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Environmental factors and apoptotic indices in patients with intrauterine growth retardation: A nested case-control study

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ABSTRACT

Background: Egypt has one of the highest incidences of IUGR. The current study investigates the effect of heavy metals toxicity as risk factors of IUGR and determines the possible role of increased apoptosis in their pathogenesis.
Methods: This study was conducted in Assiut, Egypt, included 60 women diagnosed to have IUGR. We measured lead and cadmium levels in blood besides arsenic and cadmium levels in urine. Neonatal scalp hair sample were analyzed for arsenic content. Quantitative determination of human placental Bcl-2 and caspase-3 were performed.
Results: There are significantly higher levels of heavy metals and caspase-3 and lower levels of placental Bcl-2 in the IUGR group. The levels of heavy metals were positively correlated with caspase-3 while negatively correlated (except cadmium) with Bcl-2 levels.
Conclusions: There is an alarming high level of heavy metals toxicity in Egypt that was positively correlated to IUGR. Increased placental apoptosis may be one of the possible mechanisms behind the effect.

1. Introduction

Intrauterine fetal growth restriction (IUGR) is defined as fetal birth weight below the 10th percentile, adjusted for gestational age (Resnik, 2002). The prevalence of IUGR in Egypt is about 13%, which is considered one of the highest around the world (WHO, 2011). Giving birth to a small for date baby is usually associated with higher rates of perinatal mortality and morbidity and at least carries additional costs of neonatology care (Pike et al., 2012).

Millions of environmental pollutants may pose risks during pregnancy that pertained to IUGR (Maisonet et al., 2004). Lead (Pb) is among the first studied environmental hazards with common sources that include deteriorating lead paints, lead-containing gasoline, pesticides, groundwater and lead-containing products such as cigarette smoking, newspaper and Kohl (Patrick, 2006; Rossi, 2008). Lead is crossing the

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placenta and has many adverse effects on pregnant women including IUGR (Cleveland et al., 2008).

Cadmium (Cd) is another pollutant that found in large quantity in discarded electronic waste and jewelry together with ruminants of tobacco smoking (Järup and Akesson, 2009). It is a cytotoxic and endocrinal disruptor that proved to disturb placental nutrient and calcium transport resulting in decreased fetal birth weight and premature births (Henson and Chedrese, 2004; Ronco et al., 2009). Moreover, Arsenic (As) is a heavy metal that present in pesticides, insecticides, herbicides, cosmetics, food additives and cigarette smoke (Balakumar and Kaur, 2009). It has the ability to cross the placenta, disrupts the process of embryogenesis and adversely affects the pregnancy outcome (Rahman et al., 2010).

Placental apoptosis is a normal physiological process that occurs throughout all phases of pregnancy and requires a well-balanced interaction of proapoptotic (e.g. caspase-3) and antiapoptotic factors (e.g. Bcl-2) (Khan, 2010). The apoptotic cysteine-dependent aspartate-specific proteases 3 (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. On the other hand, B-cell lymphoma-2 (Bcl-2) is an antiapoptotic protein that prevents apoptosis by preventing caspases activation or by guarding mitochondrial membrane integrity (Khan, 2010). Activation apoptotic indices have been detected in placentas from pregnancies complicated with preeclampsia and IUGR (Longtine et al., 2012).

The current study aims to compare the level of lead, cadmium and arsenic between normal and IUGR pregnancies. We also tried to explore the possible role of caspase-3 (apoptotic) and Bcl-2 (antiapoptotic) indices in the pathogenesis by which the above pollutants can lead to IUGR.

2. Materials and methods

The study was a prospective nested case-control study conducted in the Department of Biochemistry and Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Egypt in the period from March 2011 through May 2013. The study was approved from the Assiut Medical School Ethical Review Board and a written informed consent was obtained from all study participants.

All pregnant women attended the antenatal care clinic in Women's Health Hospital, Assiut during the study period were clinically examined and those with high risk of IUGR or clinically diagnosed as small for gestational age at the third trimester of pregnancy were referred for advanced Feto-maternal Unit for assessment of fetal growth.

Pregnancies diagnosed to have asymmetrical IUGR were approached for participation in the study when eligible for delivery [IUGR group (Group 1)]. 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 (Aban et al., 2004).

The antenatal cards of the study participants were marked for sample collection at the time of delivery. 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 (BMI < 20).

A full history had been taken from each participant including age, BMI, residence, working status, regularity of antenatal care, history of IUGR and passive smoking. The patient considered passive smoker if her spouse is smoking at home or she was working in an office shared with an active smoker.

Age, parity and gestational age matched women had approached to participate in the study as a [Control group (Group 2)] at the time of delivery.

2.1. Samples collection and laboratory analyses

Trained nurses of the labor ward collected the samples at the time of delivery whatever it was vaginal or by cesarean section. Six milliliters of maternal blood was collected in plastic tubes containing K_3 EDTA as anticoagulant. Two milliliters of them were collected as whole blood and the remaining 4 ml was centrifuged at 4000 rpm for 10 min and its RBCs were collected and washed three times with saline. The whole blood and RBCs were digested by using the method described by Marouf (2011) stored at 70° refrigerator were used for measurement of lead and cadmium, respectively.

First morning midstream urine samples were taken under aseptic procedure (or by a catheter in case of cesarean section) 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 till assay of urinary cadmium and arsenic. Urinary creatinine was estimated by alkaline picrate method (Bonsnes and Taussky, 1945).

Hair samples (50 mg) were taken from the back of newborn head close to scalp, cleaned and digested by using the method described by Woolf et al. (2002) for the assay of their arsenic content.

Placental samples (10 g) were collected from the fetal surface. First, we chose an area with few/no veins, and then we cut out a cube of core sample approximately ~3 cm × 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 estimation of placental Bcl-2 and caspase-3 concentrations.

2.2. Biochemical analysis

All studied heavy metals were measured by inductively coupled plasma-optical emission spectrophotometer (Thermo Fisher Scientific, ICP–OES, iCAP 6200 series), using lead, cadmium and arsenic standards (5, 10 and 10 ppm, respectively), at wavelengths 220.3, 214.4 and 193.7 nm, respectively (Akpinar-Bayizit et al., 2010).

As regards to placental samples, 1 g 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 min at room temperature with gentle shaking. The tubes containing the homogenate were centrifuged at 1000 rpm for 15 min. The supernatant was stored in different aliquots at −70 °C. Quantitative