

Original Research Paper

Studying of Toxic Effects of Zearalenone in Women Who exposed Abortion

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Abstract: Abortion describes a state in which fetuses are prematurely expelled before the natural end of gestation. Zearalenone (ZEN) is a prevalent mycotoxin contamination found in food sources worldwide, and it is a secondary metabolite of *Fusarium* fungus. Numerous investigations have revealed ZEN concentrations in food items surpassing the maximum contamination limits set by the European Union (EU) (100–200 µg/kg for unprocessed cereals and 75 µg/kg for processed cereals). This study aims to look at whether zearalenone toxin is present in the serum of women who have had abortions and those without abortions and how it affects various physiological and biochemical markers. The study was designed using a case-control study methodology. It was carried out between 1 / 10 /2023 and 1 / 3 / 2024 in the laboratories of the Department of Pathological Analysis / College of Applied Medical Sciences / University of Karbala and the laboratories of Science and Technology / Department of Environment and Water/Ministry of Science and Technology / Baghdad Governorate. A blood specimen was collected from patients with recurrent abortion and a similar sample was from pregnant women without abortion. The results showed that the percentage of women with miscarriages who carried zearalenone toxin was 56.5%. In comparison, the percentage of samples contaminated with the same toxin in women without miscarriage was (0.0%). The results of the quantitative analysis of zearalenone toxin by HPLC additionally demonstrated that the zearalenone concentration in the serum of patients with miscarriage and at the ages of (15-24), (25-34) and (35-45) years was (40.66, 42.50 and 41.82) ng/ml respectively, while the comparison groups and the three age groups were free of zearalenone toxin. The present study provides the existence of Zearalenone toxin in the blood serum of aborted women with high concentrations. The correlation between Zearalenone toxin and abortion proved that toxins that exist in blood serum lead to abortion.

Keywords: Zearalenone, Abortion, Toxic effects, Biochemical parameters

1. Introduction

An abortion is a medical operation that involves ending a pregnancy, usually during the first or second trimester [1]. Foetal chromosomal disorders are a primary cause of early pregnancy loss, and this is one of the reasons [2] or it might be carried out to protect the mother's physical or mental well-being or to end a pregnancy that carries a significant risk of serious foetal defects [3]. While there are several contributing factors to the majority of unfavourable pregnancy outcomes, such as miscarriage, maternal nutrition is one of them. Additionally, foods pregnant women eat may contain harmful contaminants such as mycotoxins [4]. Small organic compounds known as mycotoxins are produced by some fungal species as secondary metabolites, and they can infect a variety of agricultural products, including corn, cereals, nuts, and coffee beans. The main producers of these poisons are fungal genera. In cereal crops, *Aspergillus* and zearalenone (ZEA) are the most common mycotoxins [5]. Zearalenone is a mycotoxin that is largely produced by *Fusarium* species, notably *F. graminearum*, which is known to contaminate a wide range of foods and feeds [6]. ZEN causes endocrine and reproductive system disruption, which results in infertility, phenotypes similar to polycystic ovarian syndrome, and loss of pregnancy [7]. Environmental exposure to chemicals imitating endogenous estrogens, so-called xenoestrogens, may result in various detrimental consequences in diverse human organs, including the reproductive tract and the neurological system [8]. Additionally, it has hepatotoxic and nephrotoxic effects and promotes the growth of hepatocarcinoma [9].

2. Methodology

Study Design

The case-control study was conducted from October 2023 to March 2024. Samples were selected from patients attending the Obstetrics and Gynecology Teaching Hospital/Karbala.

Study Sample

A total of 100 people were studied (50) of whom were miscarriage patients and (50) of whom were a control

group. These totals are divided according to (Table 1).

Table (1) Division of study groups.

NO.	Study Groups	Characteristics Groups
1	PA ₁ TX	Patients (15-24) years with ZEN Toxin
2	PA ₂ TX	Patients (25-34) years with ZEN Toxin
3	PA ₃ TX	Patients (35-45) years with ZEN Toxin
4	PA ₁ NTX	Patients (15-24) years without ZEN Toxin
5	PA ₂ NTX	Patients (25-34) years without ZEN Toxin
6	PA ₃ NTX	Patients (35-45) years without ZEN Toxin
7	HA ₁ NTX	Healthy (15-24) years without ZEN Toxin
8	HA ₂ NTX	Healthy (25-34) years without ZEN Toxin
9	HA ₃ NTX	Healthy (35-45) years without ZEN Toxin

Biochemical Measurement

5 ml of blood was drawn from each participant's vein using a sterile syringe, 4 ml of which was placed in a Gel Tube and 1 ml in an EDTA Tube and all were brought to the main laboratory at the refrigerator. The serum was separated from the samples by centrifugation for 5 minutes at 5000 rpm after settling for 5 minutes. Before analysis, each serum sample was divided into two parts and placed in a refrigerator set at -20°C. Next, the serum was transferred with a micropipette to a plain tube for storage.

Statistical Analysis

The groups were compared on various levels using The ANOVA table and the Duncan test was used to make multiple comparisons between the groups of this study, P-values of < 0.05 and 0.01 indicate that there is a statistically significant difference between the groups.

3. Results and discussion

Measurement Qualitative of Zearalenone by TLC

Distribution of ZEN according to patients and control groups

The results showed that the percentage of samples serum collected from patients with abortion contaminated with ZEN was (56.5 %) while the percentage of samples serum collected from healthy was (0.0 %) with significant differences between them (Table 2).

Table (2): Distribution of patients and control according to ZEN's existence in blood serum detected by TLC.

Case	With Toxin	Without Toxin	P-Value
Patients	(56.5%)	(43.5%)	0.001**
Control	(0.0 %)	(0.0%)	

Distribution of ZEN according to Age groups.

While The distribution of ZEN according to age showed that the number of blood serum collected from patients at the age (of 15 - 24) year was 38% while the number of blood samples collected from women aged (25 - 34) year and (35 - 45) whose serum blood was contaminated with ZEN was 30.8% for them without significant different between all study groups (Table 3).

Table (3): Distribution of patients with abortion and exist of (ZEN) Toxin and patients with abortion without (ZEN) Toxin detected by TLC

Age groups	Patients with ZEN toxin (No and percentage)	Patients without ZEN toxin (No and percentage)	P-Value
15- 24	(38 %)	(30.0 %)	0.694
25- 34	(30.8 %)	(40.0 %)	
35- 45	(30.8 %)	(30.0 %)	

Measurement Quantitative of Zearalenone by HPLC

The results showed that the concentration of ZEN in blood serum of (PA1T) (PA2T) and (PA3T) groups were 40.66 ng/ml, 42.50 ng/ml and 41.82 ng/ml respectively while in the groups without toxins and Healthy groups (control) the blood serum of them not exist ZEN (0.00 ng/ml) with a significant difference at the 0.05 level (Table 4).

Table (4): The Comparisons between groups according to the concentration of Toxin in patients and their controls.

NO.	Groups	Mean (ng/ml)	Std. Deviation	Duncan Test	P-value
1	PA ₁ T	40.66	2.82	b	0.003**
2	PA ₂ T	42.50	4.75	b	
3	PA ₃ T	41.82	4.11	b	
4	PA ₁ NT	0.00	0.00	a	
5	PA ₂ NT	0.00	0.00	a	
6	PA ₃ NT	0.00	0.00	a	
7	HA ₁	0.00	0.00	a	
8	HA ₂	0.00	0.00	a	
9	HA ₃	0.00	0.00	a	

The difference between the litters indicates that there is a significant difference between the study groups.

Also, the results appear to be a significant difference between the patients groups with toxin compared with patients without ZEN toxin (Table 5)

Table (5): The Comparisons between groups according to the concentration of toxin in patients with toxin and patients without toxin

NO	Groups	Mean	Std. Deviation	Duncan Test	P-value
1	PA ₁ T	40.66	2.82	b	0.007
2	PA ₂ T	42.50	4.75	b	
3	PA ₃ T	41.82	4.11	b	
4	PA ₁ NT	0.00	0.00	a	
5	PA ₂ NT	0.00	0.00	a	
6	PA ₃ NT	0.00	0.00	a	

The difference between the litters indicates that there is a significant difference between the study groups.

Estimation of Correlation Coefficient (r) between Zearalenone Toxin and Abortion.

Table (6): Correlation between ZEN and abortion disease.

Disease	Zearalenone levels	P. value
	Coefficient (r)	
Abortion	0.625**	0.003

Correlation is significant at the 0.01 level.

Discussion

Measurement Qualitative of Zearalenone by TLC

Distribution of ZEN according to patients and control groups

The current study's findings are related to a study conducted in 2023 by Sirhan, who discovered that 46 (49.5%) of the serum samples collected from patients had Aflatoxin B1 contamination, while 42 (50%) of the serum samples collected from controls had Aflatoxin B1 contamination [10]. Another investigation showed that 99 (99%) of the patient samples with blood serum included Ochratoxin A, with only one patient lacking Ochratoxin A. Conversely, thirty-two (32%), blood serums were taken from the healthy [11].

Also, the results of the present study are approached with another study which found that 90 out of 100 samples rated 90% of blood samples collected from patients infected with chronic kidney disease of uncertain aetiology contaminated with Ochratoxin A have a significant difference of blood samples contaminated with Ochratoxin A (30%) they were collected from healthy. Other hand it was found the number of blood samples collected from female patients infected with Ochratoxin A was 48.8% [12].

Distribution of ZEN according to Age groups.

According to the available review, there is no study similar to this study and therefore no comparison is possible. Therefore mycotoxins are usually unable due to their small molecular weight reaching 10^4 Dalton. This means that the immune system is unable to destroy and neutralise these mycotoxins including Zearalenone. Thus, the percentage of presence of Zearalenone toxin in the blood of human, including females is dose regardless

of the category of age [13]. Another study found the highest percentage of blood samples contaminated with patulin toxin was 36% that collected from persons in the age group 70> Years [14].

Measurement Quantitative of Zearalenone by HPLC

These results approached of other studies showed that the concentration of Ochratoxin A in the blood serum of females and males infected with chronic kidney disease was 7.015 ng/ml and 7.071 ng/ml respectively [15]. Also, another study found that Aflatoxin B1 in the blood serum of females and males with diabetics and AfB1, the toxin was 4.016 ng/ml and 3.98 ng/ml respectively [16]. Another study showed that the concentration range of AfB1 in the blood serum of patients with chronic kidney disease was 0.68-8.33 ng/ml and 0.11-1.30 ng/ml for healthy (Control) [17]. Another study appearance that the concentration of Ochratoxin A in blood serum was 23.47 ng/ml [11].

Estimation of Correlation Coefficient (r) between Zearalenone Toxin and Abortion

The measurement correlation Coefficient between abortion occurrence and ZEN toxin appearance showed a moderate positive Correlation $r = 0.625$ and $P < 0.003$, as shown in (Table 6). The correlation between Zearalenone toxin and abortion proved that toxins that exist in blood serum lead to abortion.

Conclusion

The current positive association between zearalenone and miscarriage was synergistic. According to the results of the current study, patients with miscarriages were contaminated with zearalenone at a high level compared to healthy people not contaminated with the toxin

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Authors' Contributions

Hussein Mohammed Hussein: Conceptualization, methodology, formal analysis, writing -original draft preparation, investigation, visualization, resources, methods and materials. Prof. Sami Abd AL-redha Ali, Assist. Prof. Hamida Hadi Abd AL-Wahed: Supervision, project administration, writing -review & editing.

Ethics

The study procedure was authorised by the University of Karbala's College of Applied Medical Sciences Ethical Committee and Karbala Health Department. sample collection has required consent from the patients

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