة السائل المنوي، استخدمت تقنية كروماتوغرافيا سائل عالي الدقة لتحري عن وجود سم الافلا ب 1 في جميع العينات (مصل وسائل منوي). أظهرت نتائج جميع المرضى والسيطرة تحري موجب لسم الافلا ب 1 50/50 (100%) و16/ 16(100%) في المصل على التوالي بمعدل (81,08 ±3.95) جزء في البليون بمدى (114 -60) ومعدل (8,31 ± 0.63) جزء في البليون بمدى (12 -5)، على التوالي. كما تم التحري ايضا عن سم الافلا ب 1 في السائل المنوي للمرضى بمعدل 50/39 (78%) بمدى (37-0)، لكن لم يظهر سم الافلا ب 1 في أي عينة من السيطرة. وجد اختلاف احصائي عالي (P≤0.01) بين معدل مستوى سم الافلا ب 1 في الرجال العقيمين والسيطرة.

الكلمات المفتاحية: سموم فطرية 1 , العقم ,كروماتوغرافيا السائل عالي الدقة , قلة الحيامن.

INTRODUCTION

The inability to become pregnant after a year of consistent, unprotected sexual activity without the use of contraception is known as infertility (**Larsen, 2005**). "Male factor" infertility accounts for about 40–50% of male infertility, with 2% of cases having Sperm characteristics that are below ideal (**Kumar & singh ,2005**). Infertility has been associated with emotional, sociocultural, and physical difficulties (**Larsen,2005**). Additionally, a high frequency of STDs, underdeveloped or nonexistent testes, and anomalies of the pituitary and hypothalamus are linked to infertility (**Agarwal *et al.* 2021**). In Iraq there were previous study to detect AFB1 in the serum and urine of Iraqi childhood and the result shows the high level of AFB1 in the serum and urine of children (**Al-musawi,2020**).

Male infertility is most commonly caused by sperm morphology, movement issues, varicocele, difficulties ejaculating semen, low or nonexistent sperm counts, and genetic illnesses. Additionally, lifestyle decisions and environmental circumstances may have an effect (**Machen & Sandlow, 2020**). low motility of sperm (asthenozoospermia), reduced sperm quantity (oligozoospermia), reduced sperm vitality (necrozoospermia), aberrant sperm form(teratozoospermia), or any mix of these factors may be the cause (**Sharma, 2017**).

Iraq is one of countries that suffering from infertility, according to previous study in Iraq about the infertility rate, it has been estimated to be 3-4 out of 10 couples (35%) (**Al-Dujaily *et al.*,.2017**). One of strong effect on infertility mycotoxins, mycotoxins can be defined as toxic secondary metabolites naturally produced by mold (*Aspergillus*, *fusarium* and *penicillum*) (**Turner & Snyder, 2021**). The fungi synthesize toxins after infesting crops which will be transmitted to the final food products. MTs effect on human healthy and causes problems, one of this problem infertility, Numerous studies have linked the rise in male infertility to the growing presence of these MTs in the environment. (**El. Khoury *et al.*, 2019**). Studies shown that infertility rate has gotten worse from (42 to 48.5) million couples globally between 1990 and 2010, impacting 1 in 7 couples who are attempting to become pregnant. (**Stuppia *et al.*, 2015**). The toxtic category of fungal secondary metabolites known as AFs is naturally produced by *Aspergillus flavus* and *A. parasiticus,* with *A. nomius* producing them less frequently. AFs are formed at temperatures between 12 and 40 C and humidity levels between 3 and 18%. (**Arimboor, 2024**). Humans exposed to AFs run the risk of developing a number of diseases, including subacute and chronic consequences such cirrhosis, jaundice, chronic hepatitis, liver cancer, and hepatomegaly (**Hampikyan *et al.*, 2010**) and immune system (**Jawad & Alwan, 2020**).

It has been reported that the AFB1 interferes with the enzymes and their substrate that are in charge of producing various hormones, hence impairing the activity of various endocrine glands. (**Gupta & Sharma, 2011**). AFs have the capacity to produce hormonal dysregulation that causes cellular toxicity, which has an immediate impact on reproduction (**Rossi *et al.*, 2009**). According to earlier research, AFB1 disrupts the hypothalamo-pituitary testicular axis, which causes defective spermatozoa to be produced (**Supriya *et al.*, 2014**).

In Iraq there are many study about detect MTs in human, Prior research has demonstrated that AFB1 in male human's causes delayed testicular development, testicular degeneration, reduced fertility, regressive morphological abnormalities in the testis, and dysfunction of the Leydig cell (**Mohammed *et al.*, 2014**). other study shows the presence of aflatoxins in serum and urine in Iraq (**AL-Musawi, 2020**) and other study detected aflatoxin in diet (**Abu-Almaaly *et al.*,2023; Muhyaddin *et al.*, 2015**). Male infertility also effected by aflatoxins Also cause damage to the chromosome and gene by coupling to DNA, forming AFs-DNA complex lead to abnormalities in human sperm (**Pribil,2018**).

Current study aimed to detect relationship between AFB1 and male infertility.

MATERIAL AND METHOD

Collection of seminal fluid samples

Freshly ejaculated samples of human sperm were obtained from males by masturbation and collected immediately into a clean, dry, and sterile disposable plastic Petri dish in a specially designated room. These samples from 50 patients and 16 healthy men according to seminal fluid examination and information acquired from every patient. The sample extracted from Kamal Al-Samarrai Hospital in Baghdad and the period of sample collection from July 2022 to June 2023. The sample included infertile men who suffer from decrease in sperm count (oligozoospermi), sperm motile (asthenozoospermia), and abnormal sperm (teratozoospermia), while excluded from study; varicocele, azoospermia (no sperm production), viscosity samples and samples with pus cells. After 30-60 minutes, the samples were instantly delivered to the semen examination for each individual who had an acquaintance during 3-5 days of abstinence. Each semen sample was allowed to liquefy following WHO guidelines (**Catanzariti *et al.*, 2013**). Following full liquefaction, the sperm were examined macroscopically and microscopically in accordance with WHO standards from 1999 and 2010.

Collection of blood serum samples

Ten milliliters of patient's Venous blood were extracted from each patient while preserving sterility using a disposable syringe. After being put in a simple tube, the blood samples were left at room temperature for twenty minutes in order to allow them to coagulate. For 10 minutes at 3000 r.p.m., centrifugation was utilized to remove the serum from the clot. The resultant serum was divided in an Eppendorf tube and kept at -20°C until analysis to avoid repetitive cycles of freezing and thawing.

HPLC analysis

According to (**McCoy *et al.*, 2008**), the analysis was carried out in the labs of the Ministry of Science and Technology /Department of Water and Environment utilizing the HPLC technique for the detection and evaluation of mycotoxins AFB1 in serum and seminal fluid samples.

The reversed-phase HPLC (Cuknm, Germany type) on an Agilent 1100 system was linked in series with a fluorescence detector (366 nm excitation and 436 nm emission) that was

built around a diode-array UV detector at 362 nm. A C18 5 µm (150 × 4.6 mm) HPLC column (Waters Ltd., Watford, United Kingdom) was employed.

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.After the eluted peaks were integrated, the standard curve was used to quantify AFB1. At 15.5 min, authentic AFB1 was eluted. The method's detection limit was five particles per milligram.

AFB1 Standard Curve

AFB1 was measured out to be about 0.5 mg, and the volume was added to a 100 ml volumetric flask until the concentration reached 5 ng/ml. To create a calibration curve, a number of concentrations were generated and injected into the HPLC using the dilution law (C1V1=C2V2).

To determine the retention period, standard AFB1 compounds were loaded into HPLC method devices at a concentration of 0.12 ng/ml (Fig. 1).

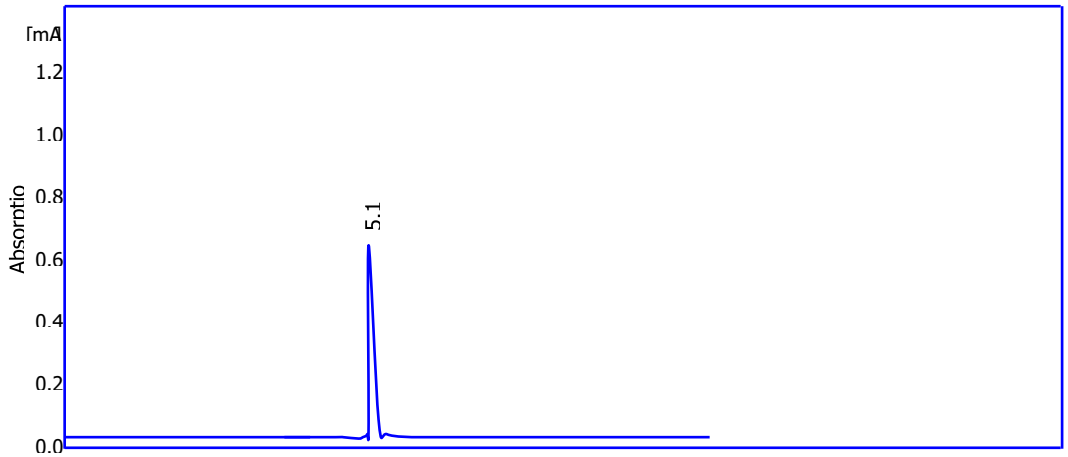

Fig. (1): Aflatoxin B1 standard curve.

STATISTICAL ANALYSIS

The tool SAS (**Statistical Analysis System, 2018)** was utilized to identify the impact of variation factors in the study parameters. The t-test was employed to compare means statistically. The chi-square test was used in this investigation to compare statistically significant percentages (0.05 and 0.01 likelihood).

RESULTS & DISSCUSSION

The present investigation is one of the few in Iraq and all world to detected the effect of aflatoxin b1 on human male fertility, there have been numerous published investigations on the identification of MTs in various food sources (**Saadullah ,2018**).

Detection of AFB1 in the serum

Table. (1) The results showed that all the patient and control were gave positive detection of AFB1 50/50 (100%)} and {16/16 (100%)} respectively, in the serum at mean (81.08 ±3.95) ng/ml at range (114-60) and (8.31 ±0.63) ng/ml at range (5-12), respectively. by HPLC technique with mean 8.31 ±0.63 ng/ml, there. were highly significantly differences * (P≤0.01). between this group, additionally, to maintain control.

Table (1): Distribution of AFB1 in the serum by HPLC technique.

Group	No of sample	Positive No. (%)	Mean by ng/ml	Range (min-mix)
Patients	50	50 (100%)	81.08 ±3.95	60-114
Control	16	16 (100%)	8.31 ±0.63	5-12
P-value	---	1.00 NS	0.0001 *	
* (P≤0.01).				

Detection of AFB1 in the seminal fluid

Table (2) show AFB1 in the seminal fluid of patients at mean 39/50(78%) ng/ml at range (37-0), but not appear of AFB1 in any sample of control. There were highly significantly differences (P≤0.01) between the mean level of AFB1 in the infertile male patients and control.

Table (2): Distribution of AFB1 in the seminal fluid by HPLC technique.

Group	No of sample	Positive No. (%)	Mean by ng/ml	Range (min-mix)
Patients	50	39 (78.00%)	15.7 ±1.04	0-37
Control	16	0 (0.00%)	0.00 ±0.00	0
P-value	---	0.0001 *	0.0001 *	
* (P≤0.01).				

This high prevalence of AFB1 in the semen of infertile group can be explained by the highest prevalence of aflatoxin B1 in different type of diet, the current observations conflict with a prior investigation by (**Tang *et al.*, 2008**) They found that, despite the paucity of research on the detection of AFB1 in human serum by HPLC technology, was the less sensitive

approach for detecting AFTs in human serum. Nevertheless, Hatem et al. (**Hatem *et al.*, 2005**). Study by (**Komsky-Elbaz, *et al*.,2018**), that study exposure of seminal fluid to AFB1 in vitro, this studied show the direct on sperm viability by damage cell membrane lead to sperm dead and by effect on mitochondria lead to dysfunction apoptosis, calcium signaling, reactive oxygen species generation, and ATP synthesis. A physiological issue linked to changes in mitochondrial function includes infertility in both males and females (**Komsky-Elbaz *et al.*, 2018**). Also effect on the DNA fragmentation lead male infertility (**Ramalho-Santos *et al.*, 2009**). Other study on model revealed Boar seminal plasma was discovered to contain a significant concentration of AFB1 residue. AFB1 can also cause testicular lesions in mice, reducing the number and quality of their sperm, and ultimately affecting male fertility (**Supriya *et al.*, 2014**). Exposure to AFB1 can lead to an imbalance between the oxidant and antioxidant enzyme systems, hence facilitating the excessive formation of reactive oxygen species (ROS) and consequent oxidative damage. (**Kannan *et al.*, 2014**). Testicular lipid and DNA oxidative damage can disrupt spermatogenesis and the testis, which is linked to low-quality semen and increased infertility (**Alahmar, 2019**). Furthermore, oxidative stress in the testis has been widely shown to cause spermatogenic cells to undergo mitochondrial damage, apoptosis, and adverse alterations in sperm motility and concentration (**Zhang *et al.*, 2020**).

CONCLUSION

Positive detection of AFB1 in the serum and seminal fluid of infertile male by using HPLC technique. This high prevalence of AFB1 in the semen of infertile group can be explained by the highest prevalence of AF B1 in different type of diet, presence of AFB1 in semen effect direct on male infertility on sperm count, sperm motility and morphology.

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