Prenatal Pesticide Exposure: Meconium as a Biomarker and Impact on Fetal Weight

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Abstract

Background: Prenatal exposure to pesticides can adversely affect fetal health. This study aims to measure levels of some pesticides in meconium obtained from newborns whose mothers were exposed to pesticides, and to identify the effect of maternal exposure to pesticides on neonatal weight.

Methods: This is a cross-sectional study. It was carried out on 190 cases (106 exposed and 84 non-exposed to pesticides). Nine pesticides (pretilachlor, DDT, lindane, chloropyrifos, diazinon, malathion, bioallethrin, α-cypermethrin and β cyfluthrin) were detected by Gas Chromatography/ Mass Spectrometry (GC/MS).

Results: The frequencies of pesticides detection in the samples were: 54.7%, 57.4%, 50%, 35.8%, 53.7%, 49.5%, 34.7%, 41.1% and 21.5% respectively. Those who reported prenatal exposure to pesticides were four times more likely to work in agricultural work (OR=4.5, 95% CI= 2.1 - 9.8). Moreover, those who reported prenatal exposure to pesticides were 1.6 times more likely to have babies with low birth weight (OR=1.59, 95% CI=1.2 - 6.3).

Conclusions: Agricultural pregnant workers in our community were significantly more exposed to several types of pesticides and this was associated with impaired fetal growth.

Keywords: Prenatal pesticides; Meconium; Gas Chromatography/ Mass Spectrometry (GC/MS), IUGR

Introduction

Intrauterine growth restriction (IUGR) is a common complication of pregnancy, affecting nearly 5-10% of newborns and is associated with increased infant mortality as well as childhood morbidity. Although the etiology of IUGR may be a consequence of several factors (such as metabolic factors, maternal infection, under nutrition, drug abuse and placental disorders), in 40% of cases, the cause is idiopathic. Environmental pollutants, oxidative stress and interaction between pollutant, free radicals and cellular system play an important role in reproductive toxicology and development of IUGR [1].

Developmental diseases such as birth defects, IUGR, and preterm delivery, account for more than 25% of infant mortality and morbidity [2]. Common risk factors such as smoking, alcohol use, older maternal age, and low socioeconomic status cause adverse fetal development [3]. Recently, it has been suggested that environmental risk factors and maternal occupation may also play an important role [4].

Pesticides are a group of environmental pollutants that pose a major risk to human health. These toxic pollutants are chemically stable and may persist as contaminants in the environment [5].

The exposure of pregnant women to pesticides is a major public health concern because these pesticides are neurotoxic to the fetus [6,7]. Moreover, some epidemiologic studies have shown that parental exposure to pesticides is a risk factor for childhood leukemia [8]. The exact cause is still unknown but chromosomal abnormalities and DNA damages are thought to be the underlying mechanisms [9].

Exposure of a pregnant woman to pesticides derives from environmental, occupational as well as domestic exposures. These pesticides may reach the fetus during gestation and after birth via breast-feeding. The detection of these pesticides has been demonstrated in maternal, umbilical cord, placental and new-born blood samples [10,11]. However pesticides in these matrices are bio-markers of short-term or recent exposure and do not show past or cumulative exposure [5].

Meconium is an ideal matrix for measuring prenatal exposure to pesticides. It is formed early in gestation and most xenobiotics and pesticides are deposited in it. Since meconium is not normally excreted in utero, pesticides deposited in meconium accumulate and increase in concentration which enhances the chance of their detection [7]. In addition, its collection is easy and non-invasive [12].

Detection of pesticides in biological samples from exposed persons is in rapid development. Recent research is focusing more on those pesticides that are considered to be endocrine-disrupting and potential risk factors for the development of leukemia and lymphoma [13]. More specifically, researches have focused on organophosphates, organochlorine pesticides, synthetic pyrethroid insecticides, triazines, chlorophenols and neurotoxic carbamates. All these compounds are of scientific interest because of their widespread use and their potential toxicology risk for human health [13].

Aims of the Study

1-To measure the levels of pesticides in meconium obtained from infants whose mothers were exposed to pesticides. 2- To identify the risk of maternal exposure to pesticides on the birth weight of the newborns.

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Subjects and Methods

Subjects

This cross sectional study was carried out on 190 delivering women from the labor ward of Women Health Hospital, Assiut University, Egypt between 2010 and 2012. These women were classified into 2 groups; 106 exposed and 84 non-exposed to pesticides. A questionnaire survey of the participants was conducted to collect general demographic information such as the type of pesticides used at home or in the farm, socioeconomic status, and source of water supply, dietary habits, educational level, maternal occupational characteristic, lifestyle habits, and maternal health.

Information about newborns was registered including gestational age, birth weight, and Apgar score. Apgar score is a score that quickly assesses the health of newborn. Scores of 7 and above were normal, 4 to 6 fairly low, and 3 and below were critically low [14].

After birth, meconium was collected from the newborns’ diapers and pooled into sterile polypropylene containers. Meconium samples were frozen at -20°C until the time of analysis of pesticides.

Mothers with serious chronic diseases such as diabetes, hypertension, anemia, renal diseases, heart diseases, thyroid disease or those who developed a serious pregnancy complication that could affect the fetal growth and development were excluded from the study. Cases with symmetrical IUGR, history of passive smoking and chronic drug intake were also excluded from the study.

An informed consent was obtained from each woman for her and her newborn’s participation in the study. The study was approved by the Local Ethics Committee of Faculty of Medicine, Assiut University.

Meconium extraction

This extraction took place according to the method described by Ostrea [15]. Meconium (0.5 g) was weighed. Five mls methanol: phosphate buffer was added, and then sonicated for about 10 min until it became homogeneous. The samples were then centrifuged at 4000 rpm for 30 min. Subsequently, 1.2 ml hexane was added, and the mixture was centrifuged again for 2 min. The mixture was settled and the organic layer was separated in vials until injected to GC/MS. Positive controls and calibrators were spiked with 100 µl of the appropriate concentration of pesticide mixture at this time and vortexed. Finally the mixture was centrifuged for 30 min at 4000 rpm and the supernatant was saved for further analysis.

Chemical preparation

Pesticide Mix in hexane was composed of: pretilachlor, DDT, lindane, chlorpyrifos, diazinon, Malathion, bioallethrin, β cyfluthrin and a cypermethrin. Pesticide Mix was custom synthesized and purchased from Sigma with cat. No. (SZE9015X, SZE9315X, SZE7038X, SZE8071X, 45428, 36143, SZE8287X, SZE7144X, and SZE5024X for the above pesticides, respectively).

Gas Chromatography-Mass Spectrometry instrumentation (GC/MS)

GC separation was performed using Gas Chromatograph from Agilent Technologies Model 7890A equipped with temperature programming capability, splitless injector, and capillary column with helium as the carrier gas at 36.796 cm/sec with a flow-rate of 1 ml min⁻¹. The injection port temperature was 250°C and interface temperature was 280°C. The DB-5ms column was used for chromatographic separation of analytes. The splitless injector (250°C) was used for injection of about 1 µl of injection volume. Detection was performed by Mass Quadruple Spectrometry detector Model 5975B. A computer data system (MSD ChemStation E.0201.1177) was used for measuring peak areas and heights.

Method validation, Quality control, analysis of laboratory reagent blanks (LRB), initial demonstration of capability, analysis of laboratory fortified blanks (LFB) were performed according to the methods described by Bielawski [12].

Calibration standards and quality control materials

Solid phase extraction was done according to the method described by Keith [16] using a solid phase extraction filter. Spiked-matrix calibration curves were constructed for the quantitation of pesticide and metabolite compounds. From the linear curve, the unknown and control sample concentrations were determined.

Statistical analysis

Data were collected, verified then analyzed using SPSS statistical package (version 16). The results were presented as mean ± SD, number and percentages. To determine the significance of non-parametric categorical variables, Chi-square test was calculated. Mann-Whitney U test analysis was carried out for non-parametric continuous variables. Multivariate logistic regression models were built to detect the most important predictors for pesticides exposure among the study sample and this was conducted using STATA (version 12.0) software. Adjusted odds ratio (AOR), Likelihood ratio test (LHR) and 95% confidence intervals (95% CIs) were calculated to assess significance in the models. Collinearity was investigated for the predictors involved in the analysis. Spearman’s rank correlations were carried out to explore any possible collinearity among predictors. To test for the validity of these results, collinearity diagnostic tests (Tolerance and Variance Inflation Factor ‘VIF’) were conducted. Multi-collinearity was assumed if the tolerance below 0.5 and/or VIF above 10. A significant P-value was considered when <0.05.

Results

Demographic characteristics

The socio-demographic data of study groups were described in (Table 1). The study included 190 subjects. There were 145 subjects who lived in rural areas and 45 who lived in urban areas. Agricultural workers were significantly associated with pesticide exposure (P<0.001). Baseline characteristics related to pregnancy and labor of the studied samples were described in (Table 2). Birth outcomes in the form of birth weight and Apgar score were shown. Low birth weight was present in 65.4% of exposed group versus 34.6% of the non-exposed group (P<0.01).

Concentration of pesticides in postpartum meconium

The levels of pesticides in postpartum meconium samples were described in (Table 3). We studied four classes of pesticides including chloroacetanilide (as pretilachlor), organochlorine (as DDT and lindane), organophosphate (as chlorpyrifos, diazinon and Malathion) and pyrethroid (as bioallethrin, β cyfluthrin and a cypermethrin). So nine types of pesticides were detected in the samples with significant rise in their levels.

As regards to the comparison of the pesticides levels in postpartum meconium in exposed and non-exposed groups, there was significant increase in the levels of DDT, lindane, diazinone, malathion,
bioallethrin and β-cyfluthrin in the exposed versus non-exposed group (Table 3). Comparing the pesticides levels in subjects living in rural and urban area, there was significant increase in the levels of lindane and Malathion in rural versus urban areas (P<0.05 and P<0.001 respectively) as shown in (Table 4).

**Concentration of pesticides and birth weight**

The comparison of pesticides levels in meconium of low and normal birth weight groups were shown in (Table 5). There was a significant increase in the mean meconium levels of pretilachlor, DDT, lindane, chlorpyrifos, diazinon, malathion and bioallethrin in the low versus normal birth weight group.

**Logistic regression model for exposure to pesticides among the studied groups**

The final logistic regression model for exposure to pesticides among studied group was described in (Table 6). This model indicated work and birth weight. Those who reported prenatal exposure to pesticides were four times more likely to work in agricultural work (OR=4.5, 95% CI= 2.1- 9.8). Similarly, those who reported prenatal exposure to pesticides were 1.6 times more likely to have babies with low birth weight (OR=1.59, 95% CI= 1.2 - 6.3).

**Discussion**

Detection of fetal exposure to environmental pesticides is so important. Many of the pesticides are neurotoxic and fetal exposure to these compounds could adversely affect prenatal and subsequent neurodevelopment. Adverse birth outcome, poor motor and cognitive development, and leukemia in childhood were reported [8,17-19]. The aim of our study was to determine fetal exposure to environmental pesticides in our community by measuring its levels in the meconium, to identify infants who are at risk at birth, and to identify whether exposure to pesticides might adversely influence the weight of the fetus.

The high rade of detection of pesticides in meconium is attributed to the repository nature of it, thus providing a wide window of exposure to xenobiotics. Meconium is formed at around the third or fourth month of gestation and most xenobiotics that the fetus is exposed to during gestation are deposited in meconium, through fetal swallowing or bile secretion from that period up to the time of birth. Compounds that deposit in meconium accumulate and increase in concentration, thus enhancing their detection [7,20]. On the other hand, pesticides in cord blood represent acute exposure and may not be readily detected due to low concentration as a result of the metabolism, excretion and deposition in tissues [7,20]. Each matrix provides specific information on pesticides exposure. Meconium and hair can indicate cumulative exposure, while amniotic fluid is an indicator of acute fetal exposure to xenobiotics [21].

The results of the present study showed that there was a significant rise in the levels of pesticides including pretilachlor, DDT, lindane, chloropyrifos, diazinon, malathion, bioallethrin, a cypermethrin and β cyfluthrin in postpartum meconium samples that were collected from newborns of mother exposed to pesticides in the agricultural area or at home. These results were in agreement with that of Wyard and Barr, 2001 who showed that, organophosphate metabolites, diazinon and chlorpyrifos could be detected in postpartum meconium in about 95% of the examined sample. These pesticides were of concern due to the fact that prenatal exposure has been linked experimentally to adverse neurodevelopmental sequel in the offspring. They added that, the level of these pesticides were higher than those detected in umbilical

### Table 1: Socio-demographic characteristics of the studied pregnant women (Exposed vs. Non-exposed)

| Parameters               | Exposed group (n=106) | Non-exposed group (n=84) | P-value |
|--------------------------|-----------------------|--------------------------|---------|
| Maternal Age in years*   | 27.7 ± 6.3            | 26.5 ± 6.5               | NS      |
| Residence                | NS                    | NS                       |         |
| Rural                    | 80 (55.2%)            | 65 (44.8%)               |         |
| Urban                    | 26 (17.9%)            | 19 (12.2%)               |         |
| Educational state:       |                       |                          |         |
| Educated                 | 29(56.9%)             | 22(43.1%)                |         |
| Non educated             | 77 (55.4%)            | 62(44.6%)                |         |
| Type of Work             |                       |                          |         |
| Agricultural             | 94 (63.1%)            | 55 (36.9%)               | <0.001* |
| Non-agricultural         | 12 (29.3%)            | 29 (70.7%)               |         |

*Mean ± SD

*Highly significant

### Table 2: Baseline characteristics related to pregnancy and labour of the studied samples (Exposed vs. Non-exposed)

| Parameters               | Exposed group (n=106) | Non-exposed group (n=84) | P-value |
|--------------------------|-----------------------|--------------------------|---------|
| Numbers of abortions*    | 0.57 ± 1.0            | 0.46 ± 0.7               | NS      |
| Gestational age in weeks*| 37.7 ± 2.1            | 37.43 ± 2.3              | NS      |
| Hospital Stay in days*   | 1.9 ± 1.3             | 1.7 ± 0.8                | NS      |
| Numbers of Deliveries    |                       |                          |         |
| Primipara                | 34 (57.6%)            | 25 (42.4%)               | NS      |
| Multipara                | 65 (54.6%)            | 54 (45.4%)               | NS      |
| Type of Delivery         |                       |                          |         |
| Vaginal delivery         | 40 (51.9%)            | 37 (48.1%)               |         |
| Cesarean section         | 66 (58.4%)            | 47 (41.6%)               |         |
| Birth Outcome            |                       |                          |         |
| Birth Weight in Kg*      | 3.09 ± 0.5            | 3.10 ± 0.7               | NS      |
| APGAR Score*             | 9.49 ± 1.01           | 9.61 ± 1.11              | NS      |
| Low Birth Weight         |                       |                          |         |
| Present                  | 17 (65.4%)            | 9 (34.6%)                | <0.01   |
| Absent                   | 89 (54.3%)            | 75 (45.7%)               |         |

*Mean ± SD

*Significant

### Table 3: Comparison between pesticides levels (µg/g) in meconium among exposed vs. non-exposed groups

| Pesticides*              | Exposed group (n=106) | Non-exposed group (n=84) | P-value* |
|--------------------------|-----------------------|--------------------------|---------|
| Chloroacetanilide        | 74.3 ±506.1           | 38.8 ±149.5              | NS      |
| Pretilachlor             |                       |                          |         |
| Organochlorine           | 510.4 ±3411.8         | 37.43 ± 59.9             | <0.001  |
| DDT                      |                       |                          |         |
| Lindane                  | 76.2 ± 138.8          | 13.7 ± 180.2             | <0.001  |
| Organophosphate          |                       |                          |         |
| Chlorpyrifos             | 81.6 ± 279.1          | 74.4 ± 216.0             | NS      |
| Diazinon                 | 287.4 ± 952.8         | 29.9 ± 81.2              | <0.001  |
| Malathion                | 389.9 ±773.7          | 86.1 ± 247.6             | <0.001  |
| Pyrethroid               |                       |                          |         |
| Bioallethrin             | 35.4 ± 5392.9         | 22.0 ± 5524.6            | <0.01   |
| β- Cyfluthrin            | 768.3 ± 952.8         | 135.7 ± 81.2             | <0.05   |
| α- Cypermethrin          | 285.7 ± 893.6         | 170.5 ± 539.7            | NS      |

*Mean ± SD

*Mann-Whitney non-parametric test was used to compare the mean difference between the two groups

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In the present study the frequencies of pesticides detection in the samples were: 54.7%, 57.4%, 50%, 35.8%, 53.7%, 49.5%, 34.7%, 41.1% and 21.5% to pretilachlor, DDT, lindane, chlorpyrifos, diazinon, malathion, bioallethrin, α-cypermethrin and β-cyfluthrin, respectively. These frequencies were higher than those reported by other studies showed that, whether in the urban or rural area, home pesticides constituted a high health risk in pregnant woman and were more likely related to its widespread and inappropriate use [15,30,31]. Poor education and inadequate labeling on the safe use of the pesticide were major reasons for its improper use [20]. Prenatal pesticide exposure in the mother could be detected and intervention measures could be initiated to minimize further exposure of the fetus to pesticides [20].

Low birth weight was more common in cases exposed to pesticides. Pesticides detected in significantly high concentrations included; chloroacetanilide (pretilachlor), organochlorines (DDT and lindane), organophosphates (chlorpyriphos, diazinon and malathion) and pyrethroid (bioallethrin). These results were in agreement with others who reported that IUGR was associated with organochlorine pesticide residue levels [1,19,32]. The underlying mechanism is unclear but mostly through endocrine disrupters [19]. The current study showed that women who reported prenatal exposure to pesticides were 1.6 times more likely to have babies with low birth weight. This in agreement with the results reported by Govarts who stated that, low level exposure of polychlorinated biphenyls impaired fetal growth and birth weight [33]. Similarly, Nieuwenhuijsen showed significant associations between environmental exposure to pesticides, chemicals, air pollution, tobacco smoke and pregnancy outcome in the form of stillbirth, decreased birth weight and congenital anomalies [34]. On the contrary, Dordevic and Ostrea reported that CNS disorders were present more in the pesticides exposed than in pesticides non-exposed newborns [9,18].
The effect of perinatal exposure to pesticides was also studied in animals [35-37]. Gomes and Lloyd studied the effect of organophosphorous pesticide on gestational outcomes in mice. They found that fetal weights were significantly lower in the exposed group compared to controls [35]. Likewise, Ahmed found that, intrauterine exposure to chloroaetonitrile decreased fetal body weight and induced malformations in the musculoskeletal system in mice [36].

In addition, Saillenfait reported that, oral administration of di-n-hexyl phthalate to rats caused developmental toxic effects, including marked embryo mortality and presence of malformation and significant decrease in fetal weight [37].

Women in pesticides exposed areas who intend to become pregnant as well as pregnant women are at high risk for adverse outcomes, thus it is important to identify occupational-related risk factors for prevention. Occupations in which women have a high exposure probability are agricultural industry and occupations involving the use of home pesticides. Since the effect of occupational exposure on fetal growth is considerable, pregnant women should be informed about the potential risk of pesticides exposure in workplace on birth weight. Further studies are needed to identify the molecular basis of the effects, to study the epigenetic effects of these exposures and to develop strategies to prevent exposure to these agents to improve birth outcome [19,33].

Conclusions

The results of the present study showed that pregnant women in our community including both rural and urban areas are significantly exposed to several types of pesticides particularly home and farm pesticides. Maternal occupational exposure is associated with impaired fetal growth but further studies are needed to confirm these findings, to evaluate the dose-response relationship and to assess post-natal complications that may occur later on.

Highlights

Pregnant women with prenatal exposure to pesticides were four times more likely to work in agricultural work

Similarly, those who reported prenatal exposure to pesticides were 1.6 times more likely to have babies with low birth weight

Occupations in which women have a high pesticide exposure probability are agricultural industry and occupations involving the use of home pesticides.

Since the effect of occupational exposure on fetal growth is considerable, pregnant women should be informed about the potential risk of pesticides exposure in workplace on birth weight.

**Table 6:** Final Logistic Regression Model for exposure to pesticides among the studied groups

| Type of Work | AOR* | 95% CI* | LRT* P-value |
|--------------|------|---------|--------------|
| Non-agricultural | 1 | | |
| Agricultural | 4.53 | 2.1 - 9.8 | < 0.001 |
| Low Birth Weight | | | |
| Absent | 1 | | |
| Present | 1.59 | 1.2 - 6.3 | < 0.05 |

*Initial model included maternal age, work status, number of deliveries, number of abortions, residence, pregnancy duration and birth weight categories

**Final model included work and birth weight only**

**Adjusted Odds Ratio, CI= Confidence Interval**

**+LRT=Likelihood Ratio Test**

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