Proses Korespondensi Artikel

Judul : The Relationship between Pesticide Exposure and Umbilical Serum IGF-1 Levels and Low-birth Weight: A Case-control Study in Brebes, Indonesia

Artikel No. : C-7

No.	Keterangan	Tanggal	Halaman
1	Submission	25 September 2019	2
2	Reviewer's Comments (major revision)	3 November 2019	3
3	Revised	20 November 2019	5
4	Accepted	27 November 2019	22
5	Publish	Januari 2020	23

1. Submission

[IJOEM] Submission Acknowledgement (Ms #1809)

Dari: Farrokh Habibzadeh, MD, (editor@theijoem.com)

Kepada: suhartono_damas@yahoo.com

Tanggal: Rabu, 25 September 2019 08.19 WIB

F. Habibzadeh, MD,
The IJOEM, Editorial Office,
Shiraz NIOC Polyclinics, Eram Blvd,
Shiraz 71438, Iran
Tel/Fax: +98-71-3225-2258
E-mail: editor@theijoem.com
Web: www.theijoem.com

Manuscript ID: 1809

Dear Suhartono Suhartono,

We wish to acknowledge receipt of your manuscript entitled "THE RELATIONSHIP BETWEEN PESTICIDE EXPOSURE AND UMBILICAL SERUM IGF-1 LEVELS AND LOW BIRTH WEIGHT: A CASE-CONTROL STUDY IN BREBES, INDONESIA" to Int J Occup Environ Med (The IJOEM). This manuscript will have the prompt attention of our Editorial Board. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal Web site:

Manuscript URL:
https://www.theijoem.com/ijoem/index.php/ijoem/author/submission/1809
Username: suhartono

If you have any questions regarding the manuscript, please contact me. It is understood that this article is submitted exclusively to the Int J Occup Environ Med (The IJOEM). Thank you for your submission.

Best Regards,

F. Habibzadeh, MD,
Editor, The IJOEM

Follow us on Twitter @TheIJOEM

2. Reviewer's Comments

[IJOEM] Decision Letter Ms #1809

Dari: Dr. Farrokh Habibzadeh (farrokh.habibzadeh@theijoem.com)

Kepada: suhartono_damas@yahoo.com

Tanggal: Minggu, 3 November 2019 16.55 WIB

Ms Title: "THE RELATIONSHIP BETWEEN PESTICIDE EXPOSURE AND UMBILICAL SERUM IGF-1 LEVELS AND LOW BIRTH WEIGHT: A CASE-CONTROL STUDY IN BREBES, INDONESIA"
Ms #1809

Dear Dr. Suhartono Suhartono,

Now, I have the comments of reviewers (summarized at the bottom of this e-mail). Please either consider their comments and revise your manuscript accordingly or provide reasonable answers to the comments. Please send us the revised version within next three weeks.

Best Regards,

F. Habibzadeh, MD,
Editor, The IJOEM

=======================
REVIEWERS' COMMENTS
=======================

1) This topic of the study is not new: the associations between serum IGF-1 and low-birth weight and between exposure to pesticides and serum IGF-1 have been reported earlier. What's new in this study?

2) What do you mean by saying "accidental sampling"? Do you mean "random sampling"? Did the authors include all births during the study period? If not, how did they select the cases and controls?

3) There are many confounders that might affect the birth weight. Those include age of mother, twin pregnancy, gestational age, smoking/drinking of mother, diabetes in mothers. What confounders were considered in this study? How did the authors match the cases and controls for confounders?

4) Please give a reference for the analytic method used for the assessment of IGF-1 level.

5) There were 29 cases and 36 controls. Why did you provide the data for only 29 controls in the Excel file and other analyses (Tables, etc)?

6) Omit Tables 3, 5, and 6. Just present the median and interquartile range (IQR) and p values in the text.

7) Omit Table 7. Just mention the variables with the adjusted ORs and their 95% CI (without p values) in the text. Furthermore, mention the variables the model was adjusted for.

8) Please provide some information about the birth weight, length and head circumference for the two studied groups.

9) Update References.

10) There are many typographical and grammatical mistakes? Use uniform terminology. For example, you use both "IGF-I" and "IGF-1". What do you mean by "70-acid"? Do you mean "70-amino-acid"? Did you use a "3-liter red cap tube without anticoagulation"? 3-liter or 3-mL? Please ask a native speaker familiar with scientific writing to extensively edit the manuscript.

3. Revised

Revised version of Ambarwati's manuscript (Ms #1809) and Response to reviewer files

Dari: Suhartono Damas (suhartono_damas@yahoo.com)

Kepada: farrokh.habibzadeh@theijoem.com

Tanggal: Rabu, 20 November 2019 14.01 WIB

Dear Dr. Farrokh Habibzadeh,

We are writing to present the revised version of our manuscript (Ms #1809) entitled "The relationship between pesticide exposure and umbilical serum IGF-1 levels and low birth weight: a case-control study in Brebes, Indonesia to The International Journal of Occupational and Environmental Medicine (IJOEM).

I would like to thank the reviewers and you for the very helpful constructive criticism of our original research article. The reviewers' comments allowed us to take a fresh look at our original submission. As a result, we have made considerable changes to the layout, language, and content of the manuscript.

Our responses to the reviewers' comments are presented in a file of **Response to Reviewers-Ambarwati's manuscript** and we have uploaded with the manuscript file in the IJOEM website.

We would also like to express my appreciation to you for providing us with a chance to revise and resubmit our manuscript.

Kind regards,

Suhartono

Manuscript_of Widyawati_revised version.docx
79.7kB

Response to Reviewers-Widyawati's manuscript.docx
24.8kB

We thank the reviewers for the thoughtful comments:

Reviewer Evaluation	**Author reply**
(1) This topic of the study is not new: the associations between serum IGF-1 and low-birth weight and between exposure to pesticides and serum IGF-1 have been reported earlier. What's new in this study?	To our knowledge, research on the relationship between pesticide exposure and LBW has been carried out frequently, but those that measure and analyze the levels of umbilical serum IGF-1 as an intermediate variable, are still very rare, perhaps never done. We have written this statement in the last paragraph of the introduction.
(2) What do you mean by saying "accidental sampling"? Do you mean "random sampling"? Did the authors include all births during the study period? If not, how did they select the cases and controls?	The method of selecting subjects to be included in a case or control group is more appropriately referred to as **consecutive sampling**, which is a non-probability sampling technique where the sample is taken easily from a researcher more like convenience sampling, with only a slight variation. Here, the researcher takes a sample or group of people and conducts a study over a period of time, collects the results, and then switches to another sample (https://www.questionpro.com/blog/consecutive-sampling/) To select research subjects, we first focus on finding cases, namely mothers giving birth in two hospitals in the study period with babies weighing less than 2500 grams. Mothers who give birth later with a normal baby weight (≥2500 grams) entered as a control group. All mothers chosen as a case or control group must meet the specified inclusion criteria, i.e., mothers who did antenatal care in the study area, had a complete maternal and child health books, singleton pregnancy, and spontaneous delivery. While the mother who had an emergency during labour, had a history of chronic diseases (diabetes mellitus, pulmonary tuberculosis, poor nutrition, etc.), and/or came from the outside Brebes Regency area were excluded. We conducted this sampling method mainly related to the technical implementation of umbilical blood sampling because of the process of blood sampling performed at the time of labor.
(3) There are many confounders that might affect birth weight. Those include age of mother, twin pregnancy, gestational age, smoking/drinking of mother, diabetes in mothers. What confounders were considered in this study? How did the authors match the cases and controls for confounders?	Several variables that have the potential to be confounding in this study have been controlled, namely: a. Age of mother: based on the results of data analysis → there was no significant difference in maternal age between the case and control groups; p=0.999 (Table 1) b. **Twin pregnancy**: one of the inclusion criteria in this study is singleton pregnancy → written in the material and method section, in the second paragraph c. **Maternal's/mother's and family member's smoking habit**: based on the results of data analysis, there was no difference between the case and control groups; all of p=1.000 d. **Mother's drinking habits:** there was no habit of drinking alcohol in mothers/women at the study site e. **Diabetes in mother**: the history of diabetes and other chronic diseases (pulmonary tuberculosis, malnutrition)

	were controlled by applying exclusion criteria (mothers who have a history of these diseases were excluded from the study subjects) f. **Gestational age**: gestational age is the only variable that cannot be controlled properly in this study, but we tried to control it at the time of analysis using a multivariate logistic regression test, the results of which showed gestational age was not proven to be a risk factor for LBW events.

(4) Please give a reference for the analytic method used for the assessment of the IGF-1 level.	We have changed the description of the method for measuring IGF-1 levels and have included a reference
(5) There were 29 cases and 36 controls. Why did you provide the data for only 29 controls in the Excel file and other analyses (Tables, etc.)?	In the material and method section (4th paragraph) has been written about the history of the determination of research subjects for both groups (case and control groups). For the control group, from 36 normal births, there were 7 subjects that had to be excluded from the study, namely 5 subjects because their blood samples were lysis and 2 subjects refused to continue their participation in this study.
(6) Omit Tables 3, 5, and 6. Just present the median and interquartile range (IQR) and p values in the text.	Tables 3 and 5 in the previous manuscript have been deleted and replaced with narration (text) that present median, IQR and p values
(7) Omit Table 7. Just mention the variables with the adjusted ORs and their 95% CI (without p values) in the text. Furthermore, mention the variables the model was adjusted for.	The results of the binary logistic regression test in Table 7 have been changed to text form (narration) by presenting adjusted-OR and 95% CI (without p-values)
(8) Please provide some information about the birth weight, length and head circumference for the two studied groups.	We have presented information on infant's anthropometric parameters, including birth weight, birth length and head circumference in Table 2
(9) Update References.	We have updated several references, i.e reference 11 (Langford et al. 1998), 12 (Holmes et al. 2000), and 18 (Youden 1950) with newer references on the same topic (Hellström et al 2016, Agrogiannis et 2014 and Florkowski 2008)
(10) There are many typographical and grammatical mistakes? Use uniform terminology. For example, you use both "IGF-I" and "IGF-1". What do you mean by "70-acid"? Do you mean "70-amino-acid"? Did you use a "3-liter red cap tube without anticoagulation"? 3-liter or 3-mL? Please ask a native speaker	Typographical and grammatical mistakes in our manuscript had been revised: 1. consistency/uniformity in the writing of the term IGF-1 2. mistakes writing '70-amino acid' word in the discussion section → has been revised 3. We have replaced the sentence "3-liter red cap tube without anticoagulation" in the

familiar with scientific writing to extensively edit the manuscript.	IGF-1 assessment method with another sentence that conforms to the IGF-1 assessment standard. To reduce the possibility of errors in writing words or sentences we have checked this manuscript with the Grammarly program

THE RELATIONSHIP BETWEEN PESTICIDE EXPOSURE AND UMBILICAL SERUM IGF-1 LEVELS AND LOW BIRTH WEIGHT: A CASE-CONTROL STUDY IN BREBES, INDONESIA

Sigit A Widyawati[1], Suhartono Suhartono[2], Mexitalia Mexitalia[3], Ariawan Soejoenoes[4]

[1)] Public Health Study Programme, Faculty of Health Science, Ngudi Waluyo University, Semarang, Indonesia

[2)] Department of Environmental Health, Faculty of Public Health, Diponegoro University, Semarang, Indonesia

[3)]Department of Pediatrics, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

[4)] Faculty of Medicine, Diponegoro University, Semarang, Indonesia

Correspondence to:
Suhartono, Department of Environmental Health, Faculty of Public Health,
Diponegoro University, Semarang, Indonesia,
Jalan Prof. Soedarto, SH, Tembalang, Semarang 1269, Indonesia.
Tel:+62-81-127-5410,
E-mail: suhartono_damas@yahoo.com

Abstract

Background: Birth weight is very important for long-term physical, mental, health and brain development. Pesticide exposure is thought to interfere with fetal growth, among others through disruption of the function of the IGF-1 hormone.

Objective: To analyze the relationship between exposure to pesticides during pregnancy and low birth weight (LBW) through the disruption of the IGF-1 hormone.

Matherials and Methods: This was a case-control study. Babies born with LBW (<2500 grams) and babies born later with normal birth weight (≥2500 grams) at two hospitals in Brebes in the study period were chosen as cases and controls, respectively. Maternal pesticide exposure was measured by interview using a questionnaire. Umbilical serum IGF-I level was tested using the ELISA method.

Results: There was a significant relationship between pesticide exposure during pregnancy and LBW (p=0.003; OR=6.8; 95% CI=2.0-22.9) and low umbilical serum IGF-1 levels (p=0.047; OR=3.6; 95% CI=1.2-11.1), and there was a significant relationship between low umbilical serum IGF-1 levels and LBW (p=0.001; OR=8.9; 95% CI=2.4-32.1).

Conclusion: There was a significant relationship between pesticide exposure during pregnancy and LBW through the umbilical serum IGF-1 reduction pathway.

Keywords: Pesticide Exposure, Umbilical Serums IGF-1, Low Birth Weight (LBW)

Introduction

Birth weight is one of the determinants of a baby's survival and will determine the quality of physical and mental growth in the future. Birth weight is even known to have a close relationship with long-term brain development.[1] Low Birth Weight (LBW), i.e. weight at birth less than 2500 grams,[2] is an important health concern, particularly in developing countries.[3] LBW is not only a major predictor of prenatal mortality and morbidity, but recent studies have found that LBW also increases the risk for noncommunicable diseases such as diabetes and cardiovascular disease later in life.[4,5]

Optimal intrauterine growth is essential for fetal development and contributes to birth weight and long-term health. Fetal growth is influenced by interactions between genetic, nutritional, hormonal, and environmental factors.[6] One environmental factor that needs attention is exposure to pesticides during pregnancy.[7,8] Pregnant women in agricultural areas are at risk of exposure to pesticides due to their involvement in agricultural activities. Several types of pesticides are often used in agricultural areas and are classified as Endocrine Disrupting Chemicals (EDC), including organophosphate, chlorpyrifos, and malathion.[9]

Exposure to toxic substances from the environment can interfere with the synthesis and secretion of the growth hormone Insulin-like Growth Hormone-1 (IGF-1).[10] IGF-1 is one of the most significant growth factors and hormones in fetal growth,[11,12] and plays a role in fetal growth especially in advanced gestational age.[13] IGF-1 is a single chain polypeptide weighing 7.5 kDa, which can promote growth both before and after birth. During pregnancy, IGF-1can affect fetal growth through effects on maternal metabolism and placenta, where IGF-1 plays a role in metabolism, mitogenesis, and differentiation of various types of cells including regulation of the development of trophoblast cells that make up the placenta.[14,15]

Research on the relationship of pesticide exposure to LBW has been conducted frequently, but those that measure and analyze umbilical serum IGF-1 levels as an intermediate variable, are still very rare. This study aims to analyze the relationship between exposures to pesticides with LBW through interference with umbilical serum IGF-1 levels.

Materials and Methods

This study used an observational design with a case-control approach. The population is mothers who gave birth in the period January-May 2018 in the Brebes Regional General Hospital and Bhakti Asih Hospital Brebes, Central Java Province.

Inclusion criteria in the selection of subjects were: mothers who did antenatal care in the study area, had a complete maternal and child health books, singleton pregnancy, and spontaneous delivery. While the mother who had an emergency during labor, had a history of chronic diseases (diabetes mellitus, pulmonary tuberculosis, poor nutrition, etc), and/or came from the outside Brebes Regency area were excluded.

Sampling was carried out by a consecutive sampling method of all deliveries at the Brebes General Hospital and Bhakti Asih Hospital in the period from January to May 2018 and fulfilled the inclusion criteria. Babies born with LBW (< 2500 grams) were included in a case group, while infants born later with normal weight (≥ 2500 grams) were included in the control group. Data on gestational age at the time of labor was obtained from the medical birth registry from the hospital. For purposes of the analysis, data on gestational age are categorized into preterm birth (<38 weeks) and term (≥ 38 weeks), because a normal pregnancy can range 38 to 42 weeks.[16]

The minimum sample size was calculated based on the case-control formula proposed by Lemeshow, et al.[17] In the research process we got 36 births with LBW (as cases) and chose 36 normal births as controls. Of 36 LBW births, 7 subjects were dropping out, because of lysis the blood samples (4 subjects), eclampsia occurs, the baby died, and refused to be the research subjects, so finally 29 LBW births were obtained as a case group. While of 36 normal births, there were 7 subjects who dropped out, because lysis of blood samples (5 subjects) and 2 subjects were refused to continue their participation in this study so that 29 subjects were obtained as a control group.

Structured questionnaires were used to collect the subject's characteristics and history of pesticide exposure. History of pesticide exposure was a composite variable of four sub-variables of exposure risks: 1) maternal involvement in agriculture before pregnancy, 2) maternal involvement in agriculture during pregnancy, 3) the maternal risk of exposure to pesticides in the home environment before pregnancy, and 4) the maternal risk of exposure to pesticides in the home environment during pregnancy. Subjects were considered to be exposed to pesticides if they had a minimum of two out of four exposure risks. Structured questionnaires were also used to obtain information about the characteristics of the subjects (age, type of occupation, level of education, maternal's smoking habit, the presence of family members who have smoking habits, etc.)

Blood sampling from the umbilical cord during labor was carried out by trained health workers (midwives). Plasma concentrations of IGF-I were measured using microplate enzymelinked immunosorbent assays (ELISA, Diagnostic Systems Laboratories, Webster, TX, USA) according to the manufacture instructions, and triplicate assays persample were performed. Absorbance was read using a microplate reader (DSL, Webster, TX, USA).[18] The cut-off value for IGF-1 level for LBW was determined using ROC curve analysis by maximizing the Youden index[19,20] which revealed a cut-off value of 32.6 ng/dL, with sensitivity and specificity of 0.862 and 0.586, respectively (Figure 1). IGF-1 levels are considered 'low' if <32.6 ng/dL.

Data were analyzed using SPSS for Windows version 16.0. Independent-sample t-test and Mann-Whitney U tests were used to compare the normally and non-normally distributed data, respectively, between cases and controls. Spearman's rho correlations were used to prove the correlation between IGF-1 serum level and the newborn's anthropometric data (birth weight, head circumference, and birth length). Chi-square test and calculation of the odds ratio (OR) and 95% Confidence Interval (CI) were used to determine the relationship between pesticide exposure and IGF-1 levels and LBW. To analyze the relationship between pesticide exposure, IGF-1 levels and, gestational age categories and LBW, the binary logistic regression test was performed. We also used the Spearman Rank Correlation test to analyze the correlation between umbilical serum IGF-1, birth weight, birth length, and head circumference.

Ethics

This study was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Diponegoro University (688/EC/FK-RSDK/XII/2017).

Results

The two groups (29 in each arm) were well matched in their basic characteristics, with the exception of the gestational age and infants anthropometric data i.e. birth weight, birth length, and head circumference (Table 1).

Table 1. Subjects characteristics. Values are either median (interquartile range, IQR) or n (%)

Subjects characteristics	**Case (*n*=29)**	**Control (*n*=29)**	***p* -value**
Age (years)	31.0 (11.0)	29.0 (11.0)	0.999 [a]
Weight (kg)	60.0 (12.0)	60.0 (11.0)	0.861 [a]
Height (cm)	155.0 (7.0)	154.0 (8.0)	0.318 [b]
Gestational age (weeks)	36.0 (3.5)	38.0 (2.5)	0.001[b]
Hemoglobin levels (g/dL)	11.0 (0.4)	11.0 (0.5)	0.415 [b]
Gravida			
-Primigravida	10 (34.5)	9 (31.0)	1.000 [c]
-Multigravida	19 (65.5)	20 (69.0)	
Parity			
-Primipara	9 (31.0)	9 (31.0)	0.523 [c]
-Multipara	20 (69.0)	20 (69.0)	
Maternal Education			
Elementary school	20 (69.0)	21 (72.4)	0.577 [c]
Junior high school	6 (20.7)	7 (24.1)	
High school	29 (100.0)	1 (3.4)	
Maternal Occupation			
Private employees	1 (3.4)	4 (13.8)	0.438 [c]
Traders	3 (10.3)	5 (17.2)	
Farmer/owner	1 (3.4)	2 (6.9)	
Farmworkers	13 (44.8)	8 (27.6)	
Housewife	10 (34.5)	10 (34.5)	
Etc	1 (3.4)	0 (0.0)	
Husband's education			
Elementary school	20 (69.0)	21 (72.4)	0.340 [c]
Junior high school	5 (17.2)	7 (24.1)	
High school	4 (13.8)	1 (3.4)	
Husband's occupation			
Private	4 (13.8)	6 (20.7)	0.900 [c]
Traders	9 (31.0)	8 (27.6)	
Farmer/owner	0 (0.0)	1 (3.4)	
Farmworkers	14 (48.3)	12 (41.4)	
Fisherman	1 (3.4)	1 (3.4)	
Etc	1 (3.4)	1 (3.4)	
Maternal's smoking habit			
Yes	0 (0.0)	0 (0.0)	1.000 [c]
No	29 (100.0)	29 (100.0)	
Family members smoking habits			
Yes	25 (86.2)	24 (82.8)	1.000 [c]
No	4 (13.8)	5 (17.2)	

[a] Independent-sample t-test, [b] Mann-Whitney U test, [c] Chi-Square test

The results of anthropometric measurements showed a significant difference in all parameters, namely birth weight, birth length, and head circumference between case and control groups (Table 2).

Table 2. The comparisons of infant anthropometric parameters between case and control groups. Values are median (IQR)

Infants anthropometrics	**Case (*n*=29)**	**Control (*n*=29)**	***p* -value**
Birth Weight (gram)	2100.0 (445.0)	3100.0 (750.0)	< 0.001 [a]
Birth Length (cm)	44.0 (2.5)	48.0 (3.5)	< 0.001 [a]
Head Circumference (cm)	30.0 (2.0)	33.0 (2.5)	< 0.001[a]

[a] Mann-Whitney U test

Maternal involvement in agricultural activities before pregnancy and the risk of pesticide exposure in the home environment during pregnancy were proved as risk factors for LBW, OR=4.9 and 6.2, respectively. The other exposure risk variables, i.e. maternal involvement in agricultural activities during pregnancy and the risk of pesticide exposure in the home environment before pregnancy, even though they are not significant, they also tend to be risk factors. The results showed the history of maternal pesticide exposure (as a composite variable) is a risk factor for LBW (OR=6.8 and 95% CI=2.0-22.9) (Table 3).

Table 3. The relationship between pesticide exposure and LBW. Values are *n* (%)

Variables	**Cases (n=29)**	**Controls (n=29)**	**OR (95% CI)**
1. Maternal involvement in agricultural activities before pregnancy			
• Yes	21 (72.4)	10 (34.5)	4.9 (1.6-15.2)
• No	8 (27.6)	19 (65.5)	
2. Maternal involvement in agricultural activities during pregnancy			
• Yes	13 (44.8)	7 (24.1)	2.5 (0.8-7.8)
• No	16 (52.5)	22 (75.9)	
3. The risk of pesticide exposure in the home environment before pregnancy			
• Yes	18 (62.1)	11 (37.9)	2.6 (0.9-7.7)
• No	11 (37,9)	18 62.1)	
4. The risk of pesticide exposure in the home environment during pregnancy			
• Yes	18 (62.1)	6 (20.7)	6.2 (1.9-20.2)
• No	11 (37.9)	23 (79.3)	
5. History of maternal pesticide exposure (composite variable)*			
• Yes	17 (58.6)	5 (17.2)	6.8 (2.0-22.9)
• No	12 (41.4)	24 (82.8)	

* Subjects were considered to be exposed to pesticides if they had a minimum of two out of four exposure risks

The Mann-Whitney U test proved that there was a significant difference in IGF-1 levels according to a history of pesticide exposure. Subjects with a history of pesticide exposure had lower IGF-1 levels than those without a history of exposure (p = 0.003), with median (interquartile range, IQR) 29.7 (17.37) and 52.7 (40.05) ng/dL respectively. Meanwhile, mothers with a history of pesticide exposure had a 3.6 times risk of having lower IGF-1 levels than those without a history of pesticide exposure (Table 4).

Table 4: The relationship between pesticide exposure and IGF-1 levels. Values are n (%)

Maternal exposure to pesticide	Umbilical serum IGF-1 levels		OR (95% CI)
	'Low' (<32.6 ng/dL)	'High' (≥32.6 ng/dL)	
• Yes (n=22)	12 (54.5)	10 (45.5)	3.6 (1.2-11.1)
• No (n=36)	9 (25.0)	27 (75.0)	

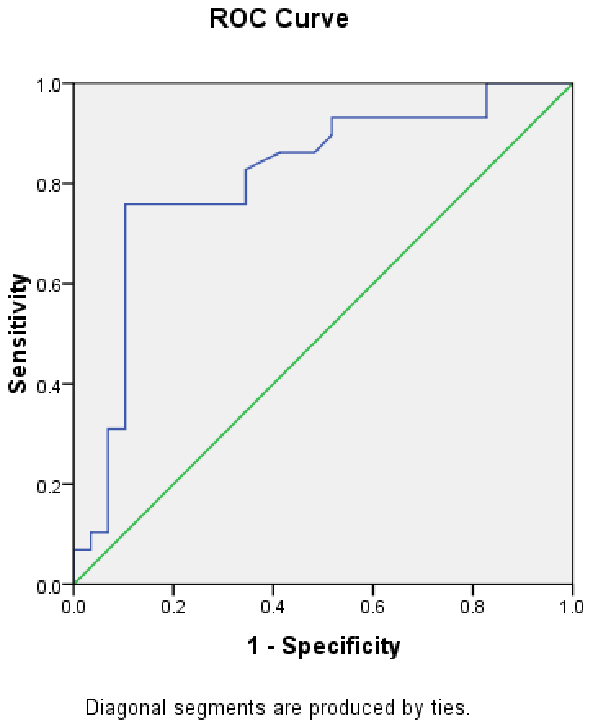

Figure 1: ROC curves for various umbilical serum IGF-1 cut-off values for LBW

The results showed IGF-1 levels in the case group were lower than the control group (Mann-Whitney U test; p <0.001), median (IQR) were 30.6 (16.88) and 57.1 (35.86) ng/dL respectively. Mothers with 'low' IGF-1 levels are at risk of giving birth to babies with low birth weight 8.9 times compared to mothers with 'high' IGF-1 levels (Table 5).

The binary logistic regression showed that of the three predictor variables, there were two variables that proved to be LBW risk factors, namely pesticide exposure (adjusted-OR=4.1; 95% CI =1.1-16.2) and low IGF-1 level (adjusted-OR =7.2; 95% CI=1.8-29.2), meanwhile gestational age less than 38 weeks was not proven to be a risk factor for LBW (adjusted-OR =2.7; 95% CI=0.7-9.8).

The results also showed a significant correlation between umbilical serum IGF-1 and all of the anthropometric parameters of the newborn, namely birth weight, head circumference and body length (all of the p-values are < 0.001) (Table 6).

Table 5: The relationships between umbilical serum IGF-1 levels and LBW. Values are *n* (%)

Umbilical serum IGF-1 levels	Cases (n=29)	Controls (n=29)	OR (95% CI)
• 'Low' (<32.6 ng/dL)	17 (58.6)	4 (13.8)	8.9 (2.4-32.1)
• 'High' (≥32.6 ng/dL)	12 (41.4)	25 (86.2)	

Table 6. Correlation between umbilical serum IGF-1, birth weight, birth length, and head circumference

Variables	Umbilical serum IGF-1		Birth weight		Birth length		Head circumference	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Umbilical serum IGF-1	-	-	0.596	<0.001	0.532	<0.001	0.445	<0.001
Birth weight	-	-	-	-	0.820	<0.001	0.777	<0.001
Birth length	-	-	-	-	-	-	0.701	<0.001
Head circumference	-	-	-	-	-	-	-	-

Discussion

The main finding of this study is the proven relationship between exposure to pesticides in pregnant women and LBW. A study in France concluded that exposure to pesticides in pregnant women has been shown to influence fetal development in the womb.[21] The involvement of pregnant women in agricultural activities puts them at risk of exposure to pesticides, for example when farmers spray plants with pesticides in the fields and pregnant women at the same time are looking for pests or pulling grass from plants. Our previous study, in the same location, proved that pesticide exposure is a risk factor for goiter,[22] hypothyroidism[23], and stunting in children.[24]

The mechanism of growth disturbance in the fetal and child phase due to pesticide exposure is because pesticides are classified as Endocrine Disrupting Chemicals (EDCs), which are chemicals in the environment that can interfere with the synthesis, secretion, transport, metabolism, binding action, and elimination of hormones in the body that function to maintain balance (homeostasis), reproductive, and growth processes.[25] One of the hormones can be disrupted by exposure to pesticides is Insulin-like Growth Factor-1 (IGF-1), a hormone that is needed in the process of the growth process. IGF-1 is a mitogenic hormone peptide with a single 70-amino acid, its structure is similar to proinsulin, which stimulates systemic body growth in various species. IGF-1 is produced in almost all organs, but the liver is the main source of circulating IGF-1.[26]

Our study showed that pregnant women with a history of pesticide exposure have lower umbilical serum IGF-1 levels than those not exposed to pesticides. Umbilical serum IGF-1 levels in the case group also proved to be much lower than the control group. These results are consistent with studies in Sudan[27] and Norway.[28] Our previous study also proved that IGF-1 levels in stunting children were lower than in normal children.[24] Analysis of all subjects (n=59) showed a significant correlation between umbilical serum IGF-1 levels and infant anthropometric parameters (birth weight, length of birth and head circumference). There is a growing body of evidence linking IGF-1 with neonate birth weight.[29] This relationship is manifested by any increase in neonate IGF-1 associated with an increase in birth weight[29] and any decrease in IGF-I associated with a decrease in birth weight.[30] In the foetus, IGF-1 and insulin, which promotes IGF-1 production,[31] rather than growth hormone is the main driver of growth.[32] IGF-1-dependent growth is mediated by the glucose–insulin axis, which allows a rapid response to nutritional fluctuations,[33] and concentrations of IGF-1 normally rise throughout mid–late gestation to support the accelerated growth that normally occurs in the third trimester in utero.[33,34]

Based on research reports on the effects of pesticides on growth hormone and IGF-1 as a result of estrogenic/anti-androgenic, it is possible that pesticides can also directly influence the growth hormone IGF-1 system.[35] Studies in animals and humans have shown that environmental pollution, such as benzopyrene, dioxin, dibenzofurans, and hexachlorobenzene can alter the normal synthesis or secretion of IGF-1.[36] Exposure to pesticides, especially organochlorine groups, can also interfere with IGF-1 function.[10,37] Pesticide exposure could be a risk factor for the occurrence of growth disorders in children living in agricultural areas.[24] Research in Spain proved that the average IGF-1 level in the female group detected by DDT metabolism in urine was lower than that which was not detected.[10] Based on the multivariate logistic regression tests it is proven that pesticide exposure and low umbilical serum IGF-1 levels (<32.6 ng/dL) together were the risk factors for LBW. During pregnancy, levels of growth factors such as IGF-1 and IGF-2, *Epidermal Growth Factor* (EGF), *Platelet-Derived Growth Factor* (PDGF), *Factor Growth Fibroblasts* (FGF-2 and FGF-4), and *Transforming Growth Factor* (TGF-ß) increase in the maternal circulation and continued elevated levels during pregnancy, this suggests that the hormone has an important role in the growth of the developing fetus.[38] Research by Karamizadeh (2008) in Iran found a positive and significant correlation between maternal IGF-1 levels and infant birth weight.[39] IGF-1 hormones play a role in growth through their role as mitogens and stimulators of cell proliferation and play an important role in the process of mild repair/regeneration.[40] Exposure to toxic materials that occur during the growth period (fetus, baby and child) is more likely to have a negative impact than exposure as adults.[41]

The limitation in this study is the measurement of the IGF-1 level that is only one time and describes the condition of the moment, so the temporality relationship in the assumption of causality has not been proven well. For further research, it is necessary to consider serial measurements of serum umbilical IGF-1, at the beginning of the first, second and third trimester, so an explanation of the role of IGF-1 as an intervening/intermediate variable of fetal growth disturbance due to pesticide exposure can be proven better.

Conclusion

There is a significant relationship between exposure to pesticides during pregnancy and LBW through the umbilical IGF-1 serum reduction pathway. However, further research is still needed, so that the explanation of the pathophysiology of LBW due to pesticide exposure can be better explained.

Conflicts of Interest: None declared.

Financial support: This research was funded by Doctoral Grant from DP2M DIKTI with grant number 032/K6/KM/SP2H/PPM/2018, February 19, 2018.

References

1. Walhovd K, Fjell A, Brown T, et al. Long-term in fl uence of normal variation in neonatal characteristics on human brain development. 2012;109(49):20089-20094. doi:10.1073/pnas.1208180109

2. World Health Organization. *Guidelines on Optimal Feeding of Low Birthweight Infants in Low-and Middle-Income Countries*.; 2011.

3. World Health Organization. *WHA Global Nutrition Targets 2025 : Low Birth Weight Policy Brief*.; 2014.

4. Risnes K, Vatten L, Baker J, et al. Birthweight and mortality in adulthood : a systematic review and meta-analysis. *Int J Epidemiol*. 2011;40:647-661. doi:10.1093/ije/dyq267

5. Larroque B, Bertrais S, Czernichow P, Leger J. School difficulties in 20-year-olds who were born small for gestational age at term in a regional cohort study. *Pediatrics*. 2001;108(1):111-115.

6. Grissa O, Yessoufou A, Mrisak I, et al. Growth factor concentrations and their placental mRNA expression are modulated in gestational diabetes mellitus : possible interactions with macrosomia. *Pregnancy and Childbirth*. 2010;10(7):1-10. doi:10.1186/1471-2393-10-7

7. Dabrowski S, Hanke W, Polanska K, Makowiec-Dabroeska T, Sobala W. Pesticide exposure and birthweight: An epidemiological study in Central Poland. *Int J Occup Med Environ Health*. 2003;16(1):31-39.

8. National Pesticide Information Center. Pesticides and Pregnancy. http://npic.orst.edu/health/preg.html. Published 2018.

9. Allsop M, Huxdorff C, Johnston P, Santilo D, Thompson K. *Pesticides and Our Health. A Growing Concern*. United Kingdom: Greenpeace Recearch Laboratories; 2015.

10. Boada L, Lara P, Álvarez-León E, et al. Serum levels of insulin-like growth factor-I in relation to organochlorine pesticides exposure. *Growth Horm IGF Res*. 2007;17:506-511. doi:10.1016/j.ghir.2007.05.004

11. Hellström A, Ley D, Hansen-pupp I, et al. Role of insulinlike growth factor 1 in fetal development and in the early postnatal life of premature infants. *Am J Perinatol*. 2016;33:1067-1071.

12. Agrogiannis G, Sifakis S, Patsouris E, Konstantinidou A. Insulin-like growth factors in embryonic and fetal growth and skeletal development (Review). *Mol Med Rep*. 2014;10:579-584. doi:10.3892/mmr.2014.2258

13. Gluckman P, Pinal C. Fetus and infant regulation of fetal growth by the somatotrophic axis. *J Nutr*. 2003;133:1741S-1746S.

14. Sferruzzi-Perri A, Owens J, Pringle K, Roberts C. The neglected role of insulin-like growth factors in the maternal circulation regulating fetal growth. *J Physiol*. 2011;1:7-20. doi:10.1113/jphysiol.2010.198622

15. Wagey F, Joice K, Indriani. IGF-1 levels related to incidence of macrosomia. *Maj Obstet Ginekol*. 2013;21(3):121-124.

16. The American College of Obstetricians and Gynecologists. *Definition of Term Pregnancy*.; 2013.

17. Lemeshow S, Hosmer Jr D, Klar J, Lwanga S. *Adequacy of Sample Size in Health Studies*. New York: John Wiley & Sons Ltd.; 1993.

18. Berrigan D, Potischman N, Dodd K, et al. Serum levels of insulin-like growth factor-I and insulin-like growth factor-I binding protein-3 : Quality control for studies of stored serum. *Cancer Epidemiol Biomarkers Prev*. 2007;16(5):1017-1022. doi:10.1158/1055-9965.EPI-07-0044

19. Florkowski C. Sensitivity, specificity, receiver-operating characteristic (ROC) curves and likelihood ratios: Communicating the performance of diagnostic tests. *Clin Biochem Rev*. 2008;29(8):S83-S87.

20. Habibzadeh F, Habibzadeh P, Yadollahie M. On determining the most appropriate test cut-off value: the case of tests with continuous results. *Biochem Medica*. 2016;26(3):297-307.

21. Petit C, Chevrier C, Durand G, Monfort C, Rouget F, Garlantezec R. Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities : a prospective cohort study in Brittany, France. *Environ Heal*. 2010;9(71):12p. doi:10.1186/1476-069X-9-71

22. Kartini A, Suhartono S, Pangestuti D, Adi M, Suratman, Rasipin. Goiter and hypothyroidism among elementary school children in lowland agricultural area, Brebes District Indonesia. *Indian J Public Heal Res Dev*. 2018;9(9):120-125.

23. Suhartono S, Kartini A, Subagio H, et al. Pesticide exposure and thyroid function in elementary school children living in an agricultural area, Brebes District, Indonesia. *Int J Occup Environ Med*. 2018;9(3):137-144. doi:10.15171/ijoem.2018.1207

24. Kartini A, Subagio H, Hadisaputro S, Kartasurya M, Suhartono S, Budiyono B. Pesticide exposure and stunting among children in agricultural areas. *Int J Occup Environ Med*. 2019;10(1):17-29. doi:10.15171/ijoem.2019.1428

25. Diamanti-kandarakis E, Bourguignon J, Giudice L, et al. Endocrine-disrupting chemicals : an endocrine society scientific statement. *Endocr Rev*. 2009;30:293-342. doi:10.1210/er.2009-0002

26. Hellstrom A, Ley D, Hansen-pupp I, et al. Insulin-like growth factor 1 has multisystem effects on foetal and preterm infant development. *Acta Paediatr*. 2016;105:576-586. doi:10.1111/apa.13350

27. Elzein A, Ali A, Hamdan H, Elhassan E, Shrif N, Adam I. Materno-foetal leptin and insulin-like growth factor in low birth weight neonates. *J Obstet Gynaecol (Lahore)*. 2016;36:31-33. doi:10.3109/01443615.2015.1030607

28. Asvold B, Eskild A, Jenum P, Vatten L. Maternal concentrations of Insulin-like Growth Factor I and Insulin-like Growth Factor Binding Protein 1 during pregnancy and birth weight of offspring. *Am J Epidemiol*. 2011:1-7. doi:10.1093/aje/kwr067

29. Vidal A, Murtha A, Murphy S, et al. Maternal BMI , IGF-I levels , and birth weight in African American and White infants. *Int J o Pediatr*. 2013:1-7. doi:10.1155/2013/191472

30. Akman I, Arioglu P, Koroglu O, et al. Maternal zinc and cord blood zinc, insulin-like growth factor-1, and insulin-like growth factor binding protein-3 levels in small-for-gestational-age newborns. *Clin Exp Obs Gynecol*. 2006;33(4):238-240.

31. LeRoith D, Yakar S. Mechanisms of Disease : metabolic effects of growth hormone and insulin-like growth factor 1. *Endocrinol Metab*. 2007;3(3):302-310. doi:10.1038/ncpendmet0427

32. Murray P, Clayton P. Endocrine Control of Growth. *Am J Med Genet Part C*. 2013;163C:76-85. doi:10.1002/ajmg.c.31357

33. Kajantie E, Dunkel L, Rutanen E, et al. IGF-I, IGF Binding Protein (IGFBP)-3, Phosphoisoforms of IGFBP-1, and Postnatal Growth in Very Low Birth Weight Infants. *J Clin Endocrinol Metab*. 2002;87(5):2171-2179.

34. Chiesa C, Osborn J, Haass C, et al. Insulin Concentrations at Birth : Is There a Relationship with Fetal Growth and Neonatal Anthropometry ? *Pediatr Clin Chem*. 2008;54(3):550-558. doi:10.1373/clinchem.2007.095299

35. Gore A, Crews D, Doan L, Merrill M, Patisaul H, Zota A. *Introduction to Endocrine Disrupting Chemicals (EDCs)-A Guide for Public Interest Organizations and Policy-Makers*. Endocrine Society; 2014. http://endocrine.org.

36. Holloway A, Petrik J, Younglai E. Influence of dichlorodiphenylchloroethylene on vascular endothelial growth factor and insulin-like growth factor in human and rat ovarian cells. *Reprod Toxicol*. 2007;24:359-364. doi:10.1016/j.reprotox.2007.05.003

37. Zumbado M, Luzardo O, Lara P, et al. Growth Hormone & IGF Research Insulin-like growth factor-I (IGF-I) serum concentrations in healthy children and adolescents : Relationship to level of contamination by DDT-derivative pesticides. *Growth Horm IGF Res*. 2010;20:63-67. doi:10.1016/j.ghir.2009.07.003

38. Romanowicz L, Galewska Z. Extracellular matrix remodeling of the umbilical cord in pre-eclampsia as a risk factor for fetal hypertension. *J Pregnancy*. 2011:9p. doi:10.1155/2011/542695

39. Karamizadeh Z, Saki S, Kashef S, Saki F. Comparison of the umbilical cord and maternal serum levels of IGF-1, leptin and cortisol in appropriate for gestational age and small for gestational age neonates. *Int J Endocrinol Metab*. 2008;2:89-94.

40. Guntur A, Rosen C. IGF-1 regulation of key signaling pathways in bone. *Bonekey Rep*. 2013;2(437):1-6. doi:10.1038/bonekey.2013.171

41. Meeker J. Exposure to environmental endocrine disruptors and child development. *Arch Pediatr Adolesc Med*. 2012:E1-E7. doi:10.1001/archpediatrics.2012.241

4. *Accepted*

[IJOEM] Decision Letter Ms #1809

Dari: Dr. Farrokh Habibzadeh (farrokh.habibzadeh@theijoem.com)

Kepada: suhartono_damas@yahoo.com

Tanggal: Rabu, 27 November 2019 01.51 WIB

Ms Title: "THE RELATIONSHIP BETWEEN PESTICIDE EXPOSURE AND UMBILICAL SERUM IGF-1 LEVELS AND LOW BIRTH WEIGHT: A CASE-CONTROL STUDY IN BREBES, INDONESIA"
Ms #1809

Dear Dr. Suhartono Suhartono,

We reviewed the revised version of your manuscript. I am pleased to inform you that the manuscript has been accepted for publication in The IJOEM. We'll edit your manuscript according to our house style and send you the galley proof of the article. In due course, you may be asked some editorial questions. Thank you very much for your contribution to the Journal.

Best Regards,

F. Habibzadeh, MD,
Editor, The IJOEM

6. Publish

The International Journal of Occupational and Environmental Medicine

The Relationship between Pesticide Exposure and Umbilical Serum IGF-1 Levels and Low-birth Weight: A Case-control Study in Brebes, Indonesia

Sigit Ambar Widyawati, Suhartono Suhartono, Maria Mexitalia, Ariawan Soejoenoes

Abstract

Background: Birth weight is very important for long-term physical, mental, health, and brain development. Pesticide exposure is thought to interfere with fetal growth, among others, through disruption of the function of the insulin-like growth hormone-1 (IGF-1) hormone.

Objective: To analyze the relationship between exposure to pesticides during pregnancy and low-birth weight (LBW) through the disruption of the IGF-1 hormone.

Methods: In a case-control study, babies born with LBW (birth weight <2500 g) and those born later with normal birth weight (≥2500 g) at 2 hospitals in Brebes were chosen as cases and controls, respectively. Maternal pesticide exposure was measured by interview using a questionnaire. Umbilical serum IGF-I level was tested using the ELISA method.

Results: There was a significant relationship between pesticide exposure during pregnancy and LBW (OR 6.8; 95% CI 2.0 to 22.9) and low umbilical serum IGF-1 levels (OR 3.6; 95% CI 1.2 to 11.1). There was a significant relationship between low umbilical serum IGF-1 levels and LBW (OR 8.9; 95% CI 2.4 to 32.1).

Conclusion: There was a significant relationship between pesticide exposure during pregnancy and LBW through the umbilical serum IGF-1 reduction pathway.

Keywords

Pesticides; Fetal blood; Infant, low birth weight; Organophosphates; Insulin like growth factor I

Full Text:

FULL TEXT (HTML) PDF [604 KB] EPUB

doi: 10.15171/ijoem.2020.1809

pISSN: 2008-6520
eISSN: 2008-6814