Aflatoxin B1 as a Threshold Concept of Uncertain Etiology of Chronic Kidney Diseases

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Abstract

A case control study was conducted in karbala province to investigate aflatoxin B1 (AFB1) exposure among the chronic kidney disease (CKD) patients and healthy control. AFB1 level were measured qualitatively by Thin layer chromatography and quantitatively by high-performance liquid chromatography.The assessment of positive AFB1 samples were evaluated along with biomarkers of renal functions tests.

The results showed that the investigated population were exposed to Aflatoxins. AFB1 was detected in 100% of uncertain CKD patients and 24%, 20% in certain CKD patients and healthy control respectively. The concentration ranges in serum samples were 0.68 –8.33 ng/mL for uncertain CKD patients, 1.21-5.60 ng/mL for certain CKD patients and 0.11- 1.30 ng/mL for healthy control. The un-certain etiology of CKD patients had a significant associations of decreasing GFR and increasing the levels of urea, creatinine with positive serum AFB1. This association was also highly interest with regard to potential interactions with Urea levels in the control group. The measurement of the AFB1 in serum samples of CKD patients and healthy control were indicated a long term exposure to the toxin which result in uncertain etiology of CKD. The effect of AFB1 exposure was confirmed through the assessment of the biochemical marker of renal tests. This study can be a good establish of a national AFs exposure monitoring programs. Also the study highlighted the needing to identify the pathogenesis of contribution AFB1 in the increasing number of uncertain CKD patients. Future study is encouraged to focus on broader areas which cover the whole of country.

Keywords: *chronic kidney disease, aflatoxins, renal damage, Idiopathic chronic kidney disease*

Introduction

Chronic kidney disease results from progressive scarring in the kidney of any cause. It is characterized by various metabolic and electrolyte abnormalities such as hyperphosphatemia, dyslipidaemia, and metabolic acidosis. However, it is often asymptomatic until the most advanced stages, when symptoms of uraemia develop [(1)].

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Diabetes and hypertension are the main causes of CKD in all high-income and middle-income countries, and also in many low-income countries [(2)].

CKD of uncertain etiology (CKDu) is a term that has been used to describe CKD that is not attributable to any traditional risk factor, such as diabetes, hypertension, or HIV. CKDu is being reported with increasing frequency across the globe, and in many parts of Central America, eastern Europe, and south Asia, it is being reported in epidemic proportions [(3).]

CKDu was suggested to be an environmentally induced disease in the first epidemiological study carried out in 2007, which aimed to identify potential risk factors of CKDu [(4)]. Environmental factors, such as heavy metal

exposures , high seasonal temperatures , agrochemical use, mycotoxins , contaminated water supplies, and snake bite , have all been studied as potential causes of CKDu [5].

Aflatoxins are poisonous by-product of fungi mainly Aspergillus which are *Aspergillus parasiticus* and *Aspergillus flavus*. It can be found in various food crops such as corn, millet rice, groundnut, sorghum and others [6].

The four major naturally known aflatoxins produced by the *Aspergillus* species of mold include AFB1, AFB2, AFG1 and AFG2 . Whereas the B designation of aflatoxins B1 and B2 result from the exhibition of blue fluorescence under UV-light, while the G designation refers to the yellow-green fluorescence of the relevant structures under UV-light [7].

Besides their occurrence in the foods and agricultural commodities, aflatoxin can be detected in biological samples, resulting from exposure through the diet. In fact, the assessment of human and animal exposure to aflatoxin, through the detection of aflatoxin biomarkers in biological samples such as in serum and urine, is significant to determine the extent and rate of aflatoxin exposure [8].

It has been reported that aflatoxins, once ingested (because of their low molecular weight), are rapidly adsorbed in the gastro-intestinal tract through a non-described passive mechanism, and then quickly appear as metabolites in blood after just 15 minutes and in milk as soon as 12 hours post-feeding [9].

Aflatoxins are mainly metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M1 [10]. The main pathway of exposure to AFB1 is through contaminated food intake [11]. However, the inhalation and dermal pathways have been reported in environmental-exposed populations [12].

It is reported that excretion of AFB1 and AFM1 occurs primarily through the biliary pathway, followed by the urinary pathway, and AFB1 could be detected in different levels in the kidney and urine of two calves with dosages of 0.8mg and 1.8mg /kg body weight, respectively [13]. However, the mechanism of the toxicity of the two AFs and their metabolites is still unclear since several mycotoxins have been identified as potential factors for nephropathy.

Therefore, the purpose of this research was to conduct a case-control study to monitoring and screening of Aflatoxin B1 levels in Karbala Province. The effect of the association between levels of Aflatoxin B1 and the standard biochemical markers of chronic kidney disease was also examined.

Materials and Methods

The present work included a case-control study ,samples were selected from the patients attending the AL-Zahraa Medical centre and consulting centre in AL-Hussain teaching hospital/ Kerbala. The sociodemographic aspects of the patients including age, gender, BMI, Genetics History of family, Stages of CKD and having any current chronic diseases such as diabetes mellitus and blood pressure). They were also exposed to medical examination for signs and symptoms of CKD by sub- specialized doctor based on the World Health Organization (WHO) criteria. For relationship purposes, patients were divided into certain and uncertain etiology of CKD. Patients groups were compared to a group who do not have a disease (apparently healthy) as a control subject. A total of 136 subjects were studied , 86 (46 male and 40 female) of them Chronic Kidney disease patients , 17 (11male and 6 female) of them were chronic kidney disease of uncertain etiology. Control group of an apparently healthy 50 subjects (28 male and 22 female) were chosen from well-known volunteers participants. For all participants (5ml) of blood was taken from the vein by sterile syringe and transported in gel tube container to the central lab. Samples were settled for 15 minutes and then centrifuged for 15 minutes at 3000 rpm to separate serum.

Kinetic colorimetric method was used to measure the concentration of Creatinine, while Enzymatic colorimetric method was used to measure concentration of urea. Albumin concentration also measured by Colorimetric method [14] [15] [16] .

Qualitative analysis of serum AflatoxinB1 was conducted According to the AL-Mosoui method (2015) [17] using thin layer chromatography (TLC) and the quantitatively identifying was performed by High Performance Liquid Chromatography (HPLC).

Results and Discussion

A new type of chronic kidney disease (CKD) not associated with diabetes or hypertension has arisen in many peri-equatorial cases over the past four decades. The cause is usually unknown and the disease is thus referred to as CKD of unknown etiology (CKDu) [18] [19]. It has been reported by the (SJW) estimates, based on two decades of community- observations, that more than 10 years of prior environmental acute exposure is possible before appearing the clinical signs of CKD [20] [21]. The clinical demographic characteristics and laboratory parameters of both patients groups and the healthy control group were summarized inTable (1). The patients group were divided into groups with different CKD stages based on the glomerular filtration rate. The descriptive table also shown an adjustment of other risk factors which were collected through the self-reported technique (student questionnaire), these factors included: age, gender, BMI, HT, DM.

Table 1 : Descriptive of the Demographic and laboratory characteristics of the study population.

	Patients Groups			Control Group
	Stage 2-3	Stage 4	Stage 5	
No. of patients	31	25	30	50
Mean Age (Years)	60	64	55	52
Gender(male/female)	(21/10)	(12/13)	(14/16)	(28/22)
BMI (Mean Kg/m2)	26.42	28.70	27.92	27.38
DM (Yes/NO)	(10/21)	(14/11)	(14/16)	0/0
HT(Yes/NO)	(15/16)	(17/8)	(17/13)	0/0
GFR (Mean ml/min/1.73m2)	42.79	20.64	9.14	127.45
Urea (Mean mg/ dL)	77.10	136.58	174.15	28.12
Creatinine (Mean mg/ dL)	1.69	3.42	6.65	0.66
Albumin (Mean g/ dL)	3.34	3.12	3.26	4.38

Figure 1, 2 and 3 were demonstrated the distribution of serum renal functions test (GFR, Urea and Creatinine) in CKD patients group compared to the healthy group.

A box plot was used to visually showing the distribution of data through displaying the data quartiles (or percentiles) and averages.

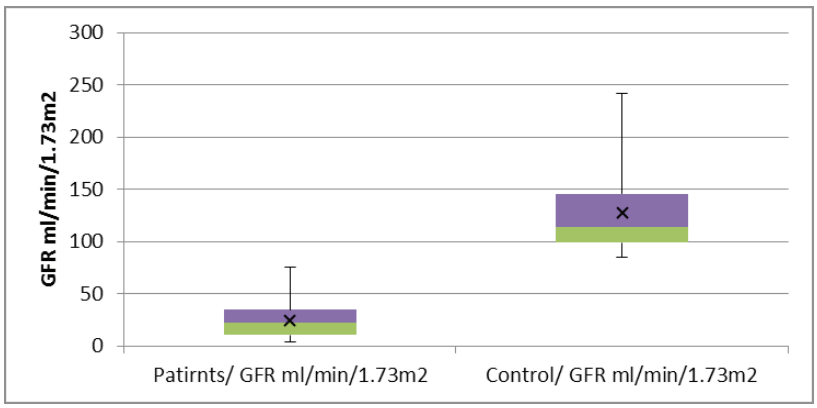

Figure 1- Distribution of GFR in CKD patients group compared to the healthy group.

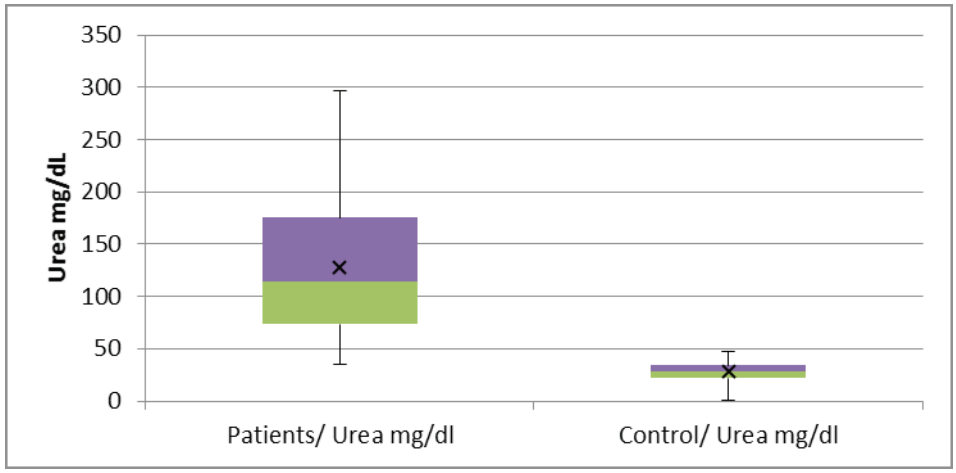

Figure 2- Distribution of serum Urea levels in CKD patients group compared to the healthy group.

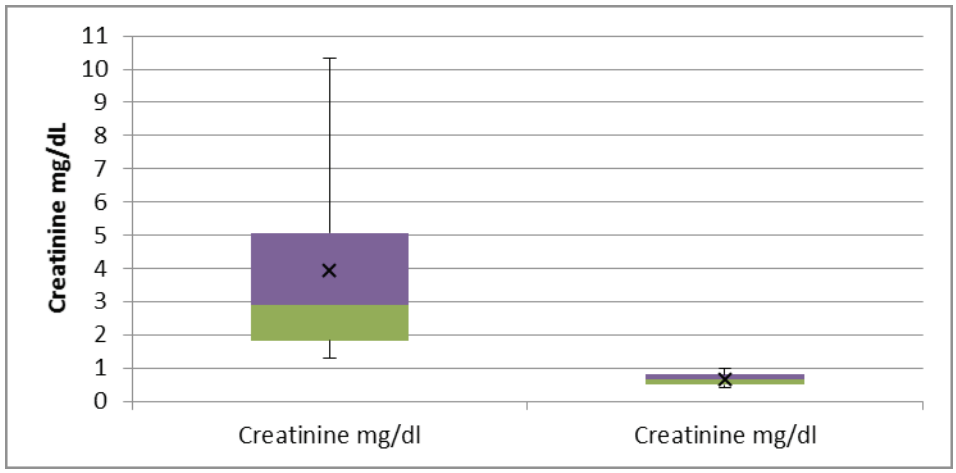

Figure 3 - Distribution of serum Creatinine levels in CKD patients group compared to the healthy group.

The serum urea and creatinine levels were increased significantly in CKD group compared to healthy control group. On the other hand, GFR was decreased in CKD group compared to healthy control group (P values shown in Table (2)).

Table 2- The confidence level of all laboratory parameters in CKD patients group compared to healthy control group.

Characteristic	Patients		Control Group Confidence Level(95.0%) Median(Lower Cl-Upper Cl)	P value (ANOVA)
	Certain Etiology Patients Confidence Level(95.0%) Median(Lower Cl-Upper Cl)	Un Certain Etiology Patients Confidence Level(95.0%) Median(Lower Cl-Upper Cl)		
BMI(Kg/m2)	27.7 (26.61-28.78)	27 (24.64-29.35)	28 (26.9- 39.19)	0.50
GFR (ml/min/1.73m2)	22.2 (18.77-25.62)	26.9 (15.25-38.56)	113.6 (102.94- 124.25)	2.59E-45
Urea (mg/ dL)	125.95 (110.61-141.28)	89.59 (59.46-119.71)	27.89 (25.14- 30.63)	3.16E-20
Creatinine (mg/ dL)	2.99 (2.31-3.66)	2.29 (0.51-4.07)	0.66 (0.61- 0.71)	8.92E-12
Albumin (g/ dL)	3.43 (3.26-3.59)	2.43 (1.92-2.93)	4.35 (4.20- 4.50)	7.39E-18

Many studies reported a significant positive relationship between serum urea and creatinine in progress CKD patients, that result from the reduction of glomerular filtration rate [22] [23]. Diminishing of glomerular filtration rate results in rise of plasma concentrations of serum creatinine and urea. This rise indicates progression of kidney disease and thus serum creatinine has greater prognostic ability compared with urea for predicting the adverse outcomes [24]. An elevated serum creatinine level is also a late sign of renal damage in essential hypertension with frankly elevated serum creatinine values predict a poor prognosis in CKD patients [24]

Assessment of AFB1-positive group of Un-Certain etiology- CKD Patients with the measured biochemical markers of renal functions:

Several investigations were examined to demonstrate the possible alteration of renal functions response to the nephrotoxin involved. the link between the CKD disease and the effect of the toxin was made by biomarkers, which provide information on factors that are causative or explanatory towards the respective condition.

In Un-Certain etiology of CKD Patients, GFR, Urea, creatinine. comparisons were performed between AFB1-positive patients groups and heathy subjects, Analysis of correlation illustrated that AFB1 levels in Un-Certain Etiology Patients has a statistically significant positive correlation with increasing Urea, creatinine levels and significant negative correlation with GFR with $P<0.05$. The correlation coefficient and p value for all measured parameters were listed in Table (3).

Table 3 - Correlation between AFB1 positive samples of Un-Certain Etiology Patients group and the biomarkers.

	Characteristic	AFB1 levels in Un-Certain Etiology Patients	P value
Variables group		Coefficient rs	
	Age(Year)	0.76	0.01
	BMI(Kg/m2)	0.43	0.22
Renal Functions	GFR (ml/min/1.73m2)	-0.51	0.013
	Urea (mg/ dL)	0.78	0.008
	Creatinine (mg/ dL)	0.72	0.01
	Albumin (g/ dL)	0.10	0.78

Recently, it has been reported that AFB1 caused obvious injury in kidney tissue, edema, cytomorphosis, and occasional severe inflammatory cell infiltration and hemorrhage [25]. Other has demonstrated the long-term administration of AFs which shown to cause renal damage and it might involve inflammation, cell necrosis, and toxicosis [26] [27].

Based on the biochemical measurements demonstrating the presence of higher concentrations of Creatinine and Urea. Together, these results confirmed that the kidney was one of the main target organs of AFs and indicate that several metabolites might be transferred, produced, or degraded in the kidney, such as proline, which was validated to be a special metabolite in kidney [25].

AFs are potent carcinogenic and genotoxic compounds, which exert toxic effects through DNA damage and mutations leading to oxidative damage. With regard to the mechanism of oxidative damage caused by

AFs, cell inactivation by proteasomes was regarded as a part of the cellular defense against oxidative stress, and AFB1 and AFM1 were reported to be the most potent activators of proteasome activity [28] [29].

Histological findings by Huiying Li et al. confirmed that aflatoxins induced oxidative stress as evidenced by peroxidation of lipids and MDA in the serum. SOD is a classical antioxidant enzyme in various organisms which converts superoxide anion radicals to hydrogen peroxide and protects organisms from oxidative injury. The former study indicate that Aflatoxins in their model resulted in releasing free radicals especially superoxide anions in kidney tissue then activate oxidative reactions which have a toxic effect on mouse kidney [25].

Interestly, no such correlations as un**certain etiology CKD Patients** were observed regarding the association between AFB1-positive of Certain Etiology CKD Patients and the measured parameters .

In spite of the positive results of presence AFB1 in group patients with Certain etiology of CKD namely due to DM and /or hypertensive, results were indicated no significant association between the level of AFB1 and the measured parameters.

It has been reported that the rate of functional decline varies based on the original disease; however, renal function often deteriorates progressively even when the original insult is controlled. Uncontrolled hypertension, regardless of the aetiology, results in more rapid renal functional decline [30].

Final Conclusion:

Aflatoxins are not only a big problem at crop production level, but also it has become a global health issue because of the consequences that the consumption of this toxin generates in animals and human beings.

Because of the recent investigations conducted in this area, it is important to take actions to prevent damage and diseases; that´s why, at first, governments supported by scientific research groups should report publicly the risks that aflatoxins consumption means by quantifying the human health impacts and the burden of disease due to the toxin exposure.

It is also important to increase laboratory detection of mycotoxins and public health response capacity of affected regions. Public health services should offer immediate attention to aflatoxicosis diagnoses and opportunistic diseases caused by them. Finally, it is important to develop response protocols to be used in an event of an outbreak of acute aflatoxicosis, which could become in an epidemic stage.

Ethical Clearance: The project of this study was taken from the ethical committee of College of Medicine / University of Kerbala.

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Conflict of Interest: Nil

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