Association between pesticide usage during pregnancy and neonatal hyperbilirubinemia requiring treatment: The Japan Environment and Children's Study

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Author contributions

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T.S., T.N. performed statistical analysis and interpretation of data.

T.S. drafted the article.

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1. What is the key message?

Our finding indicated that hyperbilirubinemia requiring phototherapy had a significant positive association with the frequent use of indoor insecticide spray during pregnancy.

2. What does it add the existing literature?

This is the first large study examining the effects of maternal exposure to pesticides or repellents on clinically relevant neonatal hyperbilirubinemia using a dataset from a nationwide birth cohort study.

3. What is the impact?

This large-scale Japanese cohort study revealed that the frequent use of indoor insecticide spray during pregnancy may increase the risk of neonatal hyperbilirubinemia requiring treatment.

Abstract

Background: Maternal exposure to pesticides during pregnancy may cause oxidative hemolysis leading to neonatal hyperbilirubinemia. This investigation examined for associations between maternal use of pesticides or repellents during pregnancy and neonatal hyperbilirubinemia requiring phototherapy.

Methods: We used the dataset from the Japan Environment and Children's Study, a large national birth cohort study registered from January 31, 2011 to March 31, 2014. The fixed data of 61,751 live births were used to evaluate the presence of neonatal hyperbilirubinemia and potential confounding factors. We employed multiple logistic regression analysis to identify correlations between the frequency of maternal pesticide or repellent use during pregnancy and clinically relevant neonatal hyperbilirubinemia.

Results: After controlling for confounding factors, there were significant associations between neonatal hyperbilirubinemia necessitating phototherapy and the frequent use of indoor insecticide spray (OR 1.21, 95% CI 1.05–1.38). For spray- or lotion-type insect repellents, an opposite relationship was observed (more than a few times a week: OR 0.70, 95% CI 0.61–0.81, up to a few times a month: OR 0.84, 95% CI 0.78–0.91).

Conclusion: The frequent use of indoor insecticide spray during pregnancy showed an increased risk of neonatal hyperbilirubinemia requiring phototherapy, which was absent for spray- or lotion-type insect repellents.

Introduction

Neonatal hyperbilirubinemia, or neonatal jaundice, is a common clinical symptom in the neonatal period and especially in the first week of life. The majority of neonatal jaundice cases are induced physiologically, with almost 60% of healthy infants considered to develop some degree of idiopathic jaundice (1). The frequency of neonatal jaundice varies according to race. For instance, the incidence of nonphysiologic hyperbilirubinemia in Asian infants is twice of that in whites and triple of that in blacks (2). Genetically, the frequency of a missense mutation in the UGT1A1 gene encoding a key enzyme of bilirubin catabolism is high in the Japanese and a proposed risk factor for neonatal hyperbilirubinemia (3). Although bilirubin has an antioxidative effect (4), infants are at risk for developing kernicterus by bilirubin deposition in the basal nucleus (5), indicating a higher risk of cerebral palsy due to neonatal hyperbilirubinemia in Asia than in other regions. Usually, neonatal hyperbilirubinemia is treated by phototherapy at first. The purpose of phototherapy is to lower the concentration of circulating bilirubin or keep it from increasing. Light energy used by phototherapy changes the shape and structure of bilirubin, converting it into molecules that can be excreted in bile or urine. It is known that phototherapy reduces severe neonatal hyperbilirubinemia requiring extensive treatment such as exchange transfusions (6).

Organophosphates, pyrethroids, carbamates, neonicotinoid insecticides and DEET (*N,N*-Diethyl-*m*-toluamide) all of which are used globally as agrichemicals and repellents, induce oxidative stress and decrease the antioxidative enzyme activities of superoxide dismutase (SOD), catalase, and glutathione reductase (7-12). The excessive oxidative stress on erythrocyte membranes induces lipid peroxidation to produce alterations in antioxidant defense mechanisms and result in oxidative hemolysis (13). As pesticide translocation through the placenta to the fetus may cause erythrocyte osmotic fragility and decrease SOD activity (14), exposure to pesticides during pregnancy is considered to increase the risk of clinically relevant neonatal hyperbilirubinemia.

There are currently no large studies on whether maternal exposure to pesticides or repellents during pregnancy is a pathogenic factor in neonatal hyperbilirubinemia. The present nationwide study therefore examined for associations between the maternal use of such chemicals and neonatal hyperbilirubinemia requiring phototherapy.

Methods

Study design and participants

Our analysis utilized the dataset from the Japan Environment and Children's Study (JECS), an ongoing cohort study initiated in 2011. In the JECS, pregnant women were recruited between January 31, 2011 and March 31, 2014. The eligibility criteria for participants were as follows: 1) residing in the study area at the time of recruitment; 2) expected delivery date after August 1, 2011; and 3) capable of comprehending the Japanese language and completing the self-administered questionnaire. Individuals who met with health care providers within the study area but who resided outside of it were excluded from the study. Details of the JECS project have been described in a previous article (15). The present investigation was based on the "jecs-ag-20160424" dataset released in June 2016 that contained information on 104,102 records. We focused on the self-described data relating to the maternal usage of repellents, pesticides, and herbicides during the second or third trimester of pregnancy along with other covariates. Target neonates were limited to normal birth weight or more (i.e., ≥2,500 g).

The JECS protocol was approved by the Institutional Review Board on Epidemiological Studies of the Ministry of the Environment, as well as by the Ethics Committees of all participating institutions: the National Institute for Environmental Studies (which leads the JECS), the National Center for Child Health and Development, Hokkaido University, Sapporo Medical University, Asahikawa Medical College, Japanese Red Cross Hokkaido College of Nursing, Tohoku University, Fukushima Medical University, Chiba University, Yokohama City University, University of Yamanashi, Shinshu University, University of Toyama, Nagoya City University, Kyoto University, Doshisha University, Osaka University, Osaka Medical Center and Research Institute for Maternal and Child Health, Hyogo College of Medicine, Tottori University, Kochi University, University of Occupational and Environmental Health, Kyushu University, Kumamoto University, University of Miyazaki, and University of Ryukyu. The JECS was conducted in accordance with the Helsinki Declaration and other nationally recognized regulations and guidelines. Written informed consent was obtained from each participant.

Data collection

Information on the frequency of maternal pesticide and repellent use was collected during the second/third trimester of pregnancy from self-reported questionnaires, which also included questions on nutritional supplementation, annual household income and maternal educational levels. Maternal complication and medication data before and during pregnancy, maternal age at delivery, and neonate information on gender, birth weight, gestational duration, Apgar score at 5 minutes from birth, and hyperbilirubinemia requiring phototherapy were collected from subject medical record transcriptions.

Outcomes, exposure, and covariates

The main outcome of this study was neonatal hyperbilirubinemia needing phototherapy. Maternal non-occupational exposure to pesticides or repellents; moth repellent for clothes; indoor insecticide spray; mosquito coils or electric mat repellents; herbicides or gardening pesticides for gardens, planters, or farms; and spray- or lotion-type insect repellents during pregnancy was assessed by the questionnaire. The survey answers which were originally grouped 6 categories ("no", "less than once a month", "1−3 times a month", "once a week", "a few times a week", "everyday") were re-grouped into 3 categories ("never", "used up to a few times a month", or "used more than a few times a week"), apart from the question relating to the usage of moth repellent for clothes, which maintained the original categorization ("never", "used from time to time", or "used continuously"). We used mothers not using pesticides or repellents during pregnancy as controls. As covariates, we also assessed maternal age at delivery, complications during pregnancy, obstetric-delivery complications, infant gender, infant Apgar score at 5 minutes from birth, supplementation during pregnancy (vitamin C, vitamin E, multivitamin, or multivitamin/mineral), annual household income and maternal educational levels.

Statistical analysis

All statistical analyses were performed using SPSS statistical software version 25 (SPSS Inc., Chicago, Illinois). Chi-square tests and Student's *t*-tests were conducted to compare covariates between each group as stratified by category as well as by frequency of use. We categorized all continuous and ordinal variables, such as maternal age (<20, 20–34, or ≥35 years), gestational duration (<37 or ≥37 weeks), Apgar score at 5 minutes from birth (<7 or ≥7), annual household income (≥6, 4–6, 2–4, or <2 million yen), and maternal educational levels (university/graduate school, professional school/junior college, high school/higher professional school, or junior high school). Logistic regression models were employed to generate adjusted odds ratios (ORs) and their 95% confidence intervals (CIs). The covariates selected in our models were based on previously published literature and biological plausibility. As it is well known that gestational duration is related to birth weight and that low birth weight infants are at high risk of hyperbilirubinemia requiring phototherapy (16,17), we only included the data of neonates having a birth weight of 2,500 g or more. After this analysis was conducted, we performed a reanalysis including low birth weight infants to evaluate for possible selection bias.

Results

Overall, 61,751 infants completed the questionnaire for analysis (Fig 1). There were 5,985 (9.7%) infants treated with phototherapy for hyperbilirubinemia. The number of expectant mothers reporting the use of moth repellent for clothes, indoor insecticide spray, mosquito coils or electric mat repellents, herbicides or gardening pesticides, and spray- or lotion-type insect repellents by the second/third trimester of pregnancy was 36,610 (59.2%), 20,352 (33.0%), 19,518 (31.6%), 5,333 (8.6%), and 15,309 (24.8%), respectively.

The participants' characteristics and frequency of insecticide or repellent use during pregnancy as stratified by treatment with phototherapy are summarized in Table 1. There were significant differences between the groups regarding for infant gender, gestational age, Apgar score at 5 minutes from birth, and obstetric complications.

In multivariate logistic regression analysis after adjustment for covariates, we observed there were significantly association between the incidence of neonatal hyperbilirubinemia and usage of indoor insecticide spray (more than a few times a week: OR 1.21, 95% CI 1.05–1.38), mosquito coils or electric mat repellents (up to a few times a month: OR 0.90, 95% CI 0.82–0.98), spray- or lotion-type insect repellents (more than a few times a week: OR 0.70, 95% CI 0.61–0.81, up to a few times a month: OR 0.84, 95% CI 0.78–0.91) as compared with controls (Table 2).

We performed reanalysis of 68,109 infants including 6,358 low birth weight infants but excluding gestational age as a covariate due to multicollinearity. There were significant associations between the incidence of neonatal hyperbilirubinemia and spray- or lotion-type insect repellents (more than a few times a week: OR 0.73, 95% CI 0.65–0.83, up to a few times a month: OR 0.85, 95% CI 0.81–0.91), which were similar to the original analysis. No significant relationships between the incidence of neonatal hyperbilirubinemia and the usage of indoor insecticide spray or mosquito coils or electric mat repellents were observed (Supplemental Table S1 and S2, online).

Discussion

This is the first large study to examine the effects of maternal exposure to pesticides or repellents on clinically relevant neonatal hyperbilirubinemia using a dataset from nationwide birth cohort study. Our finding suggested that hyperbilirubinemia requiring phototherapy had a significant positive relation with indoor insecticide spray use of more than a few times a week and negative frequent-dependent relation with spray or lotion-type insect repellents use.

Although various kinds of pesticides have been found to induce oxidative stress on living organisms, the precise relationship between the non-occupational use of these agents and hyperbilirubinemia caused by hemolysis remains unclear. There are several routes for fetal exposure to pesticides. First, such chemicals are commonly present in foods; the US Department of Agriculture reported that 26.2% of tested food samples contained residual pesticides (18). Second, pesticides sprayed on farmlands can be diffused in the air, exposing surrounding residents to chemical inhalation. In one report, atmospheric monitoring data revealed that several widely used pesticides, including chlorpyrifos, diazinon, and methyl isothiocyanate, were detected far beyond their agricultural application sites (19). Third, it was revealed that house dust was obvious to be contaminated with various types of pesticides (20). Oral intake, inhalation, and skin absorption of contaminated house dust may be a source of pesticide exposure (21,22). It is known that organophosphate pesticides are detectable in the umbilical cord blood and amniotic fluid of pregnant women exposed to pesticides (23-25), indicating a risk of transplacental fetal exposure. Other studies have confirmed associations between maternal exposure to pesticides and congenital abnormalities, mental retardation, and developmental disorders of offspring (25-28). This study did not assess pesticide exposure from foods, air and house dust. However, maternal exposure to daily-used pesticides was estimated by answer about usage and frequency.

In contrast to a possible relationship between more frequent use of indoor insecticide spray, it appeared that using moth repellent for clothes, mosquito coils or electric mat repellents, herbicides or gardening pesticides, and spray- or lotion-type insect repellents during pregnancy did not remarkably increase the incidence of neonatal hyperbilirubinemia requiring treatment. Moreover, this significant difference remained after multivariate logistic regression analysis without excluding low birth weight infants (Supplemental Table S1 and S2, online). The active ingredient in commercial repellents is commonly DEET (*N,N*-diethyl-m-toluamide) or Icaridin (1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpropylester), with the latter being less toxic and efficacious. Metabolic DEET products were identified in human urine after dermal application (29), and topical administration of DEET resulted in free radical species generation in rats (12). Furthermore, DEET was detected in cord blood (24). These findings suggested that DEET could be transferred to the fetus and exert oxidative stress on neonates. Although one report has described that daily application of DEET in the second and third trimesters of pregnancy poses low risk to birth outcomes including spontaneous abortion, stillbirth, abnormal birthweight, and neonatal growth (30), no studies have examined the oxidative stress effects of DEET or Icaridin on neonates. Excessive oxidative stress by free radicals on erythrocyte membranes induces lipid peroxidation to produce alterations in antioxidant defense mechanisms and result in oxidative hemolysis (13). Transplacental fetal exposure to these active ingredients can cause erythrocyte osmotic fragility in the fetus that may be associated with clinically relevant neonatal hyperbilirubinemia. However, there was a significant negative relationship between spray- or lotion-type insect repellent use and hyperbilirubinemia requiring phototherapy. This finding could not be explained by past study results, and the reason why this association was negative was unclear. Since repellents are applied to the skin to keep insects away instead of killing them and contain different active ingredients than do insecticides, it is believed that repellents have lower toxicity on humans, including the fetus and infants (30). In addition, mothers in the current study who frequently used spray- or lotion-type insect repellents might have had unadjusted covariates that contributed to this negative relationship.

There are several limitations to this study. First, we used a subjective questionnaire to assess maternal exposure to pesticides during pregnancy instead of objective biomarkers like blood concentration, blood and urinary metabolites, and enzyme activities. Second, although the most sensitive period for the fetus by maternal exposure to pesticides is the end of pregnancy, the investigation period varied among subjects who were in their second or third trimester. Third, there was a lack of detailed information on the diagnosis of hyperbilirubinemia requiring treatment since pediatricians and obstetricians simply recorded the use or not of phototherapy during the neonatal period. There exist two indication criteria systems for phototherapy in Japan, with one evaluating total bilirubin and the other assessing unbound bilirubin. Both criteria are determined by birth weight and age (16,17). Although total and unbound bilirubin are positively correlated, unbound bilirubin often has relatively higher values depending on the state of the infant, such as preterm birth, hypoalbuminemia, or neonatal infection (31). The discrepancy between neonatal hyperbilirubinemia diagnostic criteria accuracy is presumably slight, but misclassifications causing a bias towards the null value must be acknowledged. In the JECS protocol, misclassifications were deemed to be equal for exposures to environmental toxins because the assessors were blinded to the exposure measurements (32).

In conclusion, we examined for associations of maternal exposure to insecticides or repellents on clinically relevant neonatal hyperbilirubinemia using the data from a large nationwide birth cohort study in Japan. Our results suggest that more frequent use of indoor insecticide spray can slightly increase the risk of this complication. Further studies are required to elucidate the mechanisms underlying the effects of maternal use of indoor insecticide spray and spray- or lotion-type insect repellents on hyperbilirubinemia and other neonatal disorders and protective effects for oxidative stress by taking vitamin supplements on living organisms.

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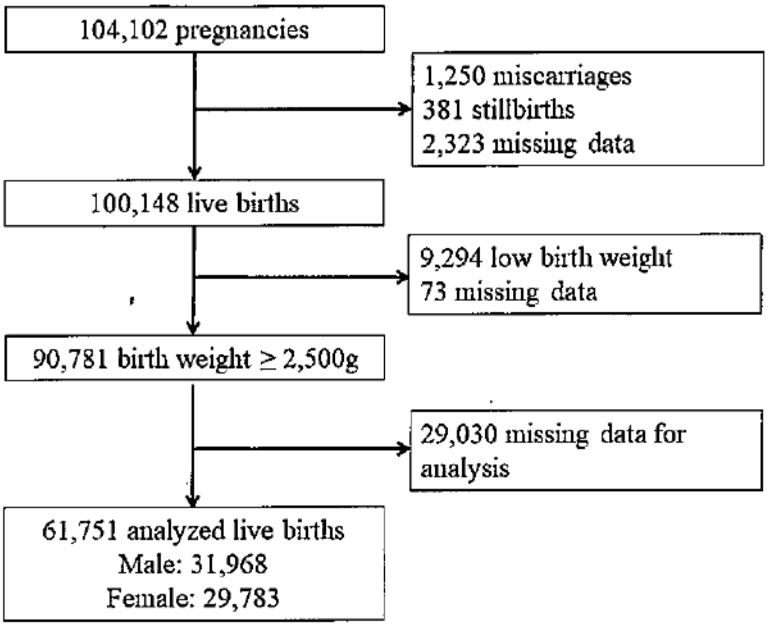

Figure 1. Case selection flowchart

Table 1. Characteristics of participants with or without phototherapy for neonatal hyperbilirubinemia

Variable	No phototherapy	Phototherapy	*P*-value
Participants, n (%)	55,766 (90.3)	5,985 (9.7)	
Maternal age, years (mean ± SD)	31.3 ± 5.0	31.2 ± 5.1	0.15[a]
Maternal age group, n (%)			0.26[b]
≥35 years	15,486 (27.8)	1,655 (27.6)	
20–34 years	39,940 (71.6)	4,283 (71.6)	
<20 years	340 (0.6)	47 (0.8)	
Frequency of using moth repellent for clothes, n (%)			0.13[b]
Continuous	12,027 (21.6)	1,306 (21.8)	
From time to time	21,092 (37.8)	2,185 (36.5)	
Never	22,647 (40.6)	2,494 (41.7)	
Frequency of using indoor insecticide spray, n (%)			0.07[b]
More than a few times a week	2,213 (4.0)	264 (4.4)	
Up to a few times a month	16,204 (29.1)	1,671 (27.9)	
Never	37,349 (67.0)	4,050 (67.7)	
Frequency of using mosquito coils or electric mat repellents, n (%)			<0.001[b]
More than a few times a week	11,060 (19.8)	1,109 (18.5)	
Up to a few times a month	6,707 (12.0)	642 (10.7)	
Never	37,999 (68.1)	4,234 (70.7)	
Frequency of using herbicides or gardening pesticides, n (%)			0.70[b]
More than a few times a week	146 (0.3)	16 (0.3)	
Up to a few times a month	4,687 (8.4)	484 (8.1)	
Never	50,933 (91.4)	5,485 (91.6)	

Frequency of using spray- or lotion-type insect repellents, n (%)			<0.001[b]
More than a few times a week	3,120 (5.6)	246 (4.1)	
Up to a few times a month	10,921 (19.6)	1,022 (17.1)	
Never	41,725 (74.8)	4,717 (78.8)	
Infant gender, n (%)			<0.001[b]
Male	28,555 (51.2)	3,413 (57.0)	
Female	27,211 (48.8)	2,572 (43.0)	
Gestational age (%)			<0.001[b]
<37 weeks	960 (1.7)	283 (4.7)	
≥37 weeks	54,806 (98.3)	5,702 (95.3)	
Apgar score at 5 minutes after birth, n (%)			<0.001[b]
<7	214 (0.4)	41 (0.7)	
≥7	55,552 (99.6)	5,944 (99.3)	
Pregnancy complications[c], n (%)			0.71[b]
Yes	8,877 (15.9)	964 (16.1)	
No	46,889 (84.1)	5,021 (83.9)	
Obstetric complications[d], n (%)			<0.001[b]
Yes	25,446 (45.6)	3,076 (51.4)	
No	30,320 (54.4)	2,909 (48.6)	
Household income (million yen), n (%)			0.81[b]
≥6	14,961 (26.8)	1,580 (26.4)	
4–6	18,378 (33.0)	1,965 (32.8)	
2–4	19,177 (34.4)	2,079 (34.7)	
<2	3,250 (5.8)	361 (6.0)	

Maternal educational level, n (%)			0.31[b]
University / Graduate school	12,608 (22.6)	1,338 (22.4)	
Professional school / Junior college	22,798 (40.9)	2,509 (41.9)	
High school / Higher professional school	17,760 (31.8)	1,882 (31.4)	
Junior high school	2,600 (4.7)	256 (4.3)	

[a]Calculated by Student's *t*-test.

[b]Calculated by the chi-square test.

[c]Pregnancy complications included hypertension, hyper/hypothyroidism, diabetes, autoimmune disease, heart disease, kidney disease, epilepsy, psychiatric disease, and others.

[d]Obstetric complications included threatened premature labor, gestational diabetes, premature rupture of membranes, placenta previa, nonreassuring fetal status, premature separation of normally implanted placenta, gestational hypertension, intrauterine infection, and others.

Table 2. Multivariate logistic regression analysis for neonatal hyperbilirubinemia treated by phototherapy based on insecticide or repellent exposure

Variable	Phototherapy (n=5,985)		
	OR	95% CI	*P*-value[a]
Exposure			
Moth repellent for clothes			
Not used (reference)	1.00		
From time to time	0.95	0.90–1.02	0.14
Continuous	1.00	0.93–1.08	0.95
Indoor insecticide spray			
Not used (reference)	1.00		
Up to a few times a month	1.01	0.95–1.08	0.76
More than a few times a week	1.21	1.05–1.38	0.007
Mosquito coils or electric mat repellents			
Not used (reference)	1.00		
Up to a few times a month	0.90	0.82–0.98	0.02
More than a few times a week	0.95	0.88–1.02	0.18
Herbicides or gardening pesticides			
Not used (reference)	1.00		
Up to a few times a month	1.00	0.90–1.10	0.95
More than a few times a week	1.03	0.61–1.74	0.91
Spray- or lotion-type insect repellents			
Not used (reference)			
Up to a few times a month	0.84	0.78–0.91	<0.001

More than a few times a week	0.70	0.61–0.81	<0.001

[a]Calculated by the chi-square test.