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RESEARCH ARTICLE

# Association between Aflatoxin B1 and Type 2 Diabetes Mellitus in Women

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**Abstract**

*Background*: Aflatoxin B1 (AFB1) is a highly toxic and carcinogenic secondary metabolite. It is produced by certain Aspergillus species, especially *A. flavus* and *A. parasiticus*. AFB1 is commonly detected in improperly stored food products, such as maize, peanuts, and grains, particularly in developing countries.
Objective: This study aimed to evaluate the presence and concentration of AFB1 in the serum of women with type 2 diabetes mellitus (T2DM) and to investigate its potential association with glycemic control parameters, specifically fasting blood sugar (FBS) and glycated hemoglobin (HbA1c).
*Methods*: A case–control study was conducted on 100 women aged 35–65 years, including 50 diabetic patients and 50 healthy controls. Serum AFB1 levels were measured using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Glycemic status was evaluated using FBS and HbA1c. Correlation analysis was performed to assess the relationship between AFB1 and glycemic indicators.
*Results*: AFB1 was detected in 90% of diabetic patients and 14% of controls using TLC, with a statistically significant difference ($P < 0.00001$). HPLC analysis showed significantly higher AFB1 concentrations in the diabetic group. Additionally, FBS and HbA1c levels were markedly elevated in diabetic patients. A significant positive correlation was observed between AFB1 concentration and both FBS and HbA1c.
*Conclusion*: The findings suggest that chronic exposure to AFB1 may be associated with poor glycemic control and possibly with the development or progression of T2DM. AFB1 may serve as a potential environmental risk factor for diabetes in vulnerable populations.

## 1. Introduction

Aflatoxin B1 (AFB1) is the most potent and prevalent member of the aflatoxin group, produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus*. It is commonly found in contaminated agricultural products such as maize, peanuts, and cereals, particularly in warm and humid climates with poor storage conditions. The International Agency for Research on Cancer (IARC) has classified AFB1 as a Group I carcinogen because of its strong association with hepatocellular carcinoma [1]. In addition to its hepatotoxic and mutagenic effects, AFB1 exhibits a wide range of systemic toxicities, such as immunosuppressive, teratogenic, and endocrine-disrupting properties. One of the key mechanisms of AFB1 toxicity involves the generation of reactive oxygen species (ROS), leading to oxidative stress, mitochondrial dysfunction, and DNA damage [2,3].

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance (IR), impaired pancreatic β-cell function, and persistent hyperglycemia. It is closely associated with obesity, physical inactivity, and chronic low-grade inflammation [4]. Oxidative stress plays a pivotal role in the pathogenesis of T2DM, as hyperglycemia-induced ROS production contributes to β-cell dysfunction, insulin resistance, and the activation of pro-inflammatory cytokines, such as (TNF-α) and (IL-6). These disturbances lead to long-term complications, such

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as cardiovascular diseases, nephropathy, retinopathy, and neuropathy [5].

The overlapping mechanisms of oxidative stress and inflammation in both AFB1 toxicity and T2DM pathophysiology have prompted investigations into a potential connection between these two conditions. Experimental evidence demonstrated that AFB1 exposure impairs insulin sensitivity and promotes β-cell dysfunction through oxidative damage [6]. In animal models, chronic AFB1 intake has been linked to altered glucose metabolism and endocrine disruption. Furthermore, epidemiological data from aflatoxin-endemic regions suggest a higher prevalence of poor glycemic control in individuals with elevated AFB1 levels [7].

## 2. Materials and Methods

### 2.1. Study design and subjects

This case–control study was conducted comprising 100 females aged 35–65 years. The participants were divided into two groups: 50 patients were diagnosed with T2DM and 50 were healthy individuals, considered as the control group. All participants provided written informed consent, and the study was approved by the local ethics committee.

### 2.2. Selection criteria of patient

Patients were selected from the Al-Hassan Metabolism Endocrine and Diabetes Center (HMEDC), Karbala, Iraq. This study comprised 50 DMT2 patients. Key sociodemographic characteristics, duration of diabetes, dietary habits, familial history of chronic illnesses, and the overall health condition were evaluated by a questionnaire.

### 2.3. Control criteria

The control group comprised 50 healthy females. They were chosen from a pool of recognized voluntary participants. Blood samples were collected from volunteers, who were free from any chronic condition. Additionally, age of the study group was convergent. The self-reported technique (student's inquiry) was also used to gather demographic data of participants.

### 2.4. Exclusion criteria

The research generally excluded people with thyroid illness, DMT1, cardiovascular diseases, and hypertension. The research excluded patients who were currently on any medication.

### 2.5. Sample collection

Venous blood samples were collected from each subject after an overnight fasting. Blood was drawn using standard venipuncture technique into serum separator tubes. The samples were allowed to clot and then centrifuged at 3,000 rpm for 10 min. The resulting serum was aliquoted and stored at -20°C. In all, 10 mL of blood was extracted from each participant utilizing a sterile syringe and thereafter transferred to the central laboratory in gel and (EDTA) tubes.

### 2.6. Biochemical Analysis

Fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) were measured using an automated clinical chemistry analyzer (Abbott Architect c4000, Abbott Diagnostics, Abbott Laboratories, USA).

### 2.7. Aflatoxin B1 Analysis

Serum levels of AFB1 were measured using high-performance liquid chromatography (HPLC) combined with thin layer chromatography (TLC) as a confirmatory technique.
TLC method: AFB1 was analyzed using TLC according to the method described by Sirhan and Al-Jumaily [7]. The analysis was performed using silica gel 60 F254 plates (Chmlab, Spain). The solvents used were chloroform and methanol of analytical grade. Standard AFB1 was used for calibration. Proteinase K kit was used during sample preparation.

HPLC method: AFB1 was quantified using HPLC (Sykam, Germany). All solvents used in HPLC analysis were of analytical grade and followed the procedure described by [8].

### 2.8. Statistical analysis

Data were analyzed using IBM SPSS Statistics v23. Descriptive statistics (mean ± SD) were used. Normality and homogeneity were assessed using Shapiro–Wilk and Levene's tests, respectively. Between-group comparisons were conducted using independent *t*-test or Mann–Whitney U test, depending on data distribution. One-way ANOVA with Scheffe's or Duncan's *post hoc* test was used for multiple group comparisons. Chi-squared ($\chi^2$) and Pearson's correlation were applied where appropriate. Receiver operative characteristic (ROC) curve analysis was performed to determine optimal cut-off values, with AUC and Youden's index used to evaluate diagnostic performance. Graphs were created using GraphPad Prism 9; $P < 0.05$ was considered statistically significant.

### 2.9. *Ethical research approval*

This study was approved by the ethics committee of the Department of Clinical Laboratories, College of Applied Medical Sciences, University of Karbala (Ref. No.: CLAMSKU/11).

Samples were collected for the study after the Ministry of Health and Environment granted permission to conduct the same. The study participants gave their permission to collect socio-demographic data as well as undertake experiments on the selected samples while maintaining patient confidentiality.

## 3. Results and Discussion

### 3.1. Qualitative detection of AFB1

The results showed a statistically significant difference ($P$ = 0.002) in AFB1 detection between patients with T2DM and healthy controls. AFB1 was detected in 90% of diabetic patients, compared to only 14% of controls, as shown in Table 1.

The findings of this investigation concurred with those of [7], who reported a prevalence of Aflatoxin B1 (AFB1) contamination of 49.5% in diabetic patients and 42% in controls, Notably, the current study suggests a higher prevalence of AFB1 contamination in females, emphasizing their relevance in the present research.

Kadhum et al. [9] also reported that the prevalence of T2DM increased with elevated concentrations of AFB1. Furthermore, the detection of AFB1 in samples from the control group suggests that exposure to this toxin in apparently healthy individuals may play a role in the onset of diabetes mellitus or contribute to the development of hepatic and renal disorders.

Furthermore, prior research has demonstrated that prolonged exposure to aflatoxins, especially AFB1, correlates with oxidative stress, inflammation, and pancreatic β-cell dysfunction, which are critical pathophysiological pathways contributing to the development of T2DM. The AFB1 is recognized for producing ROS and inducing lipid peroxidation, which eventually disrupts insulin secretion and function [10].

### 3.2. Quantitative Measurement of AFB1

Aflatoxin B1 was quantitatively determined using HPLC device to assess toxin levels in the study groups. The findings revealed a highly significant difference between the groups ($P$ = 0.00001). The highest concentration was observed in the "patient with toxin" group (4.547 ng/mL), followed by the "control with toxin" group (0.119 ng/mL). These values are considered elevated when compared to the "control without toxin" group, as illustrated in Table 2.

In this study, HPLC was chosen on the basis of its high sensitivity, specificity, and reproducibility in the detection of AFB1, as outlined by [11]. Furthermore, its effectiveness in detecting aflatoxins within biological matrices, such as serum samples, was highlighted in the research conducted by Beyene *et al.*, [12] supporting its application for accurate assessment of human exposure to aflatoxins.

Furthermore, the results of this study agreed with Sirhan and Al-Jumaily [9], who found that exposure to AFB1 was associated with the worsening of T2DM by increasing insulin resistance and promoting oxidative stress, contributing to diabetic complications. Concentration of AFB1 in the blood serum of patients with T2DM was 1.34 ng/mL.

This conclusion aligned with the findings of Mehrzad *et al.,* [13] who investigated the effects of naturally occurring concentrations of AFB1 on the pancreatic islets of various healthy mice following exposure to 10–20 ng/mL.

As a result, it is essential to monitor AFB1 contamination in diabetic patients because it may represent a modifiable environmental risk factor that exacerbates the clinical outcome of T2DM.

### 3.3. Assessment of HbA1c and fasting blood glucose test in study groups

The results revealed statistically significant differences in HbA1c levels among the studied groups. The distribution of AFB1 in the blood serum HbA1c levels was observed in the patient with toxin and control with toxin groups, with mean values of 8.644% and 5.220%, respectively. In contrast, the patient without toxin and control without toxin groups showed mean values of 8.720% and 5.430%, respectively. A highly significant difference was found between the groups ($P$ = 0.00002), as presented in Table 3.

The results also showed significant differences ($P$ = 0.00002) in FBS levels among the studied groups. The patient with toxin group exhibited the highest mean glucose level (197.378 ng/dL), followed by the patient without toxin) group (173.400 ng/dL). In contrast, both control groups with and without toxin showed comparable and significantly lower levels (104.100 ng/dL and 106.800 ng/dL, respectively), as shown in Table 4.

This study concurs with Sirhan and Al-Jumaily [9], and demonstrates that exposure to AFB1 worsens T2DM and perhaps induce pre-diabetes in healthy persons.

Recent scientific research indicates a strong correlation between exposure to AFB1 and the onset of metabolic diseases, particularly T2DM. Research done by Goessens *et al.* [14] indicates that prolonged exposure to AFB1 is

**Table (1):** Distribution of AFB1 Among Patient and Control Groups Utilizing TLC.

| Case | | samples with AFB1 | samples without AFB1 | Total | P-value |
|---|---|---|---|---|---|
| Patient | | 45 | 5 | 50 | 0.002* |
| | % | 90% | 10% | 100.0% | |
| Control | | 7 | 43 | 50 | |
| | % | 14% | 86% | 100.0% | |
| Total | | 52 | 48 | 100 | |
| | % | 52% | 48% | 100% | |

*= significant p< 0.001; is a significant difference between the study groups.

**Table (2):** Concentration measurement of AFB1 using HPLC in patient and control cohorts.

| Groups | Mean (ng/mL) | Std. deviation | Duncan's test | *P* value |
|---|---|---|---|---|
| Patient with AFB1 | 4.547 | 0.487 | B | 0.00001* |
| Patient without AFB1 | 0.000 | 0.000 | A | |
| Control with AFB1 | 0.119 | 0.008 | A | |
| Control without AFB1 | 0.000 | 0.000 | a | |

**Table (3):** Distribution of HbA1c levels in the patient and control groups.

| Groups | Mean (%) | Std. deviation (SD) | Duncan's Test | *P* value |
|---|---|---|---|---|
| Patient with AFB1 | 8.644 | 2.161 | B | 0.00002* |
| Patient without AFB1 | 8.720 | 1.504 | B | |
| Control with AFB1 | 5.220 | 0.601 | A | |
| Control without AFB1 | 5.430 | 0.600 | A | |

**Table (4):** Distribution of fasting blood glucose levels in the patient and control groups.

| Groups | Mean (mg/dL) | Std. deviation (SD) | Duncan's test | *P* value |
|---|---|---|---|---|
| Patient with AFB1 | 197.378 | 85.756 | B | 0.00002* |
| Patient without AFB1 | 173.400 | 63.948 | B | |
| Control with AFB1 | 104.100 | 12.697 | A | |
| Control without AFB1 | 106.800 | 13.270 | A | |

associated with elevated blood glucose levels, indicating its potential involvement in the development of metabolic disorders such as diabetes.

A research conducted in Iraq demonstrated a correlation between AFB1 exposure and a heightened prevalence of T2DM, revealing a statistically significant link between increasing AFB1 levels and T2DM diagnosis [9].

Akash *et al.* [15] showed that exposure to AFM1, a metabolic product of AFB1, causes oxidative stress and inflammatory reactions, which directly harm the liver and kidneys and play a role in the development of diabetes.

On the other hand, AFB1 has been shown to cause an imbalance in the gut microbiome, leading to a weakened intestinal barrier and disturbances in metabolic and immune pathways, which may contribute to increased insulin resistance and the development of T2DM [16]. This mechanism is supported by recent studies [17], which confirmed that gut microbiome dysbiosis directly contributes to the acceleration of insulin resistance in diabetic patients. A rodent study showed that oral exposure to AFB1 for 2 weeks caused significant disruption of the gut microbiota, leading to disturbances in glucose metabolism, Krebs' cycle, and lactic acid formation, supporting the relationship between AFB1 and diabetes-related metabolic changes [18].

### 3.4. Estimating the correlation coefficient between AFB1 and T2DM

The correlation coefficient revealed a statistically significant positive association between AFB1 measurements and both HbA1c levels (r = 0.347, *P* = 0.020) and FBS (r = 0.319, *P* = 0.033).

### 3.5. Assessment of receiver operative characteristic curve analysis

The overall area under the curve (AUC) values for AFB1, FBS, and HbA1c were 93.750%, 90.700%, and 96.725%, respectively. These parameters were considered reliable indicators for predicting the increased risk of developing T2DM, highlighting their value in early detection and risk assessment. The sensitivity and specificity of HbA1c were 92.000% and 97.500%, respectively. For FBS, the sensitivity and specificity were 78.000% and 97.500%, while for AFB1, they were 90.000% and 97.500%, respectively (see Table 5).

These statistically significant markers serve as diagnostic tools for identifying positive cases of the condition, as demonstrated by the ROC curves plotted based on sensitivity versus specificity.

The sensitivity and specificity of AFB1 were 92.000% and 97.500%, respectively, at a chosen cut-off point. This result confirmed that this test was an excellent indicator for detecting T2DM (Figure 1–3).

The sensitivity and specificity of FBS were 78.000% and 97.500% respectively, at a chosen cut-off point. This result also confirmed that this test was a good indicator for detecting T2DM (Figure 4).

Aflatoxin B1 toxin was considered a reasonably excellent indicator for T2DM patients with a sensitivity of 90.000% and specificity of 97.500% and at a chosen cut-off

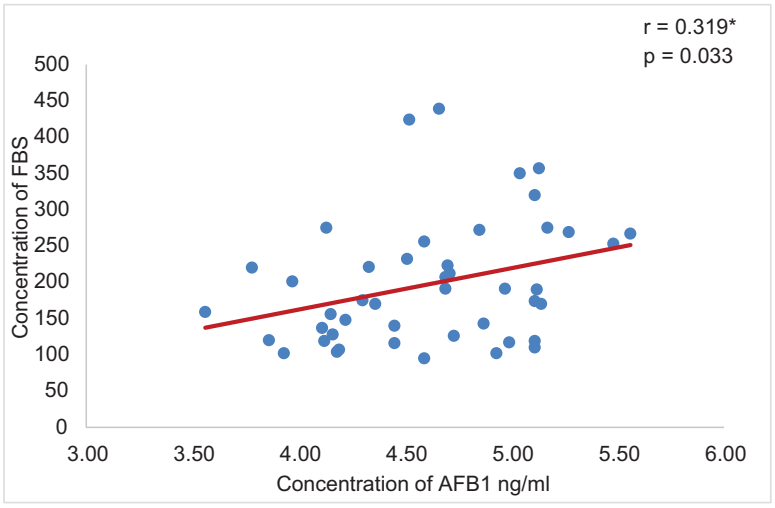


**Figure (1):** Estimation of correlation between AFB1 and FBS.

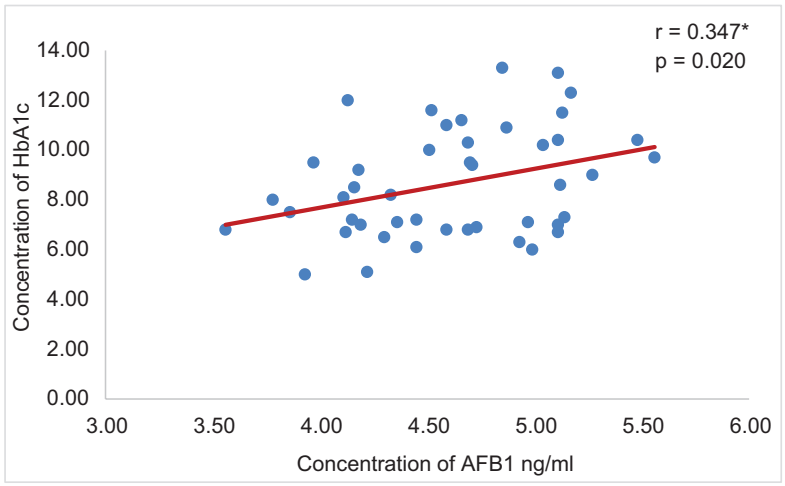


**Figure (2):** Estimation of correlation between AFB1 and HbA1c.

**Table (5):** Receiver operating characteristic curves generated based on model predictions using the studied research parameters.

| Metrics | | HBA1C | FBS | AFB1 |
|---|---|---|---|---|
| Std. error (SE) | | 0.019 | 0.031 | 0.029 |
| Asymptotic sig. | | 0.003 | 0.004 | 0.001 |
| Asymptotic 95% confidence interval (CI) | Lower bound | 0.929 | 0.846 | 0.881 |
| | Upper bound | 1.000 | 0.968 | 0.994 |
| Cut-off point | | 6.250 | 124.500 | 0.130 |
| Area under curve (AUC) | | 96.725% | 90.700% | 93.750% |
| Sensitivity | | 92.000% | 78.000% | 90.000% |
| Specificity | | 97.500% | 97.500% | 97.500% |

point; this statistically significant marker could be used to diagnose positive cases of the condition (Figure 5).

### 3.6. Study Limitations

This study has several limitations. First, the sample size was relatively small and limited to a specific geographic region, which could affect the generalizability of the findings. Second, the study relied on a single time-point measurement of AFB1 and hormonal parameters, which could not reflect long-term exposure or dynamic changes. Third, potential confounding factors, such as diet, environmental exposure, and genetic predisposition, were not fully controlled or assessed. Additionally, the cross-sectional nature of the study prevented in establishing causal relationships between AFB1 exposure and alterations in

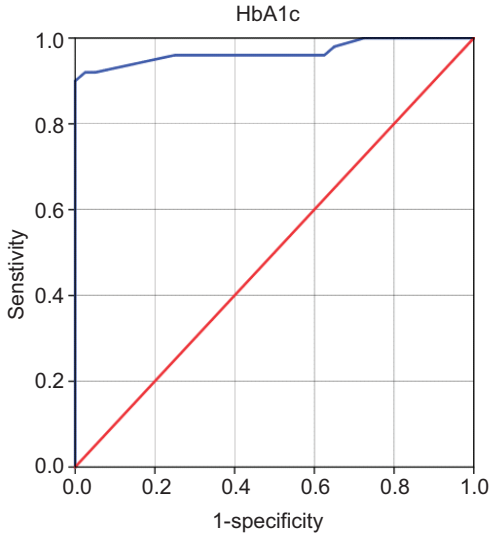


**Figure (3):** Model-predicted ROC curve based on HbA1c parameter.

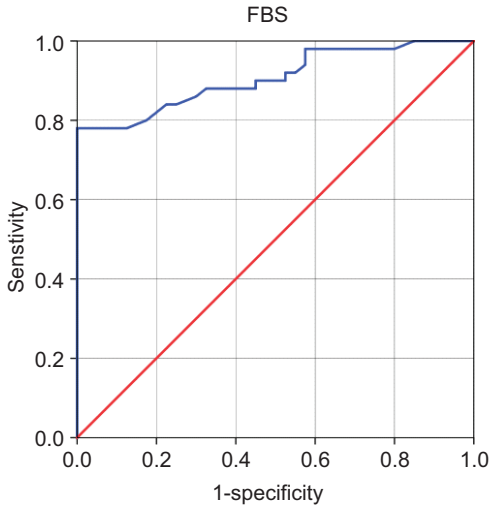


**Figure (4):** Model-predicted ROC curve based on FBS parameter.

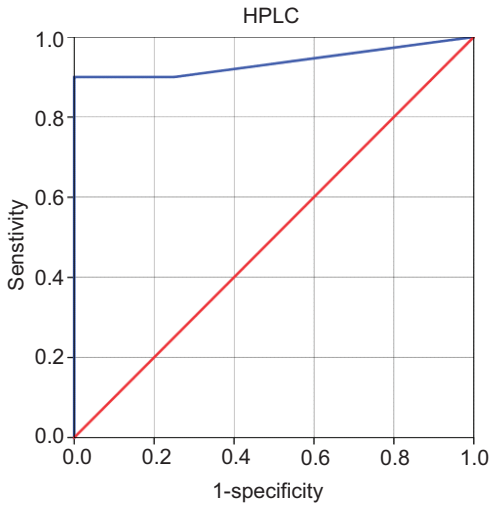


**Figure (5):** Model-predicted ROC curve based on AFB1 parameter.

glucose metabolism or hormone levels. Future studies with larger sample sizes, longitudinal designs, and broader environmental assessments are recommended to validate and expand upon these findings.

### 3.7. Recommendations

Investigating the impact of AFB1 on the human immune system is recommended as well as studying its potential role in the development of liver and kidney cancers. Since the main source of human exposure to AFB1 is the consumption of contaminated food, it is essential to ensure the safety of both local and imported food products by strictly regulating and preventing the import of items contaminated with fungi, especially those belonging to the genera Aspergillus and Fusarium. Moreover, improving the infrastructure for storing staple foods, such as wheat, rice, corn, and other cereals, is crucial to minimize fungal contamination and reduce public health risks.

## 4. Conclusions

The present study demonstrates a markedly higher rate and concentration of AFB1 among patients with T2DM, compared to healthy controls. AFB1 was detected in 90% of diabetic patients, while only 14% of the control group showed detectable levels. In addition, quantitative analysis revealed that the mean serum concentration of AFB1 in the diabetic group was 4.547 ng/mL, significantly exceeding that of the healthy group, which was 0.119 ng/mL. These findings suggested that chronic exposure to AFB1 could play a contributory role in the development or progression of T2DM, either independently or through interaction with other metabolic and environmental factors.

Given the widespread exposure to AFB1 in certain regions, these findings highlight a potential public health risk and underscore the need for routine monitoring of dietary aflatoxin levels, especially in vulnerable populations.

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