Altered Hematological and Biochemical Parameters in Schoolchildren Living in an Agricultural Community of Merida State, Venezuela

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Abstract

Epidemiological studies have found that children living in a pesticide treated farmland have higher risk of developing cancer or other serious health problems. Their exposure to pesticides could be limited by periodic health assessments; however, there are few biomarkers for pesticide exposure. The aim of this work was to examine the effects of chronic exposure to pesticides on hematological and biochemical parameters in farm-resident children compared to urban-resident control children. A cross-sectional study was conducted in 72 farm schoolchildren and 41 controls; a follow-up study was done two years later on 20 participants. The children underwent a clinical examination, nutritional assessment, and blood sampling for complete hemogram and biochemical analysis of liver and renal functions, lipid and glucose metabolism, plasma cholinesterase, thyroid hormones and total antioxidant capacity. A prevalence of malnutrition and undernutrition were found in both exposed and control children. The most important hematological results were thrombocytosis in 48% of farm children, increased mean lymphocyte and monocyte counts, and decreased mean neutrophil counts compared to control. Butyrylcholinesterase activity was diminished in 35% of farm children. Alterations in biochemical parameters were observed between exposed and control groups, however, most of the results were within normal limits, except for the significant decrease in total antioxidant capacity. Follow-up study 2 years later showed normalized platelet and lymphocyte counts, however, decreased neutrophil counts was still observed. This study demonstrates that children living in an agricultural community are exposed to the health hazards of pesticides. Undernutrition and improper dental hygiene affect children's overall health status and are likely to increase their vulnerability to toxic effects of pesticides.

Keywords: Farm children; Hematological parameters; Biochemical parameters; Pesticide exposure

Introduction

Over the last decades, several studies have dealt on the vulnerability of children to the toxic effects of pesticides because of developmental, dietary and physiological factors [1,2]. Prenatal or childhood exposure to organophosphate (OP) and organochlorine (OC) pesticides has been documented to induce neurotoxic effects, which may cause impairments in the child's neurobehavioral development [3-6]. Exposure to atmospheric OP and pyrethroid pesticides through inhalation was associated with respiratory symptoms consistent with childhood asthma [7,8]. Pesticide exposure has been implicated as a possible contributing factor in the pathogenesis of several types of childhood cancer, including those of lymphatic and hematopoietic systems, soft tissue sarcoma, brain and stomach cancer [9-12]. In children, cancer is the number one cause of death by disease; in fact, it is responsible for more deaths than all other diseases combined [13]. Leukemia is the most common childhood malignancy, representing about 30% of all cancers diagnosed in children under 15 years of age in industrialized countries [14]. The annual incidence of cancer in Venezuelan children and adolescents younger than 15 years was dominated by leukemia (37% of total cancer cases), followed by malignant brain tumors (12%) and lymphomas (11%) [15]. Thus, concern has been raised with regard children residing near pesticide-treated farmlands, or whose parents are agricultural workers, because they have higher risk of developing cancer or other serious health problems [9,11,12,16-18]. OP metabolites were seen at much higher levels for farmworkers and their children than non-farmworkers and their children during the agricultural season when OPs were in use [19].

In developing countries, the severity of pesticide exposures and toxicities are largely overlooked in the general population. The unique behavior of children and their metabolic rate often place them at risk for absorption of higher doses of pesticides from contaminated environments in comparison with adults. Their exposure to pesticides could be limited by periodic health assessments; however, there are few biomarkers for pesticide exposure. A widely accepted biomarker of exposure is the inhibition of serum cholinesterase activity caused by OPs and carbamates (CBs) [20,21]. Some studies have indicated that variations in hematological and biochemical profiles could predict and prevent health risks of pesticide exposure [22]. Ismail et al. [23] revealed significant differences in hematological, renal and hepatic indices in children and adolescent pesticide applicators compared with control group. Aside from these parameters, various studies evaluated the variation in oxidant/antioxidant status in experimental animals [24-26] as well as in aquatic organisms [27-29] to monitor the effects of chronic exposure to OP and phosphonate pesticides. Many pesticides share common cytotoxic mechanisms involving mitochondrial dysfunction,

which causes the generation of reactive oxygen species (ROS) and dysregulation of the oxidant/antioxidant system, thereby leading to oxidative stress [30,31]. The antioxidant responsive elements provide multiple defense and detoxification capacity in cells and tissues of the organism. Studies have shown an inverse association between total antioxidant capacity (TAC) and the risk of chronic diseases [32-34]; therefore, assessment of the antioxidant defense may be important for improving the health of individuals and of the general population.

In this research, we examined the effects of pesticide toxicity on hematological and biochemical parameters, plasma cholinesterase activity, thyroid gland hormones and TAC in schoolchildren living in an agricultural community of Merida state, Venezuela. The indiscriminate use of pesticides in this region has led to the contamination of both superficial and drinking water resources [35-37] as well as crops [38,39]. Previous reports from our group have confirmed the potential impact on male reproductive function of farmworkers occupationally exposed to pesticides [20,40]. Despite the widespread environmental contamination, no study has examined the health risks of children living in the region. The aim of this work was to examine the effects of chronic exposure to pesticides on hematological and biochemical parameters in farm-resident children compared to urban-resident control children.

Materials and Methods

Study area

Cross-sectional and longitudinal studies were carried out during school time periods, between 2012 and 2014, at the Municipality of Rivas Davila, Merida state, Venezuela. This region is located at 1700 m above sea level, and because of its temperate climate, farming is possible all year round. Due to intensive agricultural production, there is a great demand for pesticide use to the crops. The most frequently applied agrochemicals included: organophosphates, organochlorines, carbamates, glyphosate, pyrethroids, triazine, acetamides, benzimidazoles and bipyridyls.

Participants

For the cross-sectional study, 72 schoolchildren of Rivas Davila municipality were randomly selected in the elementary school of Las Tapias village, between 6 and 12 years old (9 ± 2 years old), 40 males and 32 females. For control group, 41 schoolchildren, 20 males and 21 females, who had no history of pesticide exposure, with similar age (9 ± 2 years old) and educational level as the exposed children, were randomly recruited in an elementary school in Merida City, located at 1600 m above sea level, Merida state, Venezuela. The longitudinal study was done two years later, to follow-up the health status of the children who resulted with altered hematological parameters. Only 59% (20/34) showed up for clinical and medical control, therefore, the variation results were estimated from the 20 children who participated in the follow-up study.

The Ethics Committee of the Council of Scientific, Humanistic, Technologic and Artistic Development of the University of Los Andes, Merida State, Venezuela, reviewed and approved the research protocol. An informed written consent was obtained from the participant children and their legal guardians.

Anthropometry and nutritional assessment

Information about demographic data of children participants were recorded and anthropometric as well as clinical measurements were collected, based on the techniques recommended by the United Nations International Biological Program [41]. An anthropometric evaluation was performed by obtaining the height, weight, waist circumference and tricipital skinfold. Body mass index (BMI) and body fat percentages were calculated to define nutritional status, based on the World Health Organization charts, adopted by the Anthropometric and Nutritional Status Assessment Workshop for the Nutrition Surveillance System (SISVAN) of the National Institute of Nutrition, which establishes cutoffs for normality between P10 and P90 percentiles [42,43]. A dietary assessment was performed through the 24-hour recalls and the frequency of food consumption [44].

Blood collection for hematological, biochemical and hormone analysis

After a 12-h overnight fast, 5 ml of blood was drawn, 2 ml of blood in EDTA vacutainers for complete hemogram and 3 ml of blood in plain vacutainers for biochemical tests. The hematological assessment included complete blood counts performed on a fully automated Mindray BC-2600 Auto Hematology Analyzer (Beckman, Inc., U.S.A.). The peripheral smears were stained with Leishman stain and studied by a hematologist. Hematological profile included number of red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), white blood cells (WBC), lymphocytes, neutrophils, and the mixed cell population of monocytes, eosinophils, basophils, blasts and other precursor white cells.

The biochemical parameters were estimations for liver involvement (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), renal function (uric acid and creatinine), lipid (cholesterol and triglycerides) and glucose metabolism (glycemia). Analyses were performed on an Omega IV, Model 3305, Semi-automatic Biochemistry Analyzer (Labomed, Inc., U.S.A.).

Plasma was collected for butyrylcholinesterase (BuChE) analysis using a portable field kit (Test-mate EQM Research, Cincinnati, OH, USA), based on the method of Ellman et al. [45], and data were expressed as $\mu mol/min/ml$ plasma (U/ml). Serum was separated and kept frozen until assayed for thyroid-stimulating hormone (TSH) and free thyroxine (FT4) by enzymatic immunoassay using ELISA kits (Right Choice Diagnostics, Muenster, Germany).

Total antioxidant capacity (TAC) was determined by the FRAP (ferric reducing antioxidant power) assay, based on the reduction of a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) form in the presence of antioxidants [46]. The end product (Fe^{2+}-TPTZ) has blue color with absorption maximum at 593 nm and the change of absorbance is related to the antioxidant capacity of the plasma.

Statistical analysis

Data are presented as means ± SD and were analyzed by One-way ANOVA or Student's t-test, followed by Dunnett's test for comparisons between control and exposed groups. P<0.05 was considered statistically significant. The magnitude of the difference between two groups was also determined by calculating Cohen's d effect size from t-tests [47,48]. Cohen's d effect sizes are classified as small (d=0.2), medium (d=0.5) and large ($d \geq 0.8$). All statistical analyses were performed using GraphPad In Stat tm, version 2.04a software.

Results

The participants showed no significant differences, in terms of mean ± SD between the exposed and control groups, in age (9 ± 2 years for both groups), weight (29 ± 10 versus 36 ± 12 kg, respectively), height (131 ± 12 versus 137 ± 12 cm, respectively), and body mass index

(16.4 ± 3.3 versus 18.4 ± 3.8 kg/m², respectively), as shown in Table 1. Most of the children had normal growth rate (75.1% versus 91.4%, between exposed and control groups, respectively). Fifteen percent of farm children showed low growth rate compared to about 2.9% of control group, and 10% of exposed children exhibited high growth rate compared to 5.7% of control. About half of participant children of both groups had normal dietary intake; however, a prevalence of malnutrition (31.4%) was observed in urban-resident children, whereas a higher percentage of undernutrition (20%) was seen in farm children compared to control.

The mean values of nine hematological parameters are summarized in Table 2. Total RBC count was significantly higher in exposed children than control group, whereas no significant difference was found in WBC count. Farm children had significantly lower (p<0.0001) Hb and HCT levels although both mean values were within the normal range. Forty eight percent of the exposed children had platelet counts above the upper limit of normality (>450 × 10³/µL). Severity of thrombocytosis was graded as follows: mild (450 × 10³/µL-700 × 10³/µL); moderate (700 × 10³/µL-900 × 10³/µL); and severe (>900 × 10³/µL) [49]. Forty six percent of farm children had mild thrombocytosis, 1% with moderate and 1%, severe thrombocytosis, whereas all control children had normal platelet counts (Table 3). Children with thrombocytosis had mean platelet count of 561 ± 113 × 10³/µL and mean platelet volume (MPV) of 9.5 ± 0.8 fL.

Mean relative lymphocyte counts of farm children were significantly higher (p<0.0012) than those of urban children, however, the absolute values were not different from control (Table 2). Both relative and absolute mean monocyte counts were significantly increased (p<0.0001; d=1.9 and 1.3, respectively) in the exposed children. Mean relative neutrophil counts were significantly decreased (p<0.0001; d=-1.2) in farm children compared to control. Although the mean absolute neutrophil counts were significantly lower (p<0.0001; d=-0.9) than control, the values were within reference limits.

In the present study, clinical examination of children participants revealed a high incidence (38%) of dental caries in the exposed group. One third (33%) of the children with tooth decay had thrombocytosis, 30% with lymphocytosis, 26% with neutropenia, and 19% had both lymphocytosis and neutropenia. The terms lymphocytosis and neutropenia refer to absolute lymphocyte and neutrophil counts, above or below normal limits by age, respectively. Based on interviews, the most common symptoms reported by farm children and their parents included headaches (33%), dizziness (19%), respiratory problems (18%), diarrhea (13%), and abdominal pains (8%); these symptoms are associated with mild exposures to OP and CB insecticides. Assay of plasma cholinesterase activity indicated that 35% of farm children had been exposed to OP and CB pesticides (Table 4). According to clinical category of enzyme inhibition, based on the study of Rajapakse et al. [50], 10% of these children had mild inhibition, with their mean BuChE activity (1.6 ± 0.2 U/ml) decreased by about 45% from mean control values (2.9 ± 0.5 U/ml). However, it should be pointed out that the agrochemicals used in the region included not only OPs and CBs but also mixtures of other chemical groups of pesticides.

For the biochemical analysis of the sera (Table 4), tests for ALT and AST were within normal ranges in both farm- and urban-resident children, however, mean ALT was significantly lower (p=0.0003) by 1.6-fold in exposed group compared with control. There was a significant difference in blood creatinine (p=0.0006) but not in uric acid concentrations between exposed and control groups, however, results were also within normal limits. Blood glucose and total lipids were comparable between the two studied groups, although, mean

triglyceride level was significantly lower (p<0.0001) by 30% in exposed children compared with control. Both TSH and FT4 levels were found within the reference ranges, however, mean FT4 concentration was significantly lower (p=0.0009) in exposed children compared with control. TAC levels were significantly different between farm- and urban-resident children (437 ± 89 µmol/L versus 1152 ± 323 µmol/L, respectively, p<0.0001, d=-3.5).

Children with platelet counts >450 × 10³/µL (thrombocytosis group) showed lymphocytosis (87%), monocytosis (43%) and neutropenia (73%). Two years later, follow-up of 20 children of thrombocytosis group showed normalized platelet counts, which was significantly reduced (p=0.0001) by 37%, from baseline levels of 575 ± 144 to 381 ± 45 × 10³/µL (Table 5). RBC parameters were within normal levels. However, significantly lower values were observed for absolute lymphocyte counts (3.2 ± 0.7 × 10³/µL versus 2.5 ± 0.4 × 10³/µL, respectively, p=0.0004), relative neutrophil (47.0 ± 8.1% versus 41.0 ± 9.0%, respectively, *p*=0.03) as well as absolute counts (3.3 ± 1.1 × 10³/µL versus 2.3 ± 0.8 × 10³/µL, respectively, p=0.002). Conversely, significantly higher values were observed for relative monocyte (7.5 ± 1.6% versus 10.5 ± 2.2%, respectively, p=0.0001) and absolute counts (0.5 ± 0.2 × 10³/µL versus 0.6 ± 0.1 × 10³/µL, respectively, p<0.05).

Discussion

In this study, we have shown alterations in various hematological and biochemical parameters of farm-resident children chronically exposed to the toxic effects of agrochemicals. Of main concern were the most frequently used pesticides such as: mancozeb, cymoxanil, glyphosate, methomyl, paraquat, chlorothalonil, chlorpyrifos, propamocarb, diazinon, carbendazim, carbofuran, malathion and atrazine, among the list of 54 active ingredients applied in the region during the study period, between 2012 and 2014. The most important hematological findings were significantly increased mean platelet counts in about half of the farm children participants in the study. These children had significantly increased lymphocyte and monocyte counts, in contrast, decreased neutrophil counts, suggesting a dysregulation of their immune system. With regard biochemical parameters, although significant differences were observed between exposed and control groups, most of the results were within normal limits. However, TAC values of farm children were significantly reduced compared to control, which could be due to dietary deficiency and to some extent, chronic exposure to adverse health effects of pesticides.

Variable	Control group (N=41)	Exposed group (N=72)
Age (years)	9 ± 2	9 ± 2
Weight (kg)	36 ± 12	29 ± 10
Height (cm)	137 ± 12	131 ± 12
Body mass index (kg/m²): †Males	19.0 ± 3.9	17.4 ± 4.4
*Females	17.8 ± 3.4	16.8 ± 3.2
Growth rate, (%):		
Normal	91.4%	75.1%
Low	2.9%	15.0%
High	5.7%	10.0%
Nutritional diagnosis, (%):		
Normal	57.1%	55.0%
Obese	17.1%	8.3%
Overweight	14.3%	6.7%
Undernutrition	11.5%	20.0%
Reference values: †16.8 – 20.3 (males); *13.9 – 21.2 (females)		

Table 1: Anthropometry and nutritional diagnosis of control and exposed groups.

Parameters	Reference Value	Control group (N=41)	Exposed group (N=72)	[†]Cohen's d	[*]p value
Red blood cells × 10^3/μL	4.0 – 5.4	5.0 (0.3)	5.2 (0.3)	0.7	0.0009
Hemoglobin g/dL	11.5 – 16.5	15.7 (1.0)	14.3 (0.8)	−1.6	<0.0001
Hematocrit (g/dl)	35.0 – 49.0	44.6 (2.5)	42.4 (2.8)	2.7	<0.0001
Platelets × 10^3/μL	150 - 450	320 (59)	460 (134)	1.3	<0.0001
Mean platelet volume (fL)	7.4 – 8.1	7.6 (0.5)	9.9 (1.1)	0.9	<0.0001
White blood cells × 10^3/μL	4.5 - 10.0	8.0 (2.2)	7.6 (1.8)	−0.2	0.3
Lymphocytes (%)	25.0 - 33.0	36.9 (8.1)	43.5 (11.1)	0.6	0.0012
Lymphocytes × 10^3/μL	1.5 – 3.0	2.9 (0.7)	3.1 (0.9)	0.2	0.28
Monocytes (%)	3.0 - 7.0	4.3 (1.1)	8.5 (2.7)	1.9	<0.0001
Monocytes × 10^3/μL	0.3 – 0.8	0.3 (0.1)	0.6 (0.2)	1.3	<0.0001
Neutrophils (%)	54.0 - 62.0	58.8 (8.3)	47.7 (9.5)	−1.2	<0.0001
Neutrophils × 10^3/μL	3.0 – 6.0	4.8 (1.9)	3.4 (1.3)	−0.9	<0.0001

[*]p: Unpaired Student's T Test, p<0.05 considered significant.
[†]Cohen's d effect size from t-tests. Sullivan and Feinn.
Table 2: Mean value (SD) of hematological parameters in control and exposed groups.

Platelet count	[*]Clinical category of thrombocytosis	Control group[a] n (%)	Exposed group[b] n (%)
>150 - 450 × 10^3/μL	Normal	41 (100)	38 (53)
>450 - <700 × 10^3/μL	Mild	0	32 (44)
>700 - <900 × 10^3/μL	Moderate	0	1 (1)
>900 × 10^3/μL	Severe	0	1 (1)

[a]N=41; [b]N=72; [*]Chiarello et al.
Table 3: Distribution of blood platelet counts in control and exposed groups.

Parameters	Reference Value	Control group (N=41)	Exposed group (N=72)	[†]Effect Size	[*]p
Serum Aspartate Aminotransferase, U/L	1 - 37	18 (7)	17 (5)	−0.2	0.4
Serum Alanine Aminotransferase, U/L	1 - 42	13 (8)	8 (6)	−0.8	0.0003
Blood Creatinine, mg/dL	0.5 – 1.5	0.6 (0.2)	0.5 (0.1)	−0.7	0.0006
Blood Uric Acid, mg/dL	3.5 – 7.2	3.3 (1.0)	3.7 (1.2)	0.4	0.07
Blood Glucose, mg/dL	70 - 110	91 (7)	91 (8)	0	1.0
Total Cholesterol, mg/dL	130 - 200	154 (24)	157 (23)	0.1	0.51
Triglycerides, mg/dL	36 - 150	95 (44)	67 (25)	−0.9	<0.0001
Thyroxine, ng/dL	0.8 – 2.0	1.6 (0.3)	1.4 (0.3)	−0.7	0.0009
Thyroid Stimulating Hormone, μIU/mL	0.3 – 8.1	2.8 (0.9)	2.5 (1.5)	0.1	0.25
Plasma Cholinesterase (U/mL)	2.4 – 3.64	[a]2.9 (0.5)	[b]2.6 (0.6)	−0.5	0.015
Total Antioxidant Capacity, μmol/L	≥ 700	1152 (323)	437 (89)	−3.5	<0.0001

[*]p: Unpaired Student's t test, p<0.05 considered significant.
[†]Cohen's d effect size from t-test
[a]N=38; [b]N=61
Table 4: Mean value (SD) of biochemical parameters in control and exposed groups.

Parameters	Baseline N=20	Follow-up N=20	Change (baseline – follow-up)	[*]p value
Red blood cells × 10^3/μL	5.1 ± 0.3	5.0 ± 0.3	0.2 ± 0.3	0.3
Hemoglobin g/dL	14.1 ± 0.6	14.8 ± 0.7	-0.7 ± 0.6	0.002
Platelets × 10^3/μL	575 ± 144	381 ± 45	194 ± 120	0.0001
White blood cells × 10^3/μL	7.0 ± 1.4	5.4 ± 1.0	1.6 ± 1.6	0.0002
Lymphocytes (%)	45.4 ± 8.1	46.9 ± 7.1	-1.5 ± 9.5	0.5
Lymphocytes × 10^3/μL	3.2 ± 0.7	2.5 ± 0.4	0.7 ± 1.0	0.0004
Monocytes (%)	7.5 ± 1.6	10.5 2.2	-3.1 ± 3.1	0.0001
Monocytes × 10^3/μL	0.5 ± 0.2	0.6 ± 0.1	-0.04 ± 0.2	0.05
Neutrophils (%)	47.0 ± 8.1	41.0 ± 9.0	6.0 ± 10.7	0.03
Neutrophils × 10^3/μL	3.3 ± 1.1	2.3 ± 0.8	1.1 ± 1.2	0.002

[*]p: Paired Student's t test; p<0.05 considered significant.
Table 5: Comparison of hematological parameters of exposed children after two years follow-up.

A high percentage (46%) of the children living in the studied agricultural community of Merida had thrombocytosis, generally considered a platelet count of more than $450 \times 10^3/\mu L$. According to Kucine et al. [51], thrombocytosis is now a common finding in the complete blood count of children. It is often transient and occurs secondary to various underlying medical disorders, usually inflammatory, because an increase in the platelet count is one aspect of the acute phase reaction. This occurs more frequently in younger children, either because of the immaturity of their innate and/or adaptive immunity, or because they have more frequent infections [51]. According to its origin, thrombocytosis is classified into primary and secondary forms. Primary thrombocytosis is an extremely rare clonal disease in childhood with an incidence of one per million children; the platelet count is generally above $1000 \times 10^3/\mu L$ and may be associated with hemorrhagic events. Secondary or reactive thrombocytosis is very common in pediatric age and is due to a variety of conditions, such as bacterial and viral infections, inflammatory diseases, recent surgery, malignancy, iron, vitamin E and B12 deficiency, hemolytic anemia, allergic reactions, nephritis, pancreatitis, and side effects of some medications [51]. From the interviews conducted with the parents of farm children, all of them were either occupationally or environmentally exposed to pesticides; hence, there was concern of the increased risk of these children to develop inflammatory or malignant diseases in childhood or later in life. Epidemiologic studies have consistently shown elevated risks of leukemia, brain cancer, Wilms' tumor, Ewing's sarcoma, and germ cell tumors in childhood, observed for children whose parents had occupational exposures to pesticides [11,12,16,17,52]. Therefore we tried to evaluate the underlying causes of altered hematological parameters in the exposed children, particularly the high incidence of secondary thrombocytosis, increased lymphocytes counts and decreased neutrophil levels, as compared with control.

The hematological results of farm children showed significantly increased mean RBC and platelet counts; in contrast, significantly decreased mean Hb and HCT concentrations compared with the control group (Table 2). It has been reported that at high altitude, adaptation mechanisms to hypoxic condition alter hematological parameters causing increments in RBC counts, Hb and HCT concentrations, while platelet counts decline with increasing altitude [53,54]. Our results were not consistent with those reported by others. Although the values were within normal limits, significant differences observed for mean RBC indices between exposed and control groups could be related to dissimilarities in their dietary intake. In this study, 20% of farm children suffered mild to severe undernutrition, 7% obesity and 6% overweight, compared to control group, which showed 12% mild undernutrition, 17% obesity and 14% overweight (Table 1). In developing countries, the coexistence of nutritional deficit and excess, or "double burden" of malnutrition, is prevalent [55]. In Venezuela, 21-28% of children between 7 and 17 years old are overweight and 15-17% are undernourished; this fact is a reflection of the "double burden", in which the prevalence of undernourished children is greater than that of overweight, particularly in rural states of lower socioeconomic strata [55]. Undernutrition, protein-energy malnutrition and micronutrient deficiencies are all associated with immune system dysfunction, which increases the vulnerability to infection through impaired immunity [56].

Iron deficiency is most commonly associated with thrombocytosis [57,58]. Elevated platelet counts, especially in the setting of iron deficiency, can lead to an increased thromboembolic risk, with clinical consequences especially in patients with inflammatory bowel disease,

chronic kidney disease or cancer [59]. Fortunately, none of the farm children showed thromboembolic manifestations during the study period. Based on RBC parameters, the mean Hb level of the farm children was within the normal limits; moreover, all detected levels were above the established cut-off levels for anemia (<11.5 g/dL) for this age group (WHO, 2001), indicating that these children were not anemic. Conversely, iron deficiency can occur with normal Hb levels and patients often show relatively few symptoms spontaneously [57]. Iron deficiency in children can affect development and lead to anemia; untreated iron deficiency can cause physical and mental delays and increases susceptibility to infections [60,61]. However, due to fund limitations, assay of micronutrient deficiency indicators, such as serum ferritin and transferrin, were not included in the study.

The most frequent cause of secondary thrombocytosis in children are bacterial or viral infections; among these infections, respiratory diseases are the most frequent, followed by gastrointestinal and genitourinary [62]. Most of the farm children participants had no symptoms of respiratory illness on the day of sampling. However, physical examination of these children showed a prevalence (38%) of dental caries, which could be a source of bacterial infection and chronic inflammation, a possible cause of secondary thrombocytosis. Dental caries is a chronic infectious disease resulting from the penetration of oral bacteria into the enamel and dentin; microorganisms subsequently trigger inflammatory responses in the dental pulp [63]. Childhood dental caries constitutes the single most common chronic disease, affecting as many as 60-90% of children worldwide between the ages of 2 and 11 years [64]. Many parents, particularly those from low socioeconomic status, are less attentive to the dental hygiene of their children; this is evidenced by the fact that, on medical follow-up two years later, children participants were still found with dental caries. In this report, one third of thrombocytosis cases is associated with dental caries and either an increased absolute lymphocyte count or a decreased absolute neutrophil count or, in a few cases, both lymphocytosis and neutropenia. The abnormal levels of these hematological parameters reflect the defensive mechanism triggered by the dental pulp immune cells against cariogenic bacterial infection into the enamel and dentin [63]. However, if tooth decay was found in only one third of thrombocytosis cases, what then is the etiology behind the elevated platelet counts in the other two thirds of the cases?

Several evidence affirm that pesticide exposure causes dysregulation of the immune system and predisposition to different types of cancers, allergies, autoimmune and infectious diseases [65,66]. Various studies on animal models exposed to specific pesticides have explored changes in hematological and biochemical parameters as possible biomarkers of pesticide exposure before adverse clinical health problems occur [24-29]. More recently, clinical studies on pesticide sprayers have focused on the immunological, hematological and biochemical statuses of these farmers [23,67-71]. However, to our knowledge, there are no published reports on biomonitoring of children living in an agricultural community, where there is a high risk of exposure to the toxic effects of pesticides.

Many of our results were consistent with those reported by others among pesticide-exposed individuals compared to controls, such as: increased levels of Hb, HCT, RBC [67]; increased lymphocyte, monocyte and platelets counts [68,70,72]; reduced mean neutrophil counts, serum acetylcholinesterase activity and T4 [70]. Evidence from some animal studies were also consistent with our findings. In rats exposed to increasing concentrations of monocrotophos, an organophosphate insecticide, increased levels of lymphocytes and reduced neutrophils

were observed [73]. Neutrophil and lymphocyte counts can be altered by various physiological and pathological processes. In farm children, the observed alterations in both lymphocyte and neutrophils levels could be caused by chronic environmental exposure to pesticides, which have the tendency to destroy neutrophils and as a consequence, overwhelm the defense mechanism of the individuals [65]. This is supported by the fact that the mean TAC level of farm-resident children was significantly reduced as compared with that of urban-resident control children. It is well accepted that stimulation of free-radical production, induction of lipid peroxidation, and disturbance of the total antioxidant capacity of the body are mechanisms of toxicity in most pesticides, including organophosphates, bipiridyl herbicides and organochlorines [74]. In the follow-up study done two years later, the most important finding was the normalization of mean platelet and absolute lymphocyte counts, however, decreased mean absolute neutrophil count was still observed. Nonetheless, the small number of re-evaluated participants strongly limit the significance of the results.

In conclusion, this study demonstrates that children living in an agricultural community are exposed to the health hazards of pesticides, as observed from alterations of hematological and biochemical parameters. Undernutrition and improper dental hygiene affect children's overall health status and are likely to increase their vulnerability to the toxic effects of pesticides.

Acknowledgements

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