The Role of Aflatoxin B1 in the Exacerbation of Type 2 Diabetes and its Effects on Liver and Kidney Functions

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Abstract

Diabetes mellitus type 2 (T2DM) is a common metabolic condition characterized by persistently elevated blood sugar levels. Aflatoxia B1 is one of the most hazardous mycotoxins and is the most poisonous and carcinogenic of the aflatoxins, more dangerous than cyanide, arsenic, and organic pesticides. AFB_1 has been classified as a group I human carcinogen by the International Agency for Research on Cancer (IARC). The purpose of this investigation is to look at AFB_1 levels in type 2 diabetics' blood and their impact on their liver and kidney functioning. A case-control investigation was carried out between November 2022 and January 2023. Patients who visited Al-Kindy Hospital, Endocrines, and Diabetes Central/Baghdad provided samples. A total of 177 patients were examined, 93 of whom had T2DM (44 men and 49 women) and 84 of whom were controls (37 men and 47 women). The results showed that both the type 2 diabetics and the control group had higher levels of AST, urea, creatinine, FBS, and HBA1c in the groups that carried the AFB_1. Elevated levels of HBA1C and FBS in the AFB_1-bearing groups in both patients and controls indicate an exacerbation of diabetes mellitus in the patients and pre-diabetes in the healthy groups. According to the study's findings, females are more sensitive to AFB_1 than males. An aggravation of diabetes mellitus in the patients and pre-diabetes in the control groups is indicated by elevated levels of HBA1C and FBS in the AFB1-bearing groups of both patients and controls.

Keywords: *GST, Glutathione S-transferases; AFB1, aflatoxin B_1; OTA, ochratoxin A.*

دور الافلاتوكسين ب1 في تفاقم السكري النوع الثاني وتاثيره على وظائف الكبد والكلى

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الخلاصة

داء السكري من النوع 2 (T2DM) هو حالة استقلابية شائعة تتميز بارتفاع مستمر في مستويات السكر في الدم. يعتبر مركب AFB1واحدًا من أكثر السموم الفطرية خطورة وهو الأكثر سمية ومسرطنًا من الأفلاتوكسين ، وهو أكثر خطورة من السيانيد والزرنيخ والمبيدات العضوية. صنفت الوكالة الدولية لأبحاث السرطان (IARC) مركب AFB1 على أنه مادة مسرطنة بشرية من المجموعة الأولى. الغرض من هذا التحقيق هو فحص مستويات الأفلاتوكسين B1 في دم مرضى السكري من النوع 2 وتأثيره على وظائف الكبد والكلى لديهم. تم إجراء تحقيق في الحالات والشواهد بين تشرين الثاني (نوفمبر) 2022 وكانون الثاني (يناير) 2023.

P-ISSN: 2664-0562
E-ISSN:2664-0554

وقدم المرضى الذين زاروا مستشفى الكندي والغدد الصماء والسكري وسط / بغداد عينات. تم فحص ما مجموعه 177 مريضا ، 93 منهم كان لديهم T2DM 44 رجلا و 49 امرأة و 84 منهم من الضوابط 37 رجلا و 47 امرأة. أظهرت النتائج أن كلا من مرضى السكري من النوع 2 ومجموعة التحكم كانت لديهم مستويات أعلى من AST واليوريا والكرياتينين و FBS و HBA1c في المجموعات التي حملت .AFB1 تشير المستويات المرتفعة من HBA1C و FBS في المجموعات الحاملة للأفلاتوكسين B1 في كل من المرضى والضوابط إلى تفاقم داء السكري في المرضى ومقدمات السكري في المجموعات الصحية. وفقًا لنتائج الدراسة ، تكون الإناث أكثر حساسية تجاه AFB1 من الذكور. تشير المستويات المرتفعة من HBA1C و FBS في المجموعات الحاملة للأفلاتوكسين B1 في كل من المرضى والضوابط إلى تفاقم داء السكري في المرضى ومقدمات السكري في المجموعات الصحية.

الكلمات المفتاحية: *الكلوتاثيون س ترانسمينيز، الافلاتوكسين ب1، الاوكراتوكسين أ.*

Introduction

Type 2 Diabetes mellitus, one of the most prevalent metabolic illnesses, is brought on by the interaction of two main factors: reduced insulin secretion by pancreatic cells and an improper insulin response in tissues with insulin receptors. Because insulin's functions and actions are essential for maintaining glucose homeostasis [1]. One of the many factors that lead to T2DM and insulin resistance is routine exposure to contaminated food, which contains a significant number of toxins [2]. Mycotoxins, which are known as secondary metabolites, have a negative impact on food quality and safety and cause significant economic losses when they are present in food [3]. The fungi Aspergillus flavus and Aspergillus parasiticus primarily create aflatoxin B_1, a type of mycotoxin, during their secondary metabolism, which among all the aflatoxins (AFs) is the most frequent and dangerous. AFB_1 is known to occur often in nature, particularly in a number of food products such as groundnuts, maize, rice, sorghum, milk, and oils [4]. The most dangerous mycotoxin, AFB_1, has negative impacts on people [5]. Nearly 4.5 billion people worldwide are at risk from AF overexposure, and these toxins are to blame for 4.6% to 28.2% of all cases of hepatocellular carcinoma [6].

Aflatoxin B_1 is known to be mutagenic, carcinogenic, and hepatotoxic. Particularly in developing nations in Asia and sub-Saharan Africa, AFB_1 is the third-leading cause of liver cancer [7]. The liver, kidney, spleen, bone marrow, gut, testis, and ovary are among the organs that AFB_1 attacks [8]. This study was carried out to determine the role of food contamination in the local markets in the exacerbation of T2DM as well as to look into the likelihood of a connection between AFB_1 and this disease, T2DM, given the prevalence of T2DM in Iraq and its numerous causes.

Material and Methods

Study design

A case-control study conducted from November 2022 to January 2023. Samples were selected from the patients attending Al-Kindy Hospital and Endocrines and Diabetes Central / Baghdad.

P-ISSN: 2664-0562
E-ISSN:2664-0554

Participant and study sample

A total of 177 subjects were studied, 93 (44 males and 49 females) of whom were T2DM and 84 (37 males and 47 females) of whom were controls. These totals are divided according to table (1).

Table (1): Division of study groups.

NO	Study groups	Means	The number of study group participants
1	M, D-2, TX	Male, type 2 diabetes with AFB_1	20
2	M, D-2, NTX	Male, type 2 diabetes without AFB_1 toxin	24
3	M, C, TX	Male, control with AFB_1 toxin	17
4	M, C, NTX	Male, control without AFB_1 toxin	20
5	F, D-2, TX	Female, type 2 diabetes with AFB_1 toxin	26
6	F, D-2, NTX	Female, type 2 diabetes without AFB_1 toxin	23
7	F, C, TX	Female, control with AFB_1 toxin	25
8	F, C, NTX	Female, control without AFB_1 toxin	22

Biochemical measurement

Each participant had 10 ml of blood collected from a vein with a sterile syringe before being transported in gel tubes to the main lab. After the samples had been allowed to settle for 15 minutes, serum was extracted from them by centrifuging them for 15 minutes at 3000 rpm. Each serum sample was split into two pieces and kept in a -20 C refrigerator prior to analysis. The serum was then transferred using a micropipette into an Eppendrof 1.5-ml container for storage.

The parameters listed below were then evaluated using these serum samples: Using an automated instrument in the hospital, measuring fasting blood sugar (FBS), HBA1c, liver function tests (AST, ALT), and renal function tests (urea, creatinine).

Statistical Analysis: The groups were compared on various levels using the ANOVA table and Duncan test. A P-value of < 0.05 indicates that there is a statistically significant difference between the groups. The chi-square test was used to compare the observed results with the expected results to reveal the relationship between the variables.

Ethical approval: Both the Baghdad Rusafa Health Department and the Ethical Committee of the College of Applied Medical Sciences at the University of Karbala gave their approval to the study protocol. The patients' permission was required in order to collect samples.

P-ISSN: 2664-0562
E-ISSN:2664-0554

Results

Measurement Qualitative of AFB_1 by TLC

The result showed that the number of sample serums collected from patients where contamination with AFB_1 was 46 (49.5%), while the number of sample serums collected from controls was 42 (50.0%), with significant differences between them, as shown in table (2).

Table (2): Distribution of AFB_1 according to patient and control groups by using TLC.

Sample		Positive	Negative	Total
P	F	46	47	93
	%	49.5%	50.5%	100.0%
C	F	42	42	84
	%	50.0%	50.0%	100.0%
Total	F	88	89	177
	%	49.7%	50.3%	100.0%
***Chi-Square Tests; F= Frequency; AFB1= AflatoxinB1 X^2 Calculate = 41.76, X^2 table (0.05) = .005;P=Patient; C=Control**				

While the distribution of AFB_1 according to sex showed that the number of males whose serum blood was contaminated with AFB1 was 37 (42%), the number of females whose serum blood was contaminated with AFB_1 was 51 (58%). Also, the number of males whose serum blood was without AFB_1 was 44 (49%) while the number of females whose serum blood was without AFB_1 was 45 (50.6%), with significant differences between them, as shown in table (3).

Table (3): Distribution of AFB_1 according to Sex by using TLC.

Sex		Positive	Negative	Total
Female	F	51	45	96
	%	58.0	50.6	54.2
Male	F	37	44	81
	%	42.0	49.9	45.8
Total	F	88	89	177
	%	100	100	100
*****Chi-Square Tests; TLC=Thin Layer Chromatography; F= Frequency X^2 Calculate = 40.27, X^2 table (0.05) = .974				

Measurement Quantitative of the AFB_1 by HPLC

AFB_1 was quantitatively measured by using an HPLC device to measure toxin concentrations in the study groups. The results showed that there was a highly significant difference between the study groups, and the *P*-value was <0.001. The highest concentration of toxin was in male patients (4.6 ng /ml) and female patients (4.5 ng/ml). While the highest concentration of toxin was in the control (0.14

P-ISSN: 2664-0562
E-ISSN:2664-0554

ng/ml) for both males and females. These concentrations are considered high compared to healthy people, as shown table (4).

Table (4): Measurement concentration AFB1 by HPLC in patients and control groups

Groups	Mean ng\ml	SD	Duncan test	P-value
M,D-2,TX	3.985	.445	A	.001*
F,D-2,TX	4.016	.401	A	
M,D-2,NTX	.005	.021	B	
F,D-2,NTX	.004	.015	B	
M,C,TX	.140	.006	B	
F,C,TX	.140	.007	B	
M,C,NTX	.010	.011	B	
F,C,NTX	.002	.001	B	
N=Number ;*= significant p< 0.005; The difference between the letters indicates that there is a significant difference between the study groups.				

Measurement of biochemical parameters in study groups

The comparisons between study groups were as follows: AFB_1 positivity was distributed among four groups. It included (M, D-2, TX), (F, D-2, TX), (M, C, TX), and (F, C, TX), compared with other groups, which included (M, D-2, NTX), (M, C, NTX), (F, C, NTX), and (F, D-2, NTX), with significant differences between them. As shown in table (5)

Table (5): Distributed AFB_1 positivity in biochemical parameters.

Groups	Biochemical parameters						
	AST (D.T)	ALT(D.T)	UREA(D.T)	CREAT(D.T)	FBS(D.T)	HBAIC(D.T)	P-value
M,D-2,TX	41.69(a)	41.92(a)	42.58(a)	1.03(a)	225.98(a)	7.645(bc)	0.001*
F,D-2,TX	41.76(a)	40.20(ab)	41.32(a)	1.00(a)	210.53(a)	7.762(b)	
M,D-2,NTX	23.52(c)	25.23(c)	32.35(b)	0.81(b)	183.88(a)	8.596(a)	
F,D-2,NTX	22.34(c)	20.02(d)	30.64(b)	0.75(b)	176.20(a)	7.178(c)	
M,C,TX	36.12(b)	39.39(ab)	32.85(b)	0.83(b)	116.91(a)	5.576(d)	
F,C,TX	36.24(b)	35.90(b)	34.56(b)	0.82(b)	118.44(a)	5.416(d)	
M,C,NTX	15.62(d)	15.45(e)	22.32(c)	0.60(c)	81.25(a)	4.665(e)	
F,C,NTX	18.12(d)	16.66(de)	23.61(c)	0.59(c)	86.89(a)	4.668(e)	

D.T= Duncan test; *=significant; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; HBA1C= Hemoglobin A1C; The difference between the letters indicates that there is a significant difference between the study groups; M,D-2,TX: Male Diabetes Type 2 With AFB1; F,D-2,TX: Female Diabetes Type 2 With AFB1; M,D-2,NTX: Male Diabetes Type 2 Without AFB1; F,D-2,NTX: Female Diabetes Type 2 Without AFB1;M,C,TX: Male Control With AFB1;F,C,TX: Female Control With AFB1; M,C,NTX: Male Control Without AFB1; F,C,NTX: Female Control Without AFB1

P-ISSN: 2664-0562

E-ISSN:2664-0554

Dissection

The results show that AFB_1 was present in the control groups, indicating that the toxin's presence in healthy individuals may cause the development of diabetes mellitus, hepatitis, or kidney disease [9]. Abd AL-Redha *et al*., who discovered a connection between toxins and patients as well as controls, were used to approach these findings [10]. The effect of AFB_1 on patients may be harmful or aggravate their disease, which is the cause. Kadhum et al. found that T2DM increased as AFB_1 concentrations increased. The results show that both males and females were impacted by AFB_1, but females were exposed to it to a greater extent. This is in line with several previous studies, including [11] and [9]. The enzyme glutathione S-transferases, whose activity in males differs from that in females, may be the cause. In a study that assessed GST activity in the human colon, Singhal *et al*., found that males had higher GST activity than females [12].

ALT and AST after ingesting AFB_1, the body quickly absorbs it and delivers it to the liver through the circulatory system [13]. This result was in agreement with the many studies included in [14-16] which shown during their tests that AFB_1-containing groups had higher ALT and AST levels than control groups. Blood urea and serum creatinine are two examples of biochemical indicators that represent the severity of a variety of kidney illnesses. Numerous studies have revealed that AFB_1 occasionally results in severe inflammatory cell infiltration and hemorrhage while also clearly damaging renal tissue. Other studies have shown that long-term administration of AFs damages the kidneys and may also result in toxicosis, cell necrosis, and inflammation. These findings showed elevated levels of urea and creatinine and showed that the kidney was one of the main organs that AFs targeted. Additionally, they suggested that certain metabolites, such as proline, which was proved to be a distinct metabolite in the kidney, might be transported, generated, or destroyed there [17-19]. result is in agreement with the many studies included in [14-17] which showed during their studies an increase in the levels of B. Urea and S. Cr compared to the control groups. The etiology of T2DM and its related metabolic problems is ultimately caused by a number of risk factors, including inflammatory reactions and oxidative stress. Alvarez *et al*. discovered a rise in glucose levels with continuous exposure to AFB_1, and our research supported their findings. When evaluating the connection between metabolic diseases and AFB_1 [20].

Unbalances in the gut microbial metabolism were observed in a recent rodent research following oral administration of AFB_1 [21]. AFB_1 was found to be able to change the gut microbiota in rats in a dose-response way, according to a similar study [21]. The researchers argued that AFB_1 can negatively alter the community structure of the gut microbiota as well as various metabolic pathways involved in gluconeogenesis, the Krebs cycle, and the formation of lactic acid [22].

P-ISSN: 2664-0562
E-ISSN:2664-0554

Conclusion

According to the study's findings, females are more sensitive to AFB_1 than males. An aggravation of diabetes mellitus in the patients and pre-diabetes in the control groups is indicated by elevated levels of HBA1C and FBS in the AFB1-bearing groups of both patients and controls.

The effects of AFB_1 on kidney and liver functions included a rise in the levels of the liver enzymes AST and ALT as well as kidney function markers urea and creatinine.

Conflict of interests

The author declares no conflict of interest.

P-ISSN: 2664-0562
E-ISSN:2664-0554

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P-ISSN: 2664-0562

E-ISSN:2664-0554