OBSTETRICS

The Correlation between Aflatoxin B1 and Placental Apoptosis in Pregnant Women with Intrauterine Growth Restriction

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ABSTRACT

Objective: To explore the effect of Aflatoxin B1 toxicity as a risk factor of intrauterine growth restriction (IUGR) and to determine the role of placental apoptotic indices in the pathogenesis of IUGR and their association with maternal risk factors including residency, working and exposure to smoking.

Materials and Methods: A case-control study was done at Women Health Hospital; Assiut University, Egypt. Sixty pregnant women with asymmetrical IUGR and a control group of 40 normal pregnancies were selected. Maternal urine samples were obtained for Aflatoxin B1 level measurement by layer chromatography. Quantitative determination of human placental Bcl-2 and caspase-3 using a monoclonal antibody-based enzyme-linked immunosorbent assay kits were performed.

Results: The results showed that aflatoxin B1 positive cases in the IUGR group had significantly higher placental caspase-3 and lower placental Bcl-2 concentrations than those which were aflatoxin B1 negative (p<0.01). The levels of placental apoptotic indices were higher in working women who lived in urban areas and those exposed to cigarette smoke than non-working women who lived in rural areas and non-smokers.

Conclusions: Aflatoxin B1 may affect the fetal growth by increasing the placental apoptosis. These results may highlight the importance of aflatoxin B1 which may contribute to the complex etiology of IUGR. Placental apoptotic indices levels were significantly affected by maternal residence, working and exposure to smoking in pregnancies complicated with IUGR.

Keywords: apoptosis; aflatoxin B1; caspase-3; IUGR; placenta

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Introduction

Intrauterine fetal growth restriction (IUGR) is defined as fetal birth weight below the 10th percentile, adjusted for gestational age\(^1\). It is considered to be an obstetric problem associated with increased perinatal mortality and morbidity\(^2\). In the last decade, the importance of IUGR has been raised again after Barker, et al have raised the importance of the fetal onset of the adult diseases “fetal programming”. They suggested that the organs of small for gestational age babies may be programmed for developing adult diseases\(^3\).

The prevalence of IUGR in Egypt was about 13%, which is considered one of the highest around the world\(^4\). About 60% of IUGR cases are idiopathic\(^5\). Millions of environmental pollutants may create risks during pregnancy that pertained to IUGR\(^6\). Many of these pollutants are proved to disrupt the reproductive development and functions in animals and man\(^7\).

Aflatoxin B1, a type of mycotoxins, was initially isolated and identified as the causative toxins in Turkey-X-disease (necrosis of the liver) in 1960 when over 100,000 turkeys died in England\(^8\). Aflatoxins are produced by different species of Aspergillus, particularly flavus and parasiticus, as well as members of the Genera Penicillium and Rhizopus. They can contaminate corn, cereals, sorghum, peanuts and other oil-seed crops\(^9\). Animal products such as milk, meat and eggs could be also contaminated\(^10\).

Aflatoxin B1 exposure may affect the human physical growth\(^11\). It is able to cross human placenta and may lead to IUGR\(^12\). Also, neonates with and without jaundice were investigated for the presence of aflatoxins in the cord blood. Jaundice and decreased birth weight was correlated with a higher concentration of aflatoxin B1\(^13\). Placental apoptosis is a normal physiological process that occurs in all phases of pregnancy requiring a well-balanced interaction of proapoptotic (e.g. caspase-3) and antiapoptotic factors (e.g. Bcl-2)\(^14\). Dysfunction of placental apoptosis results in many obstetric problems that include IUGR\(^15\). Caspases are a family of cysteine-dependent aspartate-specific proteases. Cell death is considered to follow a classical apoptotic mode if cell execution is dependent on caspases activity\(^16, 17\).

Caspase-3 is the key executioner caspase that may be activated through either death signal induced-death receptor mediated (extrinsic) pathway or mitochondria-mediated (intrinsic) pathway\(^14\). B-cell lymphoma-2 (Bcl-2) is an antiapoptotic protein that prevents apoptosis by controlling caspases activation or by guarding mitochondrial membrane integrity\(^18, 19\). The aim of our study was to compare the level of aflatoxin B1 between normal and IUGR pregnancies, and to explore the possible role of Bcl-2 and caspase-3 indices in the pathogenesis by which aflatoxin B1 can lead to IUGR. Also, the study aimed to compare the placental concentrations of caspase-3 and Bcl-2 indices in normal and IUGR pregnancies in correlation with maternal risk factors such as residency, working and exposure to smoking.

Materials and Methods

Study setting and design:

The study was a prospective case-control study that was conducted in the Departments of Medical Biochemistry and Obstetrics, Faculty of Medicine, Assiut University, Egypt from March 2012 to May 2013. Assiut Medical School Ethical Review Board approved the study and a written informed consent was obtained from all study participants.

Study Participants

The study included 100 pregnant women who were divided into 2 groups; the IUGR group: comprised of 60 singleton pregnancies with asymmetrical IUGR admitted either in labor or planned for termination of pregnancy. We excluded pregnant women with symmetric IUGR, twin pregnancies, congenitally malformed fetus, placental abnormalities, preeclampsia, chronic hypertension, diabetes mellitus, chronic debilitating and autoimmune diseases and malnutrition. The other group is the control group which comprised of 40 women with age, parity and gestational age; matched with the control group.
Study intervention
Complete and detailed medical history was taken and complete physical examinations, ultrasound evaluation and routine investigations were done for all participants to diagnose cases and exclude any other causes of IUGR.

The diagnosis of IUGR was based on three criteria: ultrasonographic deviation from the normal growth percentile, clinically detected suboptimal growth and having birth weight less than the 10th percentile of the corresponding gestational age\(^{(20)}\).

Samples collection and laboratory analyses
In Women's Health Hospital's labor ward at the time of pregnancy termination by any mean, first morning midstream urine samples were taken from the recruited women under aseptic procedure then centrifuged to separate cells and other sediments, few drops of concentrated hydrochloric acid were added to prevent contamination, and stored frozen at -70°C until the assay of aflatoxin B1 was performed. Placental samples (10 grams) were collected from the fetal surface. Firstly, we chose an area with few/no veins, and then we cut out a cube of core sample approximately 3 cm x 3 cm and 3 cm deep. The sample was washed several times with isotonic saline solution to get rid of blood, then kept in clean and labeled plastic bags and stored at (-70°C) until the measurement of placental Bcl-2 and caspase-3 concentrations.

Biochemical analysis
Detection of aflatoxin B1 in urine: 1- ml of urine sample was transferred into a glass tube then concentrated in a boiling water bath to about 1 ml. Two ml of dichloromethane and 200 mg of anhydrous sodium sulfate was added after cooling, and then shaken vigorously by vortex. The mixture was filtered through filter paper into clean glass tube. The filtrate was then evaporated to near dryness. The residue was reconstituted with 1 ml methanol and refiltered through Millipore filter (0.45 μm, USA) into eppendorf tube.

The detection of the urinary aflatoxin B1 was done by thin layer chromatography using aflatoxin standard and aluminum sheet silica gel 60 pre-coated 25 sheets 20×20 cm, layer thickness 0.2 mm (E-Merck, Darmstadt). All TLC sheets were activated at 100°C for one hour. The development solvent was a mixture of acetone-chloroform (15:85 v/v)\(^{(21)}\).

Regarding placental samples, one gram of each sample was taken, washed twice with isotonic solution and once with phosphate buffer solution (PBS). Then PBS was aspirated and 2 ml of lysis buffer were added to each specimen, then homogenized and incubated for 60 minutes at room temperature with gentle shaking. The tubes containing the homogenate were centrifuged at 1000 rpm for 15 minutes. The supernatant was stored in different aliquots at -70°C. Quantitative determination of placental Bcl-2 and caspase-3 was performed using a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) kits (Cat. No: BMS256/4 and BMS2013INST, respectively). Both kits were supplied by Bender MedSystems GmbH, Campus Vienna Biocenter 2, A-1030 Vienna, Austria, Europe.

Statistical Analysis
Collected data were reviewed and analyzed using the Statistic Package for Social Science Version 21 (SPSS 21.0) for windows. The chi-square (X²) test was used to compare qualitative data and 2-independent samples t-test was used to compare two quantitative data. Analysis of one-way variance (ANOVA) was used to compare more than two quantitative data at a time. Studying the relationship between variables was done using Pearson correlation. The level of significance was taken at p-value of ≤ 0.05.

Results
Two thousands and nine hundreds pregnant women were examined in the antenatal clinic during the study period, of them 157 (5.4%) women were diagnosed to have IUGR by ultrasonography. Ninety seven patients were excluded for various exclusion criteria resulted in 60 patients included in the study group. Forty matched age, parity and gestational age control were recruited in the control group (Fig. 1).
There were no significant differences between both groups as regard to maternal age and residence. Statistically significant differences were found between the two groups as regard to maternal working (p<0.05); with higher percentage of working women in the IUGR group. The incidence of passive smoking was significantly higher in the IUGR group (p<0.05). Furthermore, pre-pregnancy body mass indices (BMI) were significantly lower in the IUGR group (p<0.001).

The number of aflatoxin B1 detected cases and its effect on of neonatal birth weights and placental apoptotic indices concentrations within the IUGR group and the control group are shown in Table 1.
Table 1. The number of aflatoxin B1 detected cases and its effect on fetal birth weight and placental apoptotic indices in the two groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n = 40)</th>
<th>IUGR group (n = 60)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not detected (n = 39)</td>
<td>Detected (n = 1)</td>
<td></td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>3146.15 ± 338.80</td>
<td>3600</td>
<td>Ns</td>
</tr>
<tr>
<td>Placental Caspase-3</td>
<td>1.23 ± 0.32</td>
<td>1.28</td>
<td>Ns</td>
</tr>
<tr>
<td>Placental Bcl-2</td>
<td>2.05 ± 0.44</td>
<td>1.90</td>
<td>Ns</td>
</tr>
<tr>
<td>IUGR group (n = 60)</td>
<td>(n = 53)</td>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>1855.66 ± 383.14</td>
<td>1128.85 ± 152.36</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Placental Caspase-3</td>
<td>3.02 ± 0.47</td>
<td>3.63 ± 0.32</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Placental Bcl-2</td>
<td>0.74 ± 0.40</td>
<td>0.30 ± 0.11</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

SD: standard deviation.
Bcl-2 and Caspase-3 were measured in ng/mg proteins in placental tissue extract.

Table 2 presents the levels (mean±SD) of placental apoptotic indices (caspase-3 and Bcl-2) in the IUGR group as compared to the control group. Caspase-3 concentrations were significantly higher in the IUGR group than those in the control group while the levels of Bcl-2 were significantly lower in the IUGR group than those in the control group (p <0.001).

The effects of women residence on the levels of apoptotic indices is shown in Table 2. The concentrations of caspase-3 were significantly higher while Bcl-2 concentrations were significantly lower in the IUGR group than those in the control group in all residence areas (p<0.001). Within the IUGR group, there were significant differences between urban, semiurban and rural areas as regard to neonatal birth weights with lower values observed in urban followed by semiurban then rural areas (p<0.05).

Table 2. Comparison between the levels of placental apoptotic indices in the two groups with analysis of the effects of some maternal risk factors on their levels.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n = 40)</th>
<th>IUGR group (n = 60)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental apoptotic indices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental Caspase-3</td>
<td>1.23 ± 0.32</td>
<td>3.10 ± 0.50</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Placental Bcl-2</td>
<td>2.07 ± 0.45</td>
<td>0.69 ± 0.40</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Residence

Urban (n = 12) (n = 28) Ns
- Placental Caspase-3 1.31 ± 0.39 3.32 ± 0.42 < 0.001
- Placental Bcl-2 2.00 ± 0.40 0.52 ± 0.25 < 0.001

Semi-urban (n = 13) (n = 19) Ns
- Placental Caspase-3 1.15 ± 0.27 3.06 ± 0.43 < 0.001
- Placental Bcl-2 2.09 ± 0.57 0.81 ± 0.50 < 0.001

Rural (n=15) (n=13) Ns
- Placental Caspase-3 1.21±0.30 2.66±0.44 < 0.001
- Placental Bcl-2 2.08±0.39 0.89±0.38 < 0.001
Table 2. Comparison between the levels of placental apoptotic indices in the two groups with analysis of the effects of some maternal risk factors on their levels. (Cont.)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n = 40)</th>
<th>IUGR group (n = 60)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non working women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Placental Caspase-3</td>
<td>1.16 ± 0.30</td>
<td>2.92 ± 0.50</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>• Placental Bcl-2</td>
<td>2.07 ± 0.46</td>
<td>0.82 ± 0.44</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Working women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Placental Caspase-3</td>
<td>1.35 ± 0.33</td>
<td>3.22 ± 0.46</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>• Placental Bcl-2</td>
<td>2.00 ± 0.43</td>
<td>0.59 ± 0.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Exposure to smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Placental Caspase-3</td>
<td>1.22 ± 0.33</td>
<td>2.76 ± 0.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>• Placental Bcl-2</td>
<td>2.03 ± 0.49</td>
<td>0.87 ± 0.44</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Passive smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Placental Caspase-3</td>
<td>1.32 ± 0.38</td>
<td>3.28 ± 0.40</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>• Placental Bcl-2</td>
<td>2.01 ± 0.39</td>
<td>0.60 ± 0.34</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

- The levels are expressed as (mean±SD)
- Ns: non-significant; IUGR: intrauterine growth restriction
- Bcl-2 and Caspase-3 were measured in ng/mg proteins in placental tissue extract.

Moreover, the concentrations of caspase-3 were significantly higher (p<0.001) while the concentrations of Bcl-2 were significantly lower (p<0.01) in urban areas followed by semiurban then rural ones (Table 3). Within the control group, there were no significant differences between the three areas as regard to neonatal birth weights and placental apoptotic indices concentrations.

Table 3. The effects of maternal residence on the mean ± SD of neonatal birth weights, and placental apoptotic indices in the two groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Urban (n=12)</th>
<th>Semiurban (n=13)</th>
<th>Rural (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal birth weight (grams)</td>
<td>3062.50±245.99</td>
<td>3230.77±386.51</td>
<td>3130.00±352.95</td>
<td>Ns</td>
</tr>
<tr>
<td>• Placental Caspase-3</td>
<td>1.31±0.39</td>
<td>1.15±0.27</td>
<td>1.21±0.30</td>
<td>Ns</td>
</tr>
<tr>
<td>• Placental Bcl-2</td>
<td>2.00±0.40</td>
<td>2.09±0.57</td>
<td>2.08±0.39</td>
<td>Ns</td>
</tr>
<tr>
<td>IUGR group (n=60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neonatal birth weight (grams)</td>
<td>1494.64±400.31</td>
<td>1918.42±330.49</td>
<td>2150.00±165.83</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>• Placental Caspase-3</td>
<td>3.32±0.42</td>
<td>3.06±0.43</td>
<td>2.66±0.44</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>• Placental Bcl-2</td>
<td>0.52±0.25</td>
<td>0.81±0.50</td>
<td>0.89±0.38</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

- SD: standard deviation; Ns: non-significant.
- The level of significance was measured by ANOVA test.
- Bcl-2 and Caspase-3 were measured in ng/mg proteins in placental tissue extract, The levels are expressed as (mean±SD)
Placental caspase-3 concentrations were significantly higher, while Bcl-2 concentrations were significantly lower in working women in the IUGR group than working women in the control group (Table 2). Within the IUGR group, neonatal birth weights were significantly lower in working than non-working women (p<0.001). In addition, caspase-3 concentrations were significantly higher in working than non-working women; (p<0.05). Furthermore, the concentrations of Bcl-2 were significantly lower in working than non-working women (p<0.05). On the other hand, non-significant differences were found between working and non-working women in the control group (Table 4).

**Table 4.** The effects of maternal working on the mean ± SD of neonatal birth weights and placental apoptotic indices concentrations in the two groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-working woman (n = 26)</th>
<th>Working woman (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal birth weight</td>
<td>3264.00 ± 350.57</td>
<td>2970.00 ± 226.62</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>(grams)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental Caspase-3</td>
<td>1.16 ± 0.30</td>
<td>1.35 ± 0.33</td>
<td>Ns</td>
</tr>
<tr>
<td>Placental Bcl-2</td>
<td>2.07 ± 0.46</td>
<td>2.00±0.43</td>
<td>Ns</td>
</tr>
</tbody>
</table>

- SD: standard deviation; Ns: non-significant.

- Bcl-2 and Caspase-3 were measured in ng/mg proteins in placental tissue extract. The levels are expressed as (mean±SD)

As regard the effects of maternal exposure to smoking; in both passive smokers and non-smokers, the levels of caspase-3 concentrations were significantly higher, while the levels of Bcl-2 concentrations were significantly lower in the IUGR group than the control group (p<0.001 for each). Neonatal birth weights were significantly lower in passive smokers than non-smokers within the IUGR group and the control group (p<0.001 and p<0.05, respectively).

**Table 5.** The effects of maternal exposure to smoking on the mean ± SD of neonatal birth weights and placental apoptotic indices concentrations in the two groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non smokers (n = 26)</th>
<th>Passive smokers (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal birth weight</td>
<td>3256.52 ± 336.87</td>
<td>2988.24 ± 271.87</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>(grams)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental Caspase-3</td>
<td>1.22 ± 0.33</td>
<td>1.32 ± 0.38</td>
<td>Ns</td>
</tr>
<tr>
<td>Placental Bcl-2</td>
<td>2.03 ± 0.30</td>
<td>2.01 ± 0.49</td>
<td>Ns</td>
</tr>
</tbody>
</table>

Within the IUGR group, caspase-3 concentrations were significantly higher, while Bcl-2 concentrations were significantly lower in passive smokers than non-smokers; p<0.001 for caspase-3, and p<0.05 for Bcl-2 concentrations. Within the control group, placental apoptotic indices concentrations were not significantly different (Table 5).
Table 5. The effects of maternal exposure to smoking on the mean ± SD of neonatal birth weights and placental apoptotic indices concentrations in the two groups. (Cont.)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non smokers (n = 17)</th>
<th>Passive smokers (n = 43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal birth weight (grams)</td>
<td>2107.14 ± 181.86</td>
<td>1589.74 ± 420.71</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>• Placental Caspase-3</td>
<td>2.76 ± 0.48</td>
<td>3.28 ± 0.40</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>• Placental Bcl-2</td>
<td>0.87 ± 0.44</td>
<td>0.60 ± 0.34</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

- SD: standard deviation; Ns: non-significant.
- Bcl-2 and Caspase-3 were measured in ng/mg proteins in placental tissue extract. The levels are expressed as (mean ± SD).
- No active smokers were detected in the present study.

Correlation analysis between placental apoptotic indices and neonatal birth weight in the IUGR group revealed that neonatal birth weights were positively correlated with Bcl-2 concentrations ($r = 0.666$, $p<0.001$) (Fig. 2) while negatively correlated with caspase-3 concentrations ($r = -0.756$, $p<0.001$) (Fig. 3). Caspase-3 concentrations were negatively correlated with Bcl-2 concentrations ($r = -0.478$, $p<0.001$) (Fig. 4). In the control group, a negative correlations were found between neonatal birth weights and caspase-3 concentrations ($r = -0.311$, $p<0.05$), while non-significant positive correlation was found with Bcl-2 concentrations ($r = 0.110$, $p>0.05$).

Fig. 2. The correlation between placental caspase-3 concentrations and neonatal birth weights in IUGR group. Correlation is significant at the 0.01 level.
Fig. 3. The correlation between placental Bcl-2 concentrations and neonatal birth weights in IUGR group. Correlation is significant at the 0.01 level.

Fig. 4. The correlation between placental Bcl-2 concentrations and placental caspase-3 concentrations in IUGR group. Correlation is significant at the 0.01 level.
Discussion

Egypt has high prevalence of IUGR and most of the cases are idiopathic. In our study, we proved that there was a significant increase in the levels of aflatoxin B1 along with placental apoptosis in pregnant women suffering from IUGR as compared to those with normal pregnancies.

Aflatoxin B1 presents a risk to human health that is not sufficiently recognized. The main target for its toxicity and carcinogenicity is the liver (22). Aflatoxin B1 and its metabolites can cross placenta and higher levels of aflatoxin B1 in cord blood were reported in mothers living in contaminated areas (12). The precise role of aflatoxin B1 on birth outcomes remains largely unknown (23).

In the present study, there was a non significant increase in the number of aflatoxin B1 positive cases in the IUGR group (seven cases) when compared to the control group (only one case). Within the IUGR group, cases which were positive for aflatoxin B1 had significantly lower neonatal birth weight than those that were negative. These results agreed with Abdulrazzaq, et al who found a significant negative correlation between fetal birth weights and the levels of aflatoxin B1 and attributed this to maternal ingestion of aflatoxin containing foods (12). Also, Shuaib, et al found that pregnant women with high AFB1-lysine adduct in their blood were more likely to have low birth weight babies (23).

Placental apoptosis is a normal physiological process that is important for placental and fetal development, trophoblast invasion and remodeling of uterine spiral arterioles (15). This process is highly regulated and requires the harmony of a group of pro and anti-apoptotic factors (14). The exact role of apoptosis in the development of placental pathology and clinical conditions such as preeclampsia and IUGR is yet to be determined (24). However, increased placental apoptosis may deplete the syncytiotrophoblast population of the placenta resulting in impairment of placental functions especially materno-fetal exchange leading to reduction of placental perfusion and oxygenation. Attenuation of the materno-fetal exchange functions will retard fetal growth and development (25).

In the present study, placental caspase-3 concentrations were significantly higher in the IUGR group and these concentrations were negatively correlated with neonatal birth weight. Also, these concentrations were negatively correlated with placental Bcl-2 concentrations within the IUGR group. Moreover, in the present study, the concentrations of placental Bcl-2 were significantly lower in the IUGR group than those in the control group. These concentrations were positively correlated with neonatal birth weights within the IUGR group.

These results were in agreement with Longtine, et al who found an increase in extravilous trophoblast apoptosis in placenta from preeclamptic and IUGR pregnancies (26). Also, Endo, et al found an increase in the placental caspase-3 concentrations in pregnancies complicated with IUGR when compared to normal pregnancies (27). Moreover, Ishihara, et al concluded that placental Bcl-2 concentrations were low in syncytiotrophoblast of severe preeclamptic and IUGR placentas than normal pregnancies (28). On the other hand Endo, et al found no significant difference in the expression of Bcl-2 between placenta from IUGR and from normal pregnancies (27).

Aflatoxin B1 may induce apoptosis by deregulation of p53 signaling pathway and its related functions such as cell cycle, apoptosis and DNA repair (29). Deregulated p53 increases the proapoptotic/antiapoptotic ratio of Bcl-2 family, disturbs mitochondrial membrane functions, thereby favoring the release of apoptogenic proteins from the mitochondria, caspase activation and apoptosis (30).

In the present study, aflatoxin B1 positive cases in the IUGR group had significantly higher placental caspase-3 and lower placental Bcl-2 concentrations than those which were aflatoxin B1 negative. These may indicate the important role of apoptosis as a possible mechanism for aflatoxin B1 induced pathology and this agreed with Reddy, et al., who indicated that aflatoxin B1 might induce apoptosis and Meki, et al., who concluded that aflatoxin B1 could lead to activation of caspase-3 (31, 32). Furthermore, Golli-Bennour, et al.,
found that aflatoxin B1 could significantly decrease the expression of Bcl-2 in cultured Vero cells(33). The hypothesis of the possible effect of aflatoxin B1 on placental apoptosis should be confirmed by histopathological examination of placentas of IUGR and normal pregnancies.

Different residence areas may affect the birth outcomes in different ways due to the variance in the socio-economic and work conditions or in part due to different chances of exposure to environmental pollutants(34-36). In the present study, within the IUGR group, significantly lower neonatal birth weights were observed in pregnant women who lived in urban areas than those who lived in rural and semiurban areas. These results were in accord with the findings of Canadian Institute for Health Information, 2009(37).

In the last decade the proportion of working women during pregnancy has increased constantly and women are working in a broader range of occupations than before(38). Also, there are accumulating evidences that the type of work and environmental exposures in the work locations might have adverse effects on fetal development(39, 40). There are several occupations where the risk of adverse pregnancy outcomes has been found to be elevated as shoe industry workers(41), hairdressers(42), laboratory technicians(43) and agricultural workers(44).

In the present study, there was significant higher incidence of IUGR in working than non-working women and within the IUGR group the neonatal birth weights were significantly lower in working women than non-working women. These results were in agreement with Spinillo, et al, who found that the risk of IUGR was significantly higher among women reporting moderate to heavy as compared to light physical effort at work(45). Also, Thulstrup and Bonde and Ahmed and Jaakkola indicated that a large number of physical, chemical and psychosocial factors as well as physical load occurring in the workplace had been found to increase the risk of adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, LBW, birth defects and still birth(46, 47). Furthermore, the increased exposure to environmental pollutions may explain the significantly higher caspase-3 concentrations and lower Bcl-2 concentrations in employed women especially those who lived in urban areas than non-employed women and those who lived in semiurban and rural areas, respectively.

The impact of maternal exposure to smoking during pregnancy and its adverse consequences on fetal and infant development is a well-known issue(48, 49). Among other effects, smoking could impair placental development either directly due to toxic elements such as nicotine, carbon monoxide and cadmium or indirectly by reducing blood flow which can create a hypoxic environment and lead to reduced provision of oxygen and micronutrients(50).

No active smokers were detected in the present study, however, the incidence of passive smoking was higher in the IUGR group and the neonatal birth weights were significantly lower in passive smoker women than non-smoker women. These results were in agreement with, et al., who found an association between smoking and LBW, and IUGR(51). In addition, Vardavas, et al., reported that maternal smoking during pregnancy was associated with LBW, birth length and head circumference in newborns compared to those of non-smoking mothers(52). It was also reported that smoking cessation early during pregnancy significantly modified these pregnancy outcomes and was associated with substantial decrease in the incidence of low birth weights and perinatal morbidity and mortality(53). In this regard, the results of the present study indicates the necessity for the promotion of smoking cessation during pregnancy and the imperative role of primary smoking prevention, especially among women of reproductive age.

Furthermore, in the present study, caspase-3 concentrations within the IUGR group were significantly higher in passive smoker than non-smoker women while Bcl-2 showed significantly lower concentrations in passive smokers. These results agreed with Vogt-Isaksen who concluded that cigarette smoking might reduce placental blood flow and increased its apoptosis which could be one of the mechanisms that play a role in the development of IUGR(54).
Conclusions
Aflatoxin B1 may affect the fetal growth by increasing placental apoptosis. These results may highlight the importance of aflatoxin B1 which may contribute to the complex etiology of IUGR. Placental apoptotic indices levels are significantly affected by maternal residency, working and exposure to smoking in pregnancies complicated with IUGR.

Conflict of Interest
The authors declare that they have no conflict of interest.

References
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