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Study the Correlation between Aflatoxin B1 and Diabetes Type 2 and their Effect on the Human Thyroid Gland in Baghdad Province

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Abstract

Diabetes mellitus type 2 (T2DM) is a common metabolic disorder characterized by chronic hyperglycemia. One of the most harmful mycotoxins, AFB1 is more dangerous than cyanide, arsenic, and organic pesticides and is the most toxic and carcinogenic of the aflatoxins. The International Agency for Research on Cancer has evaluated AFB1 as a group I human carcinogen. This study aims to investigate aflatoxin B1 in serum from patients with T2DM and the effect of AFB1 and T2DM on thyroid hormone levels. 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. 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 Each subject had 10 ml of blood collected from a vein, and the serum was separated using centrifugation. These serum samples were subsequently used to perform ELISA tests on T3, T4, and TSH. TLC and HPLC methods for the qualitative and quantitative detection of aflatoxin B1. This study, to our knowledge, is the first to examine how AFB1 affects the thyroid gland in humans. The results demonstrated a relationship between AFB1 both the patient and control groups. Male and female patients had the highest levels of toxin (4.6 ng/ml and 4.5 ng/ml, respectively). In addition, males and females in the control group had the highest level of toxin (0.14 ng/ml). AFB1 also shown a positive correlation with T2DM (r= 0.528) p value (0.001), as well as high levels of TSH, T4, and low levels of T3. The relationship between AFB1 and T2DM was one of synergy. And according to our study findings, females are more sensitive to AFB1 than males. As well, the study found that the AFB1-borne groups had higher TSH and T4 levels and lower T3 levels.

Keywords: *Thyroid Peroxidase (TPO) enzyme, Aflatoxin B1, Triiodothyronine (T3), Thyroxin (T4), Thyroid Stimulating Hormone (TSH), Thin Layer Chromatography (TLC).*

1. Introduction

Diabetes mellitus type 2 (T2DM) is a common metabolic disorder characterized by chronic hyperglycemia. Development is generally brought about by a confluence of two main factors: impaired insulin secretion by pancreatic beta-cells and impaired insulin response in insulin-sensitive

tissues (Roden & Shulman, 2019; Hurtado & Vella, 2019). The global diabetes prevalence in 20–79-year-olds in 2021 was estimated to be 10.5% (536.6 million people), rising to 12.2% (783.2 million) in 2045. Diabetes prevalence was similar in men and women and was highest in those aged 75–79 years. Prevalence (in 2021) was estimated to be higher in urban (12.1%) than rural (8.3%) areas and in high-income countries (11.1%) compared to (5.5%) (Sun et al., 2022).

Many factors lead to T2DM and insulin resistance. One of the factors is constant exposure to contaminated food, which contains a high level of toxins (Firmin et al., 2016). Aflatoxins (AFs) are secondary metabolites produced mainly by the fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, and widely contaminate various types of crops all over the world, such as maize, peanuts, wheat, barley, and rice. Approximately 20 AFs have been identified, and four of them occur naturally, including aflatoxin B_1 (AFB1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) (Rushing & Selim, 2019; Deng et al., 2018).

AFB1 is absorbed into the digestive tract, released into systemic circulation, and affects other organs. Eraslan *et al.*, (2006) who found a significant effect of AFB1 on the thyroid gland in fish.

Thyroid hormones affect development, growth, and metabolic control, therefore being indispensable to normal development and body energy expenditure (Louzada & Carvalho, 2018). Thyroid hormone synthesis is regulated by feedback mechanisms mediated by the hypothalamus-pituitary-thyroid (HPT) axis. Thyroglobulin (Tg) synthesis, thyroid peroxidase (TPO), and active iodide uptake through the sodium/iodide symporter (NIS) are all necessary for the synthesis of thyroid hormone. Tg proteolysis results in the release of the thyroid hormones thyroxin (T4) and triiodothyronine (T3). The thyroid gland releases T4 in substantially greater quantities (in a ratio of about 14:1) than T3 (Babić et al., 2021). In humans, approximately 80% of the amount of TH secreted by the thyroid is in the form of T_4, while 20% is in the active form of T_3 (van der Spek et al., 2017). Given the prevalence of T2DM in Iraq and its many causes and effects on bodily functions, this study was conducted to ascertain the role of tainted food in local markets in the exacerbation of the disease and to assess the likelihood of a connection between AFB1 and T2DM.

2. Materials And Methods

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. 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	Groups	Characteristics Groups	Number of Groups
1	M, D-2, TX	Male, type 2 diabetes with AFB1	20
2	M, D-2, NTX	Male, type 2 diabetes without AFB1 toxin	24
3	M, C, TX	Male, control with AFB1 toxin	17
4	M, C, NTX	Male, control without AFB1 toxin	20
5	F, D-2, TX	Female, type 2 diabetes with AFB1 toxin	26
6	F, D-2, NTX	Female, type 2 diabetes without AFB1 toxin	23
7	F, C, TX	Female, control with AFB1 toxin	25
8	F, C, NTX	Female, control without AFB1 toxin	22

Blood Collection and Storage

For all participants, 10 ml of blood was taken from the vein by sterile syringe and transported in a Gell tube container to the central lab. Samples were settled for 15 minutes and then centrifuged for 15 minutes at 3000 rpm to separate serum. These serum samples were then used to measure fasting blood sugar (FBS) using the Becman-Coulter device, a fully automated device where a tube containing serum was placed in the place designated for it and then pressed on the Start button. It took 10 minutes to obtain the results. Also, qualitative and quantitative detection of serum aflatoxin B1 by TLC and HPLC techniques. According to Kareem *et al*., qualitative analysis of serum AFB_1 by thin-layer chromatography and quantitative detection of serum Aflatoxin B1 by high-performance liquid chromatography (Kareem et al., 2021). Additional, triiodothyronine (T3), thyroxin (T4), and thyroid stimulating hormone (TSH) tests were performed using ELISA techniques.

Data analysis

The IBM SPSS 26 statistical program was used to create the data analysis for this study. The ANOVA table and Duncan test were used to make multiple comparisons between the groups. A *P*-value of < 0.05 indicates that there is a statistically significant difference between the groups. The correlation coefficient (r) was used to show the correlation of the relationship and determine the continuing trend of this relationship. It is expressed as a positive or negative number between -1 and 1. The value of the number indicates the strength of the relationship, and r = 0 means no correlation. The chi-square test was used to compare the observed results with the expected results to reveal the relationship between the variables.

Ethical consideration

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.

3. Results and Discussion

Measurement Qualitative of AFB_1 by TLC

The result showed that the number of sample serums collected from patients where contamination with AFB1 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 AFB1 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 AFB1 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 AFB1 was 51 (58%). Also, the number of males whose serum blood was without AFB1 was 44 (49%) while the number of females whose serum blood was without AFB1 was 45 (50.6%), with significant differences between them, as shown in table (3).

Table 3: Distribution of AFB1 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	49.4	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

AFB1 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 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	
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	.001*
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.

Effect of AFB1 and T2DM on thyroid hormone levels

The results showed a significant decrease in T3 levels in serum groups (M, D-2, TX) and (F, D-2, TX), whose means were (7.37±1.8 and 1.6± 6.7 ng/dl), respectively, compared with (M, D-2, NTX) and (F, D-2, NTX), whose means are (7.64± 2.1 and 7.63±2.3 ng/dl), respectively. Similarly, when comparing groups of (M, C, TX), and (F, C, TX), whose means are (12.7± 1.1 ng/dl) and (12.4± 1.2 ng/dl), compare with (M, C, NTX), and (F, C, NTX), whose means are (14.11 ± 0.42 and 13.82±0.39 ng/dl), as shown in figure (1).

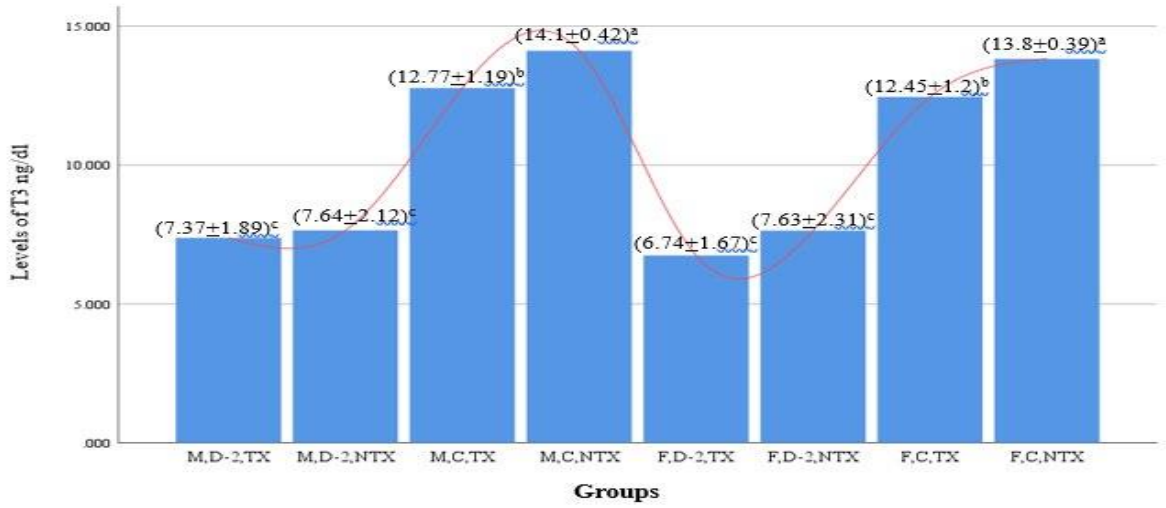

Figure 1: Distribution of T3 according to patient and control groups. The difference between the letters indicates that there is a significant difference between the study groups.

The results showed that T4 levels in the serum of the groups (M, D-2, TX) and (F, D-2, TX), whose means were (268.1 ± 40.6 and 277.9 ± 69.4 ng/dl), respectively, compare with the levels of T4 hormones in the serum of the groups (M, D-2, NTX) and (F, D-2, NTX), whose means ± SD were (241.1 ± 69.4 and 247.7 ± 83.3 ng/dl), respectively. Similarly, when comparing groups of (M, C, TX), and (F, C, TX), whose means ± SD were (180.7 ± 53.2 ng/dl) and (185.3 ± 52.5 ng/dl), respectively, with groups of (M, C, NTX), and (F, C, NTX), whose means are (173.2 ± 36.8 ng/dl) and (174.9 ± 35.9 ng/dl), respectively, with significantly increased between them, as shown in figure (2).

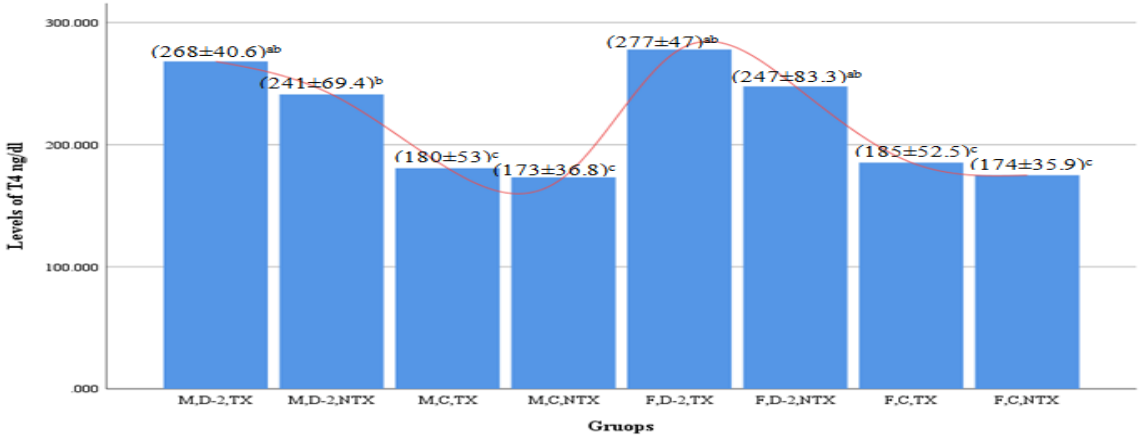

Figure 2: Distribution of T4 according to patient and control groups. The difference between the letters indicates that there is a significant difference between the study groups

Also, the results showed an increase in levels of TSH in blood serum of (M, D-2, TX), group, and (F, D-2, TX), whose means ± SD were (3.48 ± 0.37 and 4.15 ± 1.7), respectively, compare with the levels of TSH hormones in the serum of the groups (M, D-2, NTX) and (F, D-2, NTX) whose means ± SD were 3.23 ± 0.87 uIU/mL and 3.07 ± 0.59 uIU/mL, respectively. Similarly, when comparing groups of (M, C, TX), and (F, C, TX), compare with (M, C, NTX), and (F, C, NTX), with significantly increased between them, as shown in figure (3).

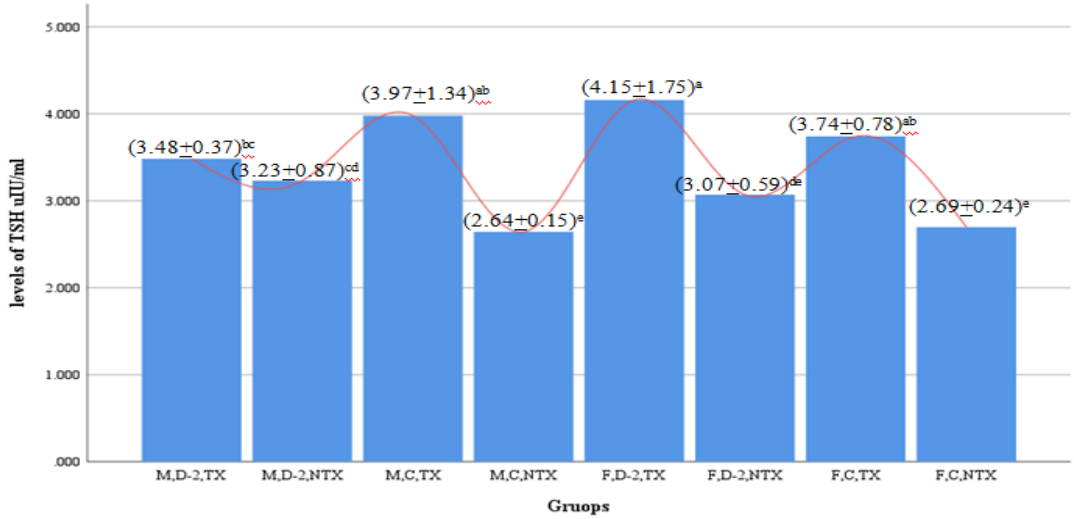

Figure 3: Distribution of TSH according to patient and control groups. The difference between the letters indicates that there is a significant difference between the study groups.

Estimation of correlation coefficient between the AFB1 and T2DM

Table (5) showed strong positive significant correlation between AFB1 and T2DM with P < 0.001 and r = 0.528.

Table 1: Correlation between T2DM and AFB1

Correlation between	Pearson Correlation	*P*-value
T2DM and AFB1	r=0.528**	0.001

Dissection

These results are approached with Abd AL-Redha *et al.*, Who found a relationship between toxins and patients as well as controls (Abd AL-Redha et al., 2017). The reason is due to the effect of AFB1 on patients, which causes damage or may exacerbate the disease. Kadhum *et al.*, reported that T2DM increased with increasing AFB1 concentrations. In the control groups, the results indicate the presence

of AFB1 in them, suggesting that the presence of the toxin in healthy people may lead to diabetes mellitus or the development of hepatitis and kidney disease (Kadhum et al., 2022).

The results indicate both males and females were affected by AFB1, but the results showed that females are more exposed than males, and this is in agreement with many of the studies, including (Abdullah & Aljumaili, 2022) and (Kadhum et al., 2022). Perhaps the reason is due to the activity of the enzyme glutathione S-transferases, whose activity in males differs from that of females.

The short-lived AFB-2,3-epoxide, also known as AFB1-8,9-epoxide (AFBO), which may form adducts with DNA and proteins and cause mutations, is the AF metabolite that gives AFs their cancer-causing effects in humans. The main liver enzymes that transform AFB1 into AFBO are Cytochromes P-450 3A4 and 1A2. The detoxifying enzyme glutathione S-transferase (GST), which catalyzes the conjugation of AFBO with glutathione, protects the liver from the toxic effects. The enzymatic conjugation of AFBO with glutathione S-transferase is one of the primary AFBO detoxification pathways (Awuchi et al., 2021). Singhal *et al*. (1992) reported that GST activity in males was higher than in females in a study that evaluated GST activity in the human colon.

Our study agreed with Alvarez *et al*. (2022) who found an increase in glucose level with continuous exposure to AFB1. When assessing the association between AFB1 and metabolic disorders (Alvarez et al., 2022). Also, another study showed a connection between AFB1 and T2DM, identified an indication that linked AFB1 to an increase in T2DM (Kadhum et al., 2022).

Many of risk factors, particularly inflammatory responses and oxidative stress, ultimately lead to the pathogenesis of T2DM and its associated Metabolic disorders. A study by Akash *et al*, who showed that human exposure to AFM1 toxins (toxins metabolized from AFB1) leads to the development of T2DM by affecting the liver and kidneys and stimulating inflammatory responses and oxidative stress (Akash et al., 2021).

The development of diabetes may, however, be influenced by mycotoxin exposure, as shown by a number of animal studies. As an illustration, a recent study in female rats found that chronic exposure to ochratoxin A (OTA), a mycotoxin related to AFB1, raises blood sugar levels while lowering insulin levels. Additionally, the pancreatic Langerhans islet cells may be harmed by OTA (Mor et al., 2017).

By altering the gut flora and resulting in dysregulation of intestinal function and weakened immune defenses, mycotoxins may also contribute to the onset of diabetes (Liew & Mohd-Redzwan, 2018). The alteration of intestinal barrier functioning and host metabolic and signaling pathways by gut dysbiosis may have a direct or indirect impact on the insulin resistance in diabetes (Wang et al., 2016). According to numerous studies, the gut microbiome's dysbiosis contributes to the quick development of insulin resistance in people with diabetes (Sharma & Tripathi, 2019). After two weeks of oral AFB1 exposure, a recent rodent study showed disturbance of the gut microbial metabolism (Wang et al., 2016). AFB1 was found to be able to change the gut microbiota in rats in a dose-response way, according to a similar study (Wang et al., 2016). The scientists hypothesized that AFB1 can cause harmful alterations in the community structure of the gut microbiota and serious disruption of numerous metabolic pathways involved in gluconeogenesis, the Krebs cycle, and the formation of lactic acid (Zhou et al., 2018).

The result of this study agrees with (Kadhum et al., 2022), who found the concentration of AFB1 in the blood serum of patients with T2DM was 1.34 ng/ml and the concentration of AFB1 in healthy blood serum was 0.13 ng/ml. In another study, our findings are in line with those of Kareem *et al*., who discovered that the concentration ranges of AFB1 in blood samples were 0.68–8.33 ng/mL for unsure CKD patients, 1.21–5.6 ng/mL for certain CKD patients, and 0.11–1.30 ng/mL for healthy controls (Kareem et al., 2021).

The reason for this is due to continuous exposure to contaminated foods, including undercooked meat, vegetables, and many other contaminated foods. Hassan *et al.*, Record high contamination of rice in Lebanon with AFB1. Exposure to AFB1 from rice consumption in Lebanon was calculated as 0.1 to 2 ng/kg of body weight per day (Hassan et al., 2022).

The results approach with Di Paola et al. (2022), who found a decrease in the level of T_3 hormone when fish were exposed to AFB1. Another study conducted on broiler chicks after AFB1 injection showed a decrease in the level of T_3 (Elwan et al., 2021). Also, another study was conducted on ducks after they were exposed to a diet containing AFB1. The results of the study showed a significant decrease in the level of the T_3 hormone (Valchey et al., 2014).

The result approach with study (Eraslan et al., 2006) who found increase in T4 levels of quail when exposed to AFB1. While other studies showed a decrease in T4 levels (Di Paola et al., 2022; Elwan et al., 2021; Valchey et al., 2014).

To the best of our knowledge, this study is the first to show how AFB1 affects thyroid gland levels in humans. The result approach with study Wang et al. (2021), who demonstrated an increase in TSH levels in zebra fish upon exposure to AFB1.

Thyroid hormones regulate many of the body's functions, including metabolism, body temperature, and heart rate. Thyroid hormones include T_3, T_4, and TSH. Malfunctions in these hormones directly affect the general situation of a living being (Shahid et al., 2022). In this study, when the effect of AFB1 toxin on thyroid hormones was examined, a decrease in the level of T3 hormone and a significant increase in T4 hormone were revealed compared to groups that did not contain AFB1. However, changes in T3 and T4 should cause a change in TSH, even if indirectly (Eraslan et al., 2006). Our study showed that TSH was significantly affected by AFB1 compared with control groups. Lower T3 and T4 concentrations stimulate the thyroid gland T3 and T4 receptors, stimulating the synthesis and release of TSH (Valchey et al., 2014). but in this study, there was an increase in levels of T4. The process of converting T4 to T3 in peripheral tissues may have slowed down. While part of the T3 that diffuses into the circulation is created in the thyroid gland, the majority of it is produced in peripheral tissues as a result of the conversion of T4, which is also synthesized in the thyroid gland, into T3. The enzyme principally in charge of this reaction is 5'-deiodinase. On the other hand, 6-phosphogluconate dehydrogenase and malic enzymes also participate in this procedure. This conversion is caused by the NAD to NADP conversion carried out by these enzymes (Noyan, 1993). Selenium plays a function in the metabolism of thyroid hormones, which are crucial for growth and development, according to the fact that type II and type III deiodinases are selenoproteins. In the liver and kidney, this enzyme catalyzes the transformation of T4 into its active metabolite, T3, and selenium deficiency causes an increase in plasma levels of T4 and a corresponding drop in levels of more active T3. When the selenium supply is insufficient, this enzyme receives selenium preferentially rather than glutathione peroxidase. Deficits in selenium and iodine interact, which has effects on livestock productivity, human and animal health, and both. The effects of concurrent iodine insufficiency in humans can either be made worse or better by selenium deficiency (Thomson, 2003). Also, thyroid peroxidase, also known as iodide peroxidase, may be affected by AFB1. TPO is an enzyme that catalyzes the oxidation of iodide to produce iodine atoms, which are then attached to tyrosine residues on thyroglobulin to produce T4 or T3, or thyroid hormones (Habza-Kowalska et al., 2019).

Kalra *et al.,* (2019) who found that T2DM impairs the conversion of thyroxine (T4) to triiodothyronine (T3) in the peripheral tissues. Both hypothyroidism and hyperthyroidism are more common in type 2 diabetes mellitus (T2DM) patients than in healthy humans. Perros *et al*. (1995), who found a decrease in TSH levels in T2DM, whereas our study reported an increase in TSH levels (Perros et al., 1995). Perhaps the reason for this is the low level of T3, which in turn stimulates TSH through the receptors. On the other hand, perhaps because the sample serum from patients collected in this study was newly diagnosed with type 2 diabetes and the proportions of toxins indicated by the HPLC (0.005, 0.004 ng/dl) were influencing the level of TSH in both diabetic males and females. Additionally, BMI was evaluated in patient groups.

4. Conclusion

The relationship between AFB1 and T2DM was one of synergy. According to our study findings, females are more sensitive to AFB1 than males. As well, the study found that the AFB1-borne groups had higher TSH and T4 levels and lower T3 levels.

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Conflict of interest:
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References:

Abd AL-Redha, S., Falah, Z., Ahmed, F., Falah, G., & Hasson, A. (2017). Investigation of Ochratoxin A in blood and it's relationship with Cancer diseases.

Abdullah, H. A. R., & Aljumaili, S. A. R. (2018). Investigation of Patulin in samples blood of persons in Karbala province.

Akash, M. S. H., Haq, M. E. U., Qader, A., & Rehman, K. (2021). Biochemical investigation of human exposure to aflatoxin M1 and its association with risk factors of diabetes mellitus. *Environmental Science and Pollution Research*, *28*, 62907-62918. https://doi.org/10.1007/s11356-021-14871-w

Alvarez, C. S., Rivera-Andrade, A., Kroker-Lobos, M. F., Florio, A. A., Smith, J. W., Egner, P. A., ... & Groopman, J. D. (2022). Associations between aflatoxin B1-albumin adduct levels with metabolic conditions in Guatemala: A cross-sectional study. *Health Science Reports*, *5*(1), e495. https://doi.org/10.1002/hsr2.495

Awuchi, C. G., Ondari, E. N., Ogbonna, C. U., Upadhyay, A. K., Baran, K., Okpala, C. O. R., ... & Guiné, R. P. (2021). Mycotoxins affecting animals, foods, humans, and plants: Types, occurrence, toxicities, action mechanisms, prevention, and detoxification strategies—A revisit. *Foods*, *10*(6), 1279. https://doi.org/10.3390/foods10061279

Babić Leko, M., Gunjača, I., Pleić, N., & Zemunik, T. (2021). Environmental factors affecting thyroid-stimulating hormone and thyroid hormone levels. *International journal of molecular sciences*, *22*(12), 6521. https://doi.org/10.3390/ijms22126521

Deng, J., Zhao, L., Zhang, N. Y., Karrow, N. A., Krumm, C. S., Qi, D. S., & Sun, L. H. (2018). Aflatoxin B1 metabolism: Regulation by phase I and II metabolizing enzymes and chemoprotective agents. *Mutation research/reviews in mutation research*, *778*, 79-89. https://doi.org/10.1016/j.mrrev.2018.10.002

Di Paola, D., Iaria, C., Capparucci, F., Arangia, A., Crupi, R., Cuzzocrea, S., ... & Peritore, A. F. (2022). Impact of mycotoxin contaminations on aquatic organisms: Toxic effect of aflatoxin B1 and fumonisin B1 mixture. *Toxins*, *14*(8), 518. https://doi.org/10.3390/toxins14080518

Elwan, H., Xie, C., Miao, L. P., Dong, X., Zou, X. T., Mohany, M., ... & Elnesr, S. S. (2021). Methionine alleviates aflatoxinb1-induced broiler chicks embryotoxicity through inhibition of caspase-dependent apoptosis and enhancement of cellular antioxidant status. *Poultry Science*, *100*(8), 101103. https://doi.org/10.1016/j.psj.2021.101103

Eraslan, G., Akdoğan, M., Liman, B. C., Kanbur, M., & DELİBAŞ, N. (2006). Effects of dietary aflatoxin and hydrate sodium calcium aluminosilicate on triiodothyronine, thyroxine, thyrotrophin and testosterone levels in quails. *Turkish Journal of Veterinary & Animal Sciences*, *30*(1), 41-45.

Firmin, S., Bahi-Jaber, N., & Abdennebi-Najar, L. (2016). Food contaminants and programming of type 2 diabetes: recent findings from animal studies. *Journal of Developmental Origins of Health and Disease*, *7*(5), 505-512. https://doi.org/10.1017/S2040174416000210

Habza-Kowalska, E., Gawlik-Dziki, U., & Dziki, D. (2019). Mechanism of action and interactions between thyroid peroxidase and lipoxygenase inhibitors derived from plant sources. *Biomolecules*, *9*(11), 663. https://doi.org/10.3390/biom9110663

Hassan, H. F., Kordahi, R., Dimassi, H., El Khoury, A., Daou, R., Alwan, N., ... & Karam, L. (2022). Aflatoxin B1 in Rice: Effects of Storage Duration, Grain Type and Size, Production Site, and Season. *Journal of Food Protection*, *85*(6), 938-944. https://doi.org/10.4315/JFP-21-434

Hurtado, M. D., & Vella, A. (2019). What is type 2 diabetes?. *Medicine*, *47*(1), 10-15. https://doi.org/10.1016/j.mpmed.2018.10.010

Kadhum, G. M., AL_Jumaili, S. A., & AL_Hashemi, H. A. (2022). Investigation of Aflatoxin B1 in blood of diabetes mellitus type 2 patients. *HIV Nursing*, *22*(2), 3632-3634.

Kalra, S., Aggarwal, S., & Khandelwal, D. (2019). Thyroid dysfunction and type 2 diabetes mellitus: screening strategies and implications for management. *Diabetes Therapy*, *10*, 2035-2044. https://doi.org/10.1007/s13300-019-00700-4

Kareem, S. A., Hameed, R. M., AL-Redha, S. A., & Al-Khatt, H. (2021). Aflatoxin B1 as a Threshold Concept of Uncertain Etiology of Chronic Kidney Diseases. *Indian Journal of Forensic Medicine & Toxicology*, *15*(3).

Liew, W. P. P., & Mohd-Redzwan, S. (2018). Mycotoxin: Its impact on gut health and microbiota. *Frontiers in cellular and infection microbiology*, *8*, 60. https://doi.org/10.3389/fcimb.2018.00060

Louzada, R. A., & Carvalho, D. P. (2018). Similarities and differences in the peripheral actions of thyroid hormones and their metabolites. *Frontiers in endocrinology*, *9*, 394. https://doi.org/10.3389/fendo.2018.00394

Mor, F., Sengul, O., Topsakal, S., Kilic, M. A., & Ozmen, O. (2017). Diabetogenic effects of ochratoxin A in female rats. *Toxins*, *9*(4), 144. https://doi.org/10.3390/toxins9040144

Noyan, A. (1993). Yaflamda ve Hekimlikte Fizyoloji. *Hormonlar*, *977*, 1033.

Perros, P., McCrimmon, R. J., Shaw, G., & Frier, B. M. (1995). Frequency of thyroid dysfunction in diabetic patients: value of annual screening. *Diabetic medicine*, *12*(7), 622-627. https://doi.org/10.1111/j.1464-5491.1995.tb00553.x

Roden, M., & Shulman, G. I. (2019). The integrative biology of type 2 diabetes. *Nature*, *576*(7785), 51-60. https://doi.org/10.1038/s41586-019-1797-8

Rushing, B. R., & Selim, M. I. (2019). Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. *Food and chemical toxicology*, *124*, 81-100. https://doi.org/10.1016/j.fct.2018.11.047

Shahid, M.A., M.A. Ashraf, and S. Sharma (2022). *Physiology, Thyroid Hormone.* StatPearls.

Sharma, S., & Tripathi, P. (2019). Gut microbiome and type 2 diabetes: where we are and where to go?. *The Journal of nutritional biochemistry*, *63*, 101-108. https://doi.org/10.1016/j.jnutbio.2018.10.003

Singhal, S. S., Saxena, M., Awasthi, S., Ahmad, H., Sharma, R., & Awasthi, Y. C. (1992). Gender related differences in the expression and characteristics of glutathione S-transferases of human colon. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression*, *1171*(1), 19-26. https://doi.org/10.1016/0167-4781(92)90135-M

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., ... & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes research and clinical practice*, *183*, 109119. https://doi.org/10.1016/j.diabres.2021.109119

Thomson, C.D., (2003). *SELENIUM | Physiology*, in *Encyclopedia of Food Sciences and Nutrition (Second Edition)*, B. Caballero, Academic Press: Oxford. p. 5117-5124.

Valchev, I., Lazarov, L., Hristov, T. S., Kanakov, D., Binev, R., & Nikolov, Y. (2014). Blood triiodothyronine, thyroxine and thyroid-stimulating hormone concentrations in mulard ducks with experimental aflatoxicosis. *Bulgarian Journal of Veterinary Medicine*, *17*(3).

van der Spek, A. H., Fliers, E., & Boelen, A. (2017). The classic pathways of thyroid hormone metabolism. *Molecular and cellular endocrinology*, *458*, 29-38. https://doi.org/10.1016/j.mce.2017.01.025

Wang, J., Tang, L., Glenn, T. C., & Wang, J. S. (2016). Aflatoxin B1 induced compositional changes in gut microbial communities of male F344 rats. *Toxicological Sciences*, *150*(1), 54-63. https://doi.org/10.1093/toxsci/kfv259

Wang, Y., Wang, Q., Ji, C., Guo, X., Yang, G., Wang, D., ... & Chen, C. (2021). Mixture toxic impacts and the related mechanism of aflatoxin B1 and deoxynivalenol on embryonic zebrafish (Danio rerio). *Environmental Sciences Europe*, *33*(1), 1-15. https://doi.org/10.1186/s12302-021-00529-4

Zhou, J., Tang, L., Wang, J., & Wang, J. S. (2018). Aflatoxin B1 disrupts gut-microbial metabolisms of short-chain fatty acids, long-chain fatty acids, and bile acids in male F344 rats. *Toxicological sciences*, *164*(2), 453-464. https://doi.org/10.1093/toxsci/kfy102