Investigation of ochratoxin A in blood of chronic kidney disease of uncertain etiology

LTC. Wael .S.Hasan, Prof.Sami.A.Ali

Republic of Iraq, Ministry of Interior, College of Applied medical Science/university of Karbala
College of Applied medical Science/university of Karbala

Abstract

The aim of the study was to detect Ochratoxin A in blood samples of people suffering from chronic kidney disease. Samples were collected from Hariri Gas Hospital / Medical City / Baghdad Health Department / Rusafa. It included a number of (200) samples, (100) of which were for patients suffering from chronic kidney disease, and the other (100) were for healthy people(control).

The results of detecting the presence of Ochratoxin A by 90% in the blood plasma of nephropathy disease patients, while the percentage of its presence in the blood plasma of healthy patients was 3% with a significant difference. Its percentage among male patients, as the percentage among females is 44%, while the percentage among males is 46%.

The results also showed a clear superiority in the concentration rates of Ochratoxin A in the blood plasma of patients relative to healthy person, as its average concentration in female and male patients was 7.015 ng/ml and 7.071 ng/ml, respectively, while it was in both healthy females and males Was 0.1 ng/ml and 0.09 ng/mL, respectively.
Materials and Methods

1- Blood Collection And Storage

For all participants, 5ml of blood was taken from the vein by sterile syringe and transported in a 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. The serum was then transported by micropipette to the sterile container (Eppendorf1.5ml tube) for storage which was conducted by divided each serum into two parts and then stored at -20c until analyses. stored serum samples then used to measure of ochratoxin A.

2- Extraction of Ochratoxin A in serum samples

Serum samples in the size of 500uL were taken in a sterile test tube and added to each one of them 50uL from proteinase K solution and left to react for 10 minutes. After that the mixture was exposed to Centrifugation for 15 minute at 3000rpm, then from each sample the failtarte was taken and the precipitate was neglected. Chloroform (1ml) was added to each filtrate (twice its size ) and shake vigorously in the electric shaker device, where it formed two layers(chloroform layer and serum layer ), chloroform layer was separated by separating funnel and put in sterile other glass tube and let to evaporate.
3- Evaluation of Ochratoxin A level in blood Serum of human conducted according to Skarkova et al., (2013). As the following step.

HPLC model SYKAMN (Germany) was used to analyze add detection of Thiamethoxam. The mobile phase was an isocratic acetonitrile :D.W:formic acid (50:47:3) at flow rate at 1.0mL/min, column was C18-ODS (25 cm* 4.6mm) and the detector Florescent (Ex=365 nm, Em =445 nm).

Ochratoxin was extracted from samples (20ml) by homogenization with 20 mL acetonitrile: H2O (6:4,v=v) for 2 minutes. The extract was filtered and 4mL of the filtrate was diluted with 25mL phosphate buffer saline pH 7.4 (PBS). The samples were degassed in a sonic bath for 30 minutes then the PH was adjusted to 7.2 using 2 M sodium hydroxide 5 mL of acetonitrile is added to the sample and then stored until the analysis is performed.

The concentration of Ochratoxin A was calculated by adopting a curve on the absorption values of standard poison concentrations, The concentration was calculated according to the retention time of detention estimated using the following equation:

\[
\text{substance concentration} = \frac{\text{Standard substance concentration} \times \text{model space}}{\text{Standard material area}}
\]

\[ppb = \frac{\text{dilution factor}}{\text{volume} \times \text{weight}}\]
Introduction

In human blood levels in Tunisia has demonstrated a likely rate of human contamination 82% of positive sample in the general population (3.5 ± 6.8 ng/ml) at the detection limit of (0.1ng/ml) and 100% in cases of whatever the etiology these percentages are lower when the detection limit is set to (1ng/ml). These results led us to search for a possible association of the presence of OTA in human blood with cases of nephropathy as has been found in the Balkan peninsula. The first study was performed in the Sahel region in Tunisia surrounding the city of Monastir. It showed that a certain number of people having chronic interstitial nephropathy of unknown etiology were OTA positive (Achour et al., 1993).

In Italy Breitholtz-Emanuelsson et al., (1994) found significantly higher OTA concentration in blood of patients with renal stones or cysts. Get 1.4 ng/ml while it was in chronic renal insufficiency 0.60 ng/ml but in the blood of healthy persons was 0.53 ng/ml.

Otherhand the OTA concentration in blood of dialysed patients was 1.97 ng/ml but in blood of healthy persons was 0.71 ng/ml (Jimenez et al. 1998).

In the blood of patients with bladder cancer Ozcelik et al., (2001) found significantly higher concentration of OTA 2.1 ng/ml than in control 0.4 ng/ml. while Grosso et al., (2003) did not find this in a group of patients with urinary tract tumours of different localization.
In France, in two patients with chronic renal failure, OTA found in the blood at very high concentration get 205 ng/ml and 367 ng/ml in blood (Fillastre, 1997).

Wafa et al., (1998) showed that the maximum OTA level in healthy controls was 0.91 ng/ml and 10.15 ng/ml in patients with nephropathic syndromes in Egypt.

Malir et al., (2001) illustrated that OTA concentration in blood were higher in patients with chronic renal insufficiency treated with dialysis than in healthy individuals indicting that OTA accumulation due to renal failure cannot be eliminated with dialysis. This is confirmed by the lack of the difference in OTA concentration when measured before and after dialysis in patients with chronic renal insufficiency.

Fuchs and peraica (2005) showed that Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin that is thought to be implicated in the aetiology of endemic nephropathy in the Balkans (BEN). The frequency of this human deadly illness in Bosnia and Herzegovina, Bulgaria, Croatia, Romania, Serbia, and Monte Negro is linked to a relatively high incidence of usually uncommon renal pelvis and ureter urothelial tumors. Despite the fact that OTA was identified more frequently and/or in greater concentration in the food and blood of residents in BEN-affected areas than in other areas, the role of OTA in the development of BEN remains unknown. Dialysis patients with chronic renal failure had a higher blood factor.
Results and Discussion

The results showed 90 out of 100 samples (90%) of blood collected for patients infected with chronic kidney disease of uncertain etiology were contained OTA with a significant difference of number blood samples contaminated with OTA (only 3%) that collected from a healthy person (Table 1).

Also, this study illustrated the number of blood samples collected from Females and males patients borne the OTA was 44 (48.8%) and 46 (51.1%) respectively with no significant difference between them. (Table 3-2)

The results of this study agreement with many studies. AL-Musoui (2015) found (23.07%) of patients’ blood specimens had OTA in their blood get 10% and showed the males highly infected (87.5%) with OTA while in Females were (70%) Micco et al., (1995) illustrated that 22 out of 111 breast milk samples were contamination with OTA in range 0.1-12 ng/ml.

Breithoitz- Emanuelsson et al., (1993) found that concentration of OTA in human milk was approximately lo fold lower than in human blood.
Table (1) Effect of nephropathy disease in number and percentage OTA level in blood serum

<table>
<thead>
<tr>
<th>Case</th>
<th>NO.of persons with OTA</th>
<th>NO.of persons without OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>90(90%)</td>
<td>10(10%)</td>
</tr>
<tr>
<td>Control healthy persons</td>
<td>3(3%)</td>
<td>97(97%)</td>
</tr>
</tbody>
</table>

$x^2$ calculate=152

$x^2$ table=3.84

In our study accepted the alternative hypothesis (Ha) and reject the null hypothesis (HO) in other words the presence of OTA is related with nephropathy
(Table 2) Effect of Gender in number and percentage of patients with nephropathy and borne the OTA in blood serum

<table>
<thead>
<tr>
<th>Gender</th>
<th>NO.of patients with OTA</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>44</td>
<td>48.8</td>
</tr>
<tr>
<td>Males</td>
<td>46</td>
<td>51.1</td>
</tr>
</tbody>
</table>

\[ x^2 \text{ calculate}=0.4 \]
\[ x^2=\text{table(at 0.05)}3.84 \]

In our study accepted the null hypothesis (Ho) in other words the presence of OTA is related with Gender

**Effect of nephropathy and Gender in the concentration of OTA blood Serum**

The results revealed that the level of OTA in blood serum rose in Females patients with nephropathy were 7.015 ng/ml female with a significant difference \((p<0.001)\) from the level of OTA in blood serum in the control group.

As well as, the level of OTA in male blood serum patients with nephropathy and borne of OTA get 7.071 ng/ml with a significant difference \((p<0.001)\) from the level of OTA in blood serum in the control group get 0.09 ng/ml (Table 3-3)

The result of this study agreement with Achour et al., (1993) who found OTA in blood serum of 82 persons with (3.5 - 6.8) ng/ml concentration between (3.5 ± 6.8) ng/ml and detection of OTA in blood of 100 other persons (concentration 1 ng/ml)
our results have allowed use to establish the presence of OTA in human plasma in Chile in both populations, the incidence of positive values for OTA in blood were over 50% with respect to the levels found, the averages were similar to those reported in the countries except in San Vicente de Tagua – Tagua where the women’s group presented values higher than other reports (Grosso et al., 2003). The OTA concentration in the blood of patients with different kidney disease and healthy subjects was compared in several countries in all studies the concentration of OTA in the blood of the patient with chronic renal insufficiency treated with dialysis was significantly higher than in the blood of control subjects. (Ozcelik et al., 2001)

The increased OTA concentration was not found in cases of other acute renal insufficiency that had developed on the basis of other diseases without pre-existing nephropathy (Malir et al., 2001)

Breitholtz – Emanuels et al., (1994) Found significantly higher concentration of OTA than in control.
Table (3) Effect of nephropathy and Gender in the concentration of OTA ng/mml blood Serum

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control (Mean ± SD)</th>
<th>Patients (Mean ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0.100 ± 0.000</td>
<td>7.015 ± 0.623</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>Male</td>
<td>0.09 ± 0.028</td>
<td>7.071 ± 0.644</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>Total</td>
<td>0.093 ± 0.0208</td>
<td>7.043 ± 0.630</td>
<td>0.0001 **</td>
</tr>
</tbody>
</table>

* means significance differences (P <0.05)  ** means high significances differences (P <0.001)

References


• Jalil, A. A. T. EPIDEMIOLOGY OF CERVICAL CANCER AND HIGH RISK OF HUMAN PAPILLOMA VIRUS IN PATIENT. ББК 28.6 3, 85(7).


• Breitholtz- Emanuelsson, A., Minervini, F., Hult, K. and Visconti, A., 1994. Ochratoxin A in human serum samples collected in southern Italy from
