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Original Article

Risk of prenatal pesticide exposure to anemia and stunted birth in horticultural areas of West Lampung Regency, Indonesia

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ABSTRACT

Introduction: The massive pesticide use in agriculture has raised concerns about their effects on human health, including fetal growth. This study aims to evaluate the risk of prenatal pesticide exposure to anemia and stunted birth in a horticulture center in Lampung Province, Indonesia.

Methods: This study was meticulously conducted as a prospective cohort study, spanning from August 3rd, 2020 to June 30th, 2021. We closely followed the pregnancies of 100 participants, categorizing them into two groups: those exposed to pesticides (Pregnant women farmer/WF=50) and those unexposed (Pregnant women not farmer/WNF=50). Our observations included work methods, working hours, and personal protection used. We also measured cholinesterase, hemoglobin, energy, and protein intake. Birth length measurements were taken at all births. We employed chi-square and stratification tests to assess the risk of exposure to outcomes and applied Relative Risk (RR) with 95% CI.

Results: Among the 100 participants, 80 (80.0%) were aged 21-35, and 87 (87.0%) were low-income. Most WF 42 (84.0%) work more than 5 hours daily, and 38 (76.0%) have incomplete PPE. The study found that pregnant women with pesticide poisoning were 5.30 times more likely to have anemia (RR: 5.30; 95% CI: 2.02-13.90) than those not poisoned. However, pesticide exposure had no statistically significant effect on stunted babies (p-value > 0.05).

Conclusion: This study has proven that pesticide exposure is a risk factor for anemia. However, there is insufficient evidence to explain its effect on birth length. Examination of pesticide poisoning, nutritional monitoring during pregnancy, and social safety nets are recommended.

Keywords: Anemia, Horticulture, Pesticides, Pregnant, Stunting

Introduction

The massive use of pesticides in agriculture often causes health problems. Every year, it is estimated that there are up to 5 million cases of pesticide poisoning, with deaths reaching 220,000 people.[1] The main effects of pesticide poisoning are symptoms of the nervous system, such as headaches, dizziness, paresthesia, tremors, discoordination, and seizures. Pesticides will inhibit the acetylcholinesterase enzyme, leading to acetylcholine accumulation in nerve tissue and vector organs. The chronic effects are weight loss, anemia, anorexia, impaired liver function, and delayed neuropathy.[1–6] The most significant use of pesticides is in horticultural agriculture, where they are used in large doses continuously during the growing season.[7–9] Pesticide exposure occurs during spraying activities, preparing equipment for spraying, including mixing pesticides, washing equipment/clothing used when spraying, removing grass from plants, looking for pests, watering plants, and harvesting.

Anemia in pesticide poisoning is caused by the formation of sulfhemoglobin and methemoglobin, which interferes with hemoglobin function.[6,10–14] During pregnancy, anemia will increase the risk of bleeding, premature birth, stillbirth, impaired fetal growth resulting in low birth weight and short babies (stunting), perinatal death, and reduced body defenses.[15–20]

West Lampung Regency, the largest horticultural agricultural area in Lampung Province, is not just a geographical location for our study but a crucial hub of agricultural activity. With the most significant income from the agricultural food and horticulture sector (53.81% GRDP), horticultural farming spans approximately 1,254 hectares, producing a staggering 237,500 tons of vegetable crops.[21] The prevalence of anemia in pregnant women in 2016 and 2017 was 7.7% and 5.8%, more significant than the province in the same year (4.7% and 4.9%).[22] This data underscores the urgency and relevance of our research in this specific location.

While many studies have reported the relationship between pesticide poisoning and various factors, such as the use of personal protective equipment, work methods, length of time worked, and years of service there needs to be a significant gap in our understanding.[4,7,8,23,24] To our knowledge, few studies have been conducted in Indonesia to assess pesticide exposure among pregnant women working in horticulture. This study aims to fill this crucial gap and evaluate the risk of prenatal pesticide exposure to anemia and stunted birth in a horticulture center in Lampung Province, Indonesia.

Methods

Our prospective cohort study conducted between August 3rd, 2020 and June 30th, 2021 in Liwa City, West Lampung Regency, is designed to be comprehensive and rigorous. All pregnant women who lived in the study area for at least six months were included, ensuring a diverse and representative sample. Pregnant women with physical disabilities, diseases, and blood disorders were excluded from this study to maintain the integrity of our data. The exposed group (Pregnant women farmer/WF) were pregnant women who worked permanently in non-organic horticulture and used or had used pesticides for six months before becoming pregnant. The unexposed group (Pregnant women not farmers/WNF) were pregnant women who worked other than horticultural farmers, had never worked on horticultural farming, and did not use or had used pesticides for six months before becoming pregnant. This comprehensive approach ensures the validity and reliability of our findings.

The sample size is:

$$n = \frac{(Z_{\propto}\sqrt{2PQ} + Z_{\beta}\sqrt{P_1 Q_1 + P_2 Q_2})^2}{(P_1 - P_2)^2}$$

α = 2-side significance level (0.05)

1- β = Power of test (0.80)

RR = Relative Risk (2.0)

P_2 = Probability of anemia in the unexposed group (0.3)[24]

P_1 = Probability of anemia in the exposed group (RR x P_1)

P = $(P_1 + P_2)/2$

Q_1 = $1 – P_1$

Q_2 = $1 – P_2$

Q = 1 - P

So, the total sample size (n)=100

This study was conducted after obtaining approval from the Health Research Ethics Committee of the Tanjung Karang Health Polytechnic (No. 261/KEPK-TJK/V/2020) and permission from the West Lampung District Health Office. Guided by the Helsinki protocol, participant consent was taken, and data handling was confidential. There is no risk of harm to the participant, who has the right to withdraw during the study. All research procedures were explained prior to interviews and biological sampling.

Data were collected from measurements, observations, and interviews using a questionnaire. All participants were included in the study from the start of their pregnancy and were medically tested positive for pregnancy. Blood sampling to measure Hemoglobin and Cholinesterase levels and a 24-hour diet recall to assess energy and protein intake were carried out three times to represent each trimester of pregnancy.

The baby's birth length is the body length measured shortly after birth (in centimeters) using a length measuring board in a sleeping position and grouped into "stunted" (if < 46.1 cm for male, and < 45.4 cm for female) and "normal" (if ≥ 46.1 cm for male, and ≥ 45.4 cm for female). Cholinesterase in the participants' blood was measured by the kinetic method using a spectrophotometer (405 nm) and then grouped into "poisoned" (if < 3,990 μ/L) and "normal" (if ≥ 3,990 μ/L). Hemoglobin in the blood, expressed in units of grams percent (gr%), was measured using the Point of Care Testing (POCT) method. Examination results are grouped into "anemia" (if < 11 gr%) and "normal" (if Hb blood ≥ 11 gr%).

Determination of the adequacy of energy and protein intake for pregnant women refers to the Regulation of the Minister of Health.[25] Energy intake is the amount of maternal intake during pregnancy, assessed using the Food Frequency Questionnaire (FFQ). The energy adequacy limit value for pregnant women ages 19-29 years is 2550 kcal per day, and for ages 30-49 years is 2450 kcal per day. Grouped into "low" (if it is less than the adequacy limit value for age) and "adequate" (if it is equal to or more than the adequacy limit value for age).

Protein intake is the value of maternal protein intake during pregnancy, assessed using the FFQ. According to the Regulation of the Minister of Health, the protein adequacy limit value for pregnant women aged 19-29 years is 76 grams per day, and for those aged 30-49 years is 77 grams per day. Grouped into "low" (if it is less than the adequacy limit value for age) and "adequate" (if it is equal to or more than the adequacy limit value for age).

The working hour is the average time the participants work in the garden daily. They grouped into "> 5 hours per day" and "≤ 5 hours per day". Using masks, gloves, hats, shoes, shirts, and trousers assesses the completeness of Personal Protective Equipment (PPE). They are grouped into "incomplete" and "complete."

The collected data were entered into the Statistical Package for Social Sciences (SPSS) version 24 after being checked for completeness and coded. Univariate analysis to describe the proportion of each variable. The Chi-square test was used for bivariate analysis to assess group proportion differences. However, if there are cells in the contingency table with an Actual Count value equal to zero or cells with an expected count value of less than 5, then the Fisher exact test is used. Calculation of Adjusted Relative Risk (RR) with 95% CI was applied to assess the risk of exposure to the outcome.

This study also applied a stratification test to assess the chances of pregnant women with pesticide poisoning becoming anemic and giving birth to stunted babies. Stratification tests were performed with Epi Info version 7.

Results

Table 1 shows that most participants (WF and WNF) were aged 21-35 years, namely 41 (82.0%) and 39 (78.0%). The majority of the education level of the WF group had graduated from primary school 14 (28.0%) and junior high school 13 (26.0%), while the WNF group had graduated from senior high school 34 (68.0%). Almost all participants (WF and WNF) have an income of less than 134.33 (USD) per month, namely 46 (92.0%) and 41 (82.0%).

Based on the history of pregnancy (gravidity), the majority of participants (WF and WNF) had been pregnant 1-3 times, respectively, 45 (90.0%) and 43 (86.0 %). Based on parity, 14 (28.0%) participants in the WF group had never given birth, while 40 (80%) in the WNF group had given birth 2-3 times.

The measurement of working time in the garden and using PPE was only for the pesticide-exposed group (WF). The study results found that most WF worked more than five hours a day 42 (84.0%) and worked with incomplete PPE 38 (76.0%). Only 12 (24.0%) WF use complete PPE, namely gloves, masks, hats, shoes, and long clothes.

Table 1. Participant's characteristics (n= 100)

Variables	WF n (%)	WNF n (%)
Age		
< 21	6 (12.0)	2 (4.0)
21-25	15 (30.0)	10 (20.0)
26-30	16 (32.0)	18 (36.0)
31-35	10 (20.0)	11 (22.0)
36-40	3 (6.0)	6 (12.0)
>40	-	3 (6.0)
Education		
Primary school	14 (28.0)	9 (18.0)
Junior high school	13 (26.0)	7 (14.0)
Senior high school	23 (46.0)	34 (68.0)
Income per month (USD)		
< 67.16	11 (22.0)	20 (40.0)
67.16 - 134.33	35 (70.0)	21 (42.0)
134.33 - 201.49	3 (6.0)	4 (8.0)
>201.,49	1 (2.0)	5 (10.0)
Gravidity		
1 - 3	45 (90.0)	43 (86.0)
> 3	5 (10.0)	7 (14.0)
Parity		
Never	14 (28.0)	8 (16.0)
2-3	36 (72.0)	40 (80.0)
> 3		2 (4.0)
Working-hour		
≥ 5 hours per day	42 (84.0)	-
< 5 hours per day	8 (16.0)	-
PPE		
Incomplete	38 (76.0)	-
Complete	12 (24.0)	-

Table 2 shows that most participants had low energy intake in the WF 35 (70.0%) and WNF 36 (72.0%). Likewise, for protein intake, half of WF 25 (50.0%) and 32 (64.0%) WNF also had low protein intake. The statistical analysis showed no difference in the proportion of energy intake (p-value = 0.826) and protein intake (p-value = 0.226) between the WF and WNF groups.

Table 2 shows around 19 (38.0%) WF pesticide poisoning status, while in the WNF group, there was 5 (10.0%). Statistical analysis showed a significant difference in the proportion of poisoning status between the WF and WNF (p-value = 0.002). The WF was 3.80 times more likely to be poisoned than the WNF (RR= 3.80; 95% CI 1.54-9.38). Table 2 shows 17 (34.0%) WF with anemia, while WNF is around 5 (10.0%) with anemia. Statistical analysis showed a significant difference in the proportion of anemia between the WF and WNF (p-value = 0.008). The WF group was 3.40 times more likely to have anemia than the WNF (RR= 3.40; 95% CI 1.36-8.50).

In the WF group, 3 (6.0%) babies born were in the stunted category. While in the WNF, there was 1 (2.0%) stunted baby. The statistical analysis results did not show a difference in the proportion of the birth length between the WF and WNF (p-value = 0.617).

Table 2. Variables distribution based on occupation (n= 100)

Variables	WF n (%)	WNF n (%)	p-value	RR (95% CI)
Energy intake				
Low	35 (70.0)	36 (72.0)	0.826	0.97 (0.75-1.25)
Adequate	15 (30.0)	14 (28.0)		1
Protein intake				
Low	25 (50.0)	32 (64.0)	0.226	0.78 (0.55-1.10)
Adequate	25 (50.0)	18 (36.0)		1
Cholinesterase				
Poisoning	19 (38.0)	5 (10.0)	0.002	3.80 (1.54-9.38)
Normal	31 (62.0)	45 (90.0)		1
Hemoglobin				
Anemia	17 (34.0)	5 (10.0)	0.008	3.40 (1.36-8.50)
Normal	33 (66.0)	45 (90.0)		1
Birth length				
Stunted	3 (6.0)	1 (2.0)	0.617	3.00 (0.32-27.87)
Normal	47 (94.0)	49 (98.0)		1

We conducted a stratification analysis by grouping participants based on occupation (WF and WNF) to investigate the relationship between poisoning status from pesticide exposure with anemia and stunted childbirth. Table 3 shows that 13 (68.4%) participants from the WF (exposed) group who were poisoned by pesticides had anemia status. While in the WNF (unexposed) group, none of the participants with pesticide poisoning had anemia status. The stratification analysis showed that the pregnant women (WF) poisoned by pesticides were 5.30 times more likely to experience anemia than those not (RR: 5.30; 95% CI: 2.02-13.90; p-value= 0.001).

Table 4 shows that 3 (18.0%) poisoned WF gave birth to stunted babies. Meanwhile, all WNFs who were poisoned gave birth to normal babies. However, the analysis did not provide sufficient evidence to show the effect of pesticide exposure on stunting babies (p-value > 0.05).

Table 3. Stratification analysis of poisoning and anemia based on occupation

Poisoning by occupation	Anemia n (%)	Normal n (%)	p-value	RR (95% CI)
WF				
Poisoning	13 (68.4)	6 (31.6)	0.0001	5.30 (2.02-13.90)
Normal	4 (12.9)	27 (87.0)		1
WNF				
Poisoning	0 (0.0)	5 (100.0)	0.576	-
Normal	5 (11.1)	40 (88.9)		

Crude RR (95% CI) = 4.57 (2.24-9.35); p-value = 0.0006
Adjusted RR (95% CI) = 3.99 (1.63-9.79); p-value = 0.001
Test of homogeneity (M-H): p-value = 0.029

Table 4. Stratification analysis of poisoning and stunting based on occupation

Poisoning by occupation	Stunted n (%)	Normal n (%)	p-value	RR (95% CI)
WF				
Poisoning	3 (18.8)	16 (84.2)	0.049	-
Normal	0 (0.0)	31 (100.0)		
WNF				
Poisoning	0 (0.0)	5 (100.0)	0.900	-
Normal	1 (2.2)	44 (97.8)		
Crude RR (95% CI) = 9.50 (1.03-87.13); p-value = 0.042				
Adjusted RR (95% CI) = 18.60 (0.32-1074.78); p-value = 0.150				
Test of homogeneity (M-H): p-value = 0.135				

Discussion

This study's findings are significant, revealing that 19 (38.0%) pregnant women working in horticulture farming (WF) experienced pesticide poisoning. The statistical analysis further underscores the gravity of the situation, showing a significant difference in the proportion of poisoning status between WF and WNF (p-value = 0.002). The alarming data indicates that pregnant women who work in horticultural agriculture (exposed to pesticides) are 3.80 times more likely to be poisoned by pesticides than pregnant women who are not farmers (RR= 3.80; 95% CI: 1.54-9.38). The study also found a strong correlation between pesticide poisoning and the length of pesticide exposure, with 42 (84.0%) WF working more than 5 hours daily. This findings is similar to Kapeleka et al. farmers with long working hours have a probability of about three times poisoning (OR= 3.497; 95% CI: 1.080; 11.322).[9]

In addition to working hours, the incomplete use of PPE significantly increases pesticide exposure among farmers. It is concerning to note that the majority, 38 (76.0%) of WF, worked with incomplete PPE. Similar to Jyoti S et al. 42(56.8%) farmers had poor practices in using PPE.[26] According to Damalas et al. using PPE is crucial for personal protection against exposure because most pesticides are highly lipid-soluble agents that are well absorbed from the skin.[27] Thus, the poor use and quality of PPE is a significant cause of exposure among farmers.[9]

The use of PPE when in contact with pesticides dramatically affects the quantity of pesticides that enter the farmer's body. Several route pesticides into the body (portal of entry) are known: skin, inhalation, and digestion. However, skin exposure is the most common method, especially if there are skin disorders and sweating. According to Kurniasih et al. over 90% of poisoning cases worldwide are caused by skin contamination, followed by inhalation.[7]

Observations also found that farmers used non-indicative pesticides. Even though there are no pests, farmers believe that continuous use of pesticides is one way to prevent plant pests. These three variables (length of exposure, dose, and completeness of PPE) determine pesticide poisoning in farmers.[9,27–30]

Anemia is one of the chronic effects of pesticide poisoning.[4,16,17,19,28] The results showed (Table 2) that 17 (34.0%) of WF had anemia. Similar to Yushananta et al. and other studies (Table 3), we found that pregnant women (WF) who were poisoned from pesticide exposure were 5.30 times more likely to have anemia than pregnant women who were not exposed (RR: 5.30; 95% CI: 2.02-13.90).[4,7,8,23,28] Anemia in pesticide poisoning sufferers is due to the formation of sulfhemoglobin and methemoglobin in red blood cells. Sulfhemoglobin occurs due to pesticide sulfur content, thus forming bonds. Sulfhemoglobin causes hemoglobin to become abnormal and unable to carry out its function.[3,4,6,11,12,14] Methemoglobin is formed when the iron in Hb is oxidized from ferrous to ferric, or nitrite bonds with Hb. Methemoglobin causes Hb to be unable to bind oxygen. Sulfhemoglobin and Methemoglobin in red blood cells cannot be converted back into normal hemoglobin. The presence of Sulfhemoglobin and Methemoglobin in blood cells will cause a decrease in hemoglobin levels in red blood cells, resulting in hemolytic anemia. Hemolytic anemia due to contact with pesticides is caused by enzymatic defects in red blood cells and the amount of toxic substances that enter the body.[3,6,10–12,14,30]

Anemia is one variable of nutritional status for pregnant women. Anemia during pregnancy will increase the risk of bleeding, premature birth, infant death in the womb, and impaired fetal growth, resulting in low birth weight babies and stunted.[16,17,19,31] Stunting is a height mismatch based on age, below minus 2 Standard Deviations (<-2SD) from the WHO median standard.[32] Stunting indicates chronic malnutrition that can occur from fetal growth.[33] The effects of stunting include increasing infant morbidity and mortality, impaired cognitive, motor, and verbal development, increased risk of obesity, and decreased reproductive health.[33,34]

Table 2 shows that most participants had low energy intake at WF 35 (70.0%) and WNF 36 (72.0%). Likewise, for protein intake, half of WF 25 (50.0%) and 32 (64.0%) WNF had low protein intake. However, the analysis results did not show any difference in the proportions of the two variables between WF and WNF. This condition can be caused by poverty and low education. Table 1 shows that most participants have a monthly income of less than 134.33 (USD) and a maximum of high school graduates. According to Adedeji et al. and other research, malnutrition in pregnant women is related to socio-economic inequality, household food insecurity, and education.[35–37]

Table 4 shows that 3 (18.0%) WF who were poisoned gave birth to stunted babies. Meanwhile, no WNF gave birth to stunted babies. However, in this study, there is not enough evidence to conclude the effect of pesticide exposure on the birth of stunted babies (p-value > 0.05). These findings are similar to Wang et al. in China and Chiu et al. in the US, who did not find a relationship between pesticide exposure and birth length.[38,39] Likewise, Harley et al. in the US and Soesanti et al. in Indonesia also found no association between pesticide exposure and birth weight and length.[40,41] However, Widyawati et al. in Indonesia, Naksen et al. in Thailand, and Jaacks et al. in Bangladesh concluded that pesticide exposure during pregnancy was associated with low birth weight.[42,43,44]

According to Ferguson et al. pesticide exposure is associated with reduced fetal size (length and weight) in mid-pregnancy but not during delivery.[45] Furthermore, increases in exposure biomarkers from early and mid-gestation showed associations with the most precise growth measures. Dries et al. also concluded that pesticide exposure is associated with impaired growth in early pregnancy.[46]

Fortunately, two intervention strategies can be implemented to reduce the risk of anemia and stunting in mothers working in horticultural agriculture. The first is exposure prevention, which can be achieved using complete PPE to protect against pesticides entering the skin, inhalation, and mouth. The second strategy is improving nutrition. This can be done by increasing nutritional intake through foods high in protein and iron (such as meat, fish, chicken, liver, and eggs) and vegetables and fruits with a high vitamin C content to increase iron absorption. Supplementation of Fe tablets in pregnant women, monitoring nutritional status during pregnancy, and testing for poisoning are other necessary actions that must be taken. The government's role through a social safety net for people experiencing poverty is a program that must be implemented to reduce family food insecurity. If implemented effectively, these strategies can significantly reduce the risk of anemia and stunting, offering hope for a healthier future for these mothers and their children.

While this study provides valuable insights, it is essential to acknowledge its limitations, including small sample sizes, pesticide ambient exposure, and daily food recall. Given these results' potential public health implications, additional studies must be conducted to confirm or refute these findings and evaluate the continued risks associated with continued insecticide use. This ongoing research is essential to ensure the health and safety of pregnant women working in horticultural agriculture.

Conclusions

This study has proven that pregnant women with pesticide poisoning were 3.99 times more likely to have anemia than those who were not poisoned (RR: 3.99; 95% CI: 1.63-9.79). However, there is not enough evidence to conclude the effect of pesticide exposure on the birth length (p-value > 0.05). Poor working methods, long working hours, and incomplete use of PPE increase the number of pesticides that enter the body. For this reason, increasing knowledge and skills in pesticide use must be done to avoid pesticide exposure. Research recommendations include examining pesticide poisoning, monitoring nutrition during pregnancy, and social safety nets for low-income people.

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