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ALTERAÇÕES HEMATOLÓGICAS E BIOQUÍMICAS EM INDIVÍDUOS EXPOSTOS A AGROTÓXICOS: IMPACTO DA EXPOSIÇÃO DIRETA *VERSUS* INDIRETA

HAEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS IN PESTICIDE-EXPOSED INDIVIDUALS: DIRECT EXPOSURE IMPACT VERSUS INDIRECT

ALTERACIONES HEMATOLÓGICAS Y BIOQUÍMICAS EN INDIVIDUOS EXPUESTOS A PESTICIDAS: IMPACTO DE LA EXPOSICIÓN DIRECTA VERSUS INDIRECTA

Vinícius Gonçalves de Souza[1]
Jhennefer Sonara Aguiar Ramos[2]
Thays Millena Alves Pedroso[3]
Felipe de Araújo Nascimento[4]
Daniela de Melo e Silva[5]
Michelle Rocha Parise[6]

RESUMO

Sabe-se que indivíduos expostos diretamente a agrotóxicos são mais suscetíveis a alterações em parâmetros hematológicos e bioquímicos. No entanto, inexistem estudos que comparam os efeitos da exposição direta e indireta a agrotóxicos em tais parâmetros. Este estudo objetivou verificar se indivíduos indiretamente, mas ainda massivamente expostos a agrotóxicos por residirem ou trabalharem em áreas agrícolas com intensa aplicação de agrotóxicos, apresentam alterações hematológicas e bioquímicas. As análises foram realizadas em 62 indivíduos expostos a agrotóxicos residentes em um município localizado na região Centro-Oeste do Brasil, sendo metade trabalhadores rurais expostos diretamente (ocupacionalmente) e, o restante, expostos indiretamente. Além dos parâmetros hematológicos e bioquímicos, foram analisados parâmetros sociodemográficos, clínicos e ocupacionais para descartar vieses no estudo. Não foram encontradas diferenças significativas quanto ao tipo de exposição nos parâmetros hematológicos. Já nos parâmetros bioquímicos, os indivíduos expostos diretamente apresentaram maior frequência de níveis alterados de colinesterase, bem como de níveis elevados de AST, ALT e GGT em mulheres em relação as expostas indiretamente a agrotóxicos ($p<0,05$), estes últimos também em relação aos parâmetros de normalidade. O colesterol total, VLDL e triglicerídeos, por sua vez, foram significativamente elevados em ambos os grupos, talvez pelo sobrepeso. Sendo assim, este estudo mostrou que os indivíduos indiretamente expostos aos agrotóxicos, ainda que consideravelmente expostos a grandes quantidades de agrotóxicos diariamente, sofrem menos alterações bioquímicas que os trabalhadores expostos diretamente. No entanto, estes resultados não excluem a necessidade de monitoramento destes indivíduos, nem a possibilidade de alterações por exposição indireta a agrotóxicos. Mais estudos envolvendo comparação entre populações rurais expostas direta e indiretamente a agrotóxicos devem ser realizados em outras populações, uma vez que o perfil de exposição pode diferir de cada local devido a diferenças na carga de agrotóxicos e frequências de aplicação empregadas.

PALAVRAS-CHAVE

Agroquímicos. Biomarcador. Contagem de Células Sanguíneas. Colinesterase. Testes de Função Hepática.

ABSTRACT

It is known that individuals directly exposed to pesticides are more susceptible to changes in hematological and biochemical parameters. However, there are no studies comparing the effects of direct and indirect exposure to pesticides on such parameters. This study aimed to verify if individuals indirectly, but still massively exposed to pesticides due to residing or working in agricultural areas with intense pesticide application, exhibit hematological and biochemical alterations. Analyses were conducted on 62 individuals exposed to pesticides residing in a municipality located in the Midwest region of Brazil, with half of them being rural workers directly exposed (occupationally) and the remaining indirectly exposed. Besides hematological and biochemical parameters, sociodemographic, clinical, and occupational parameters were analyzed to discard biases in the study. No significant differences were found regarding the type of exposure in hematological parameters. However, in biochemical parameters, individuals directly exposed to pesticides showed a higher frequency of altered levels of cholinesterase, as well as higher levels of AST, ALT, and GGT in women compared to those indirectly exposed to pesticides ($p<0.05$), the latter also in relation to normality parameters. Total cholesterol, VLDL, and triglycerides were significantly elevated in both groups, possibly due to overweight. Therefore, this study showed that individuals indirectly exposed to pesticides, although considerably exposed to large quantities of pesticides daily, undergo fewer biochemical alterations than workers directly exposed. However, these results do not exclude the need for monitoring these individuals, nor the possibility of alterations due to indirect pesticide exposure. Further studies involving comparison between rural populations exposed directly and indirectly to pesticides should be conducted in other populations, as exposure profiles may differ from each location due to differences in pesticide loads and application frequencies employed.

KEYWORDS

Agrochemicals; biomarker; blood cell count; cholinesterase; Liver Function Tests.

RESUMEN

Se sabe que los individuos expuestos directamente a plaguicidas son más susceptibles a alteraciones en parámetros hematológicos y bioquímicos. Sin embargo, no existen estudios que comparen los efectos de la exposición directa e indirecta a plaguicidas en tales parámetros. Este estudio tuvo como objetivo verificar si individuos expuestos de manera indirecta, pero aun masivamente, a plaguicidas debido a que residen o trabajan en áreas agrícolas con intensa aplicación de estos, presentan alteraciones hematológicas y bioquímicas. Se realizaron análisis en 62 individuos expuestos a plaguicidas residentes en un municipio ubicado en la región Centro-Oeste de Brasil, siendo la mitad trabajadores rurales expuestos directamente (ocupacionalmente) y el resto expuestos indirectamente. Además de los parámetros hematológicos y bioquímicos, se analizaron parámetros sociodemográficos, clínicos y ocupacionales para descartar prejuicios en el estudio. No se encontraron diferencias significativas en cuanto al tipo de exposición en los parámetros hematológicos. Sin embargo, en los parámetros bioquímicos, los individuos expuestos directamente presentaron una mayor frecuencia de niveles alterados de colinesterasa, así como niveles elevados de AST, ALT y GGT en mujeres en comparación con las expuestas indirectamente a plaguicidas ($p<0,05$), estos últimos también en relación con los parámetros de normalidad. El colesterol total, VLDL y triglicéridos, por su parte, estaban significativamente elevados en ambos grupos, posiblemente debido al sobrepeso. Por lo tanto, este estudio mostró que los individuos expuestos indirectamente a plaguicidas, aunque están considerablemente expuestos a grandes cantidades de estos diariamente, sufren menos alteraciones bioquímicas que los trabajadores expuestos directamente. Sin embargo, estos resultados no excluyen la necesidad de monitorear a estos individuos, ni la posibilidad de alteraciones por exposición indirecta a plaguicidas. Se deben realizar más estudios que involucren la comparación entre poblaciones rurales expuestas directa e indirectamente a plaguicidas en otras poblaciones, ya que el perfil de exposición puede diferir en cada lugar debido a diferencias en la carga de plaguicidas y frecuencias de aplicación utilizadas

PALABRAS CLAVE

Agroquímicos; biomarcador; recuento de células sanguíneas; colinesterasa; pruebas de función hepática.

1 INTRODUCTION

Pesticides are widely used by farmers to increase the amount and quality of production by controlling pests and plant diseases (TUDI *et al*., 2021). Brazil is a country in which occurs the greatest use of pesticides, bearing the register of 308 formulated pesticide products (NASCIMENTO *et al*. 2020; RAMOS *et al*., 2021, BRASIL, 2022).

Although the benefits of pesticide use in rural production, people exposed to these compounds are more susceptible to several health disorders, such as immunological and haematological changes, DNA damage, nervous and endocrine diseases, cancer, as well as changes in reproductive function (GARCÍA-GARCÍA *et al*., 2016; NICOLOPOULOU-STAMATI *et al*., 2016; MREMA *et al*., 2017; GODOY *et al*., 2019; RAMOS *et al*., 2021; NASCIMENTO *et al*., 2022; PEDROSO *et al*., 2022).

Specially regarding haematological and biochemical alterations due to pesticides exposure, alterations such as increased counts of erythrocytes, leukocytes, white blood cells and platelet counts, anisocytosis of red blood cells (RBC), neutrophils to lymphocytes ratio (NLR), platelets and hemoglobin, as well as decreased creatinine, total cholesterol, triglyceride and alkaline phosphatase have been reported (GARCÍA-GARCÍA *et al*., 2016; NEJATIFAR *et al*., 2022).

Assessment of haematological parameters (i.e. complete blood count) and clinical biochemical parameters (i.e. liver serum enzymes, lipidic profile) is a great way of biomonitoring since these are low cost and accessible tools which easily and properly accounts for helping monitoring occupationally exposed rural workers health status (NEJATIFAR *et al*., 2022).

Pesticide exposure can be divided into direct exposure, in the case of exposure in the workplace, and indirect exposure, which includes exposure through air, water, soil, food chain, as well as by the contact with contaminated clothes (TUDI *et al*., 2022).

In this regard, the previous find of our research group that indirect exposure leads to higher DNA damage levels and immune cell lines alterations when compared to unexposed individuals (RAMOS *et al*., 2021), associated to the fact that the effects of indirect (but still significantly) exposure to pesticides on haematological and biochemical is not still explored, when considering the residents in agricultural areas nearby crops and the rural workers involved in agricultural activities that leads to indirect exposure to pesticides, this study aimed to compare the effects of direct and indirect pesticide-exposure towards haematological and biochemical parameters to identify if the different way of expressive exposure to pesticides implies in significant distinct patterns of haematological and biochemical alterations.

2 METHODOLOGY

2.1 STUDY POPULATION AND DATA COLLECTION

It is a cross-sectional study that was conducted with 62 Brazilian individuals from a municipality of intense agricultural activity located in the middle west of Brazil. Our aim was to compare two different types of exposure by evaluating individuals who lived in the same place (same length of stay at the same rural area) and who, therefore, shared the same source of water, food, lifestyle habits, which, if different between the groups, could be responsible for biases in the parameters evaluated.

In order to assure this, the recruitment and selection of these individuals was carried out with the help of Municipal Health Department of the municipality of Jataí, state of Goiás, which has basic health units in rural areas that frequently serves both directly and indirectly pesticide exposed individuals from the same location.

The individuals included in this study had to have an age equal to and/or over 30 years old and equal to and/or less than 65 years old and also had to present a history of exposure to pesticides (direct or indirect); Even if they attended the required inclusion criteria, individuals were excluded from the study if they had a previous or current history of cancer and use of chemotherapy; a history of exposure to therapeutic or occupational radiation; of using genotoxic drugs, immunosuppressants or any substance thar could interfere directly or indirectly with the results of hematological and biochemical tests; or if did not agree to participate of the study and sign the TCLE.

The individuals were categorized into two groups – directly exposed (pesticide-exposed rural workers that are occupationally involved in activities such as storage, mixing, loading, and spraying of pesticides), n=31; and indirectly exposed (people that are also exposed to considerable amounts pesticides but in an environmental way, specially by living nearby crops and thus being exposed through air dispersion of pesticides), n=31.

The individuals in the study were informed about all the procedures and the aims of the study. They also signed an informed written consent before participation. This research was approved by the Research Ethics Committee of the Federal University of Goias (#2.648.494) and all the procedures were performed according to Resolution nº 466/12 of the National Health Council, which approves the regulatory guidelines for research involving humans in Brazil.

A questionnaire with open-ended and closed questions was applied to all the participants to collect sociodemographic data and life habits (age, sex, smoking, alcohol use, overweight, and sedentarism), medical, as well as occupational data (comorbidities, adequate use of personal protective equipment [PPE] and acute intoxication history).

2.2 SAMPLE COLLECTION

All the subjects were submitted to a collection of 15 mL of peripheral blood divided into EDTA (ethylenediaminetetraacetic acid) and clot activator vacuum tubes. Samples were transported to the Mutagenesis Laboratory of the Federal University of Goias at 4ºC and were processed, frozen, and stored at -20ºC.

2.3 HAEMATOLOGICAL AND BIOCHEMICAL ANALYSIS

The blood count and all the biochemical tests were done by a public laboratory of the local Secretary of Health. The automated hematological counter ABX micros 60 (Horiba ABX Diagnostics, France) and commercial tests for biochemistry analysis were used. Table 1 shows the referential values employed as the parameter of normality.

Table 1 – Referential values of normality for laboratory tests

	Referential values	
	Men	Women
Erythrocytes (mi/mm³)	4.5 – 5.9	4.5 – 5.1
Hemoglobin (g/dL)	14.0 – 17.5	12.3 – 15.3
Hematocrit (%)	41.5 – 50.4	35.9 – 44.6
MCV (fL)	80 – 96	
MCH (pg)	27.5 – 33.2	
MCHC (%)	33.4 – 35.5	
RDW (%)	12.2 – 14.6	
Reticulocytes (/µL)	25000 - 75000	
Leukocytes (/µL)	4400 – 11000	
Lymphocytes (/µL)	1000 – 4800	
Monocytes (/µL)	0 – 800	
Neutrophils (/µL)	1800 – 8500	
Eosinophils (/µL)	0 – 450	
Basophils (/µL)	0 – 200	
Platelet count (*10³/mm³)	150 – 450	
Total cholesterol (mg/dL)	< 190	
HDL (mg/dL)	> 40	
LDL (mg/dL)	< 130	
VLDL (mg/dL)	< 30	
Triglycerides (mg/dL)	< 150	
Cholinesterase (U/L)	4900 – 11900	
AST (U/L)	< 35	< 31
ALT (U/L)	< 42	< 32
GGT (U/L)	7 – 45	5 - 27
Glucose levels (mg/dL)	< 126	

AST: aspartate aminotransferase. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. HDL: high-density lipoproteins. LDL: low-density lipoproteins. MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. MCHC: mean corpuscular hemoglobin concentration. RDW: red cell distribution width. VLDL: very low-density lipoprotein.
Source: Research Data.

2.4 STATISTICAL ANALYSIS

Categorical variables were presented as absolute and relative frequency, while continuous variables were presented as mean and standard deviation. Statistical analysis was performed by the software SPSS version 26.0. Shapiro-Wilk and Levene's tests were done to check normality and homoscedasticity. The student´s t test was employed for continuous variables and the Chi-square test was used for categorical variables. A p value lower than 0.05 indicates statistical significance.

3 RESULTS

Clinical parameters of the 31 directly and 31 indirectly pesticide-exposed individuals are presented in Table 2.

Table 2 – Clinical parameters of pesticides-exposed rural workers of Jataí, GO, Brazil.

	Type of exposure		*p*-value
	Direct (n=31)	**Indirect** (n=31)	
Sex[1]			
Men	26 (83.9)	19 (61.3)	0.046
Women	5 (16.1)	12 (38.7)	
Age[2]	50.5 ± 11.5	47.1 ± 14.2	0.390
Comorbidities[1]			
Allergies	4 (12.9)	9 (29.0)	0.119
Dyslipidemia	2 (6.5)	5 (16.1)	0.229
High blood pressure	9 (29.0)	8 (25.8)	0.776
Diabetes mellitus	6 (19.4)	3 (9.7)	0.279
Thyroid diseases	3 (9.7)	1 (3.2)	0.301
Infectious diseases	1 (3.2)	1 (3.2)	1.000
Heart disease	1 (3.2)	0 (0.0)	0.313
Osteoarticular disease	1 (3.2)	3 (9.7)	0.301

	Type of exposure		*p*-value
	Direct (n=31)	**Indirect** (n=31)	
Harmful habits[1]			
Overweight	18 (58.1)	25 (80.6)	0.054
Smoking	5 (16.1)	3 (9.7)	0.449
Alcohol use	19 (61.3)	18 (58.1)	0.796
Sedentarism	13 (41.9)	13 (41.9)	0.912
Adequate use of PPE[1]	11 (35.5)	6 (19.4)	0.155
Acute intoxication history[1]	6 (19.4)	4 (12.9)	0.490

PPE: Personal protective equipment

[1] Value expressed as absolute frequency (relative frequency)

[2] Value expressed as mean ± standard deviation

Source: Research Data.

Regarding the adequate use of PPE, it is important to point that in both directly or indirectly pesticide-exposed groups, a low adherence to the use of PPE was reported, thus predisposing these individuals to a greater health risk due to pesticide exposure. The used PPEs reported by them were masks (34%), gloves (30%), glasses (23%), boots (23%) and overalls (18%). Concerning pesticides, exposed individuals reported frequent use of 269 herbicides and in second place, insecticides and fungicides. The most commonly used pesticides were glyphosate, 2,4-D, cypermethrin, deltamethrin, and atrazine. The most common crops were soybean and corn (RAMOS *et al*., 2021).

By analyzing the sex of these 62 individuals distributed throughout the two studied groups, men were predominant in both direct and indirect pesticide-exposed groups (n=45, 72,58%). However, if we analyze exclusively the number of women, a greater number was found in the indirectly pesticide-exposed group (38.7%, $p < 0.05$) when compared to the amount of women in the directly pesticide-exposed group. The absolute values of haematological and biochemical tests are presented in Table 3.

Table 3 – Comparison of absolute values of hematological and biochemical tests of direct and indirect pesticides-exposed rural workers of Jataí, GO, Brazil.

	Type of exposure		*p*-value
	Direct (n=31)	**Indirect** (n=31)	
Red cells count			
Erythrocytes (mi/mm³)			
Men	5.04 ± 0.63	5.03 ± 0.38	0.889
Women	4.49 ± 0.15	4.69 ± 0.34	0.130

	Type of exposure		*p*-value
	Direct (n=31)	**Indirect** (n=31)	
Hemoglobin (g/dL)			
Men	15.02 ± 1.63	15.26 ± 1.11	0.654
Women	13.43 ± 1.66	13.75 ± 1.23	0.703
Hematocrit (%)			
Men	45.52 ± 4.90	46.50 ± 3.78	0.486
Women	40.75 ± 3.78	41.69 ± 3.68	0.624
MCV (fL)	90.87 ± 4.83	89.87 ± 5.73	0.631
MCH (pg)	29.79 ± 2.30	29.47 ± 2.23	0.231
MCHC (%)	33.10 ± 0.74	32.81 ± 0.62	0.144
RDW (%)	13.13 ± 0.92	12.97 ± 0.80	0.522
Reticulocytes (/µL)	67349.35 ± 30058.90	72084.19 ± 37025.38	0.735
White cells count			
Leukocytes (/µL)	6764.52 ± 2282.04	6980.65 ± 2065.17	0.762
Lymphocytes (/µL)	2066.39 ± 587.49	2269.97 ± 618.08	0.278
Monocytes (/µL)	380.71 ± 137.29	397.74 ± 164.19	0.921
Neutrophils (/µL)	4216.61 ± 1887.69	4274.16 ± 1759.82	0.916
Eosinophils (/µL)	100.81 ± 69.41	135.23 ± 160.08	0.304
Basophils (/µL)	0.00 ± 0.00	0.00 ± 0.00	1.000
Platelet count (*10^3/mm^3)	222.74 ± 52.95	247.74 ± 60.82	0.108
Total cholesterol (mg/dL)	200.61 ± 44.28[@]	202.87 ± 46.19[@]	0.730
HDL (mg/dL)	43.55 ± 12.06	47.97 ± 16.75	0.287
LDL (mg/dL)	123.25 ± 38.37	118.46 ± 37.69	0.550
VLDL (mg/dL)	33.82 ± 22.51[@]	36.44 ± 29.90[@]	0.860
Triglycerides (mg/dL)	169.10 ± 112.53[@]	167.52 ± 115.76[@]	0.751
Cholinesterase (U/L)	6068.26 ± 2129.36	5370.19 ± 2962.16	0.321
AST (U/L)			
Men	32.18 ± 27.40	29.08 ± 9.79	0.969
Women	55.00 ± 43.5^{4}@	22.85 ± 7.01	0.023*

	Type of exposure		*p*-value
	Direct (n=31)	**Indirect** (n=31)	
ALT (U/L)			
Men	33.03 ± 37.12	28.41 ± 33.40	0.787
Women	44.25 ± 34.7^{1}@	17.77 ± 5.90	0.023*
GGT (U/L)			
Men	59.30 ± 78.91$^{@}$	42.75 ± 25.29	0,817
Women	118.25 ± 106.92$^{@}$	22.08 ± 10.49	0.002*
Glucose levels (mg/dL)	112.74 ± 39.29	105.29 ± 26.24	0.342

The values are expressed as mean ± standard deviation. *p<0.05, significant in relation to the indirectly exposed group. @ indicates a mean value higher than the standard values. AST: aspartate aminotransferase. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. HDL: high-density lipoproteins. LDL: low-density lipoproteins. MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. MCHC: mean corpuscular hemoglobin concentration. RDW: red cell distribution width. VLDL: very low-density lipoprotein.
Source: Research Data.

The absolute and relative frequency of altered parameters in individuals in each evaluated group are shown in TABLE 4.

Table 4 – Frequency of alterations in hematological and biochemical tests of direct and indirect pesticides-exposed rural workers of Jataí, GO, Brazil.

	Type of exposure		*p*-value
	Direct (n=31)	**Indirect** (n=31)	
Anemia	10 (32.3)	8 (25.8)	0.576
Reticulocytes			
High	11 (35.5)	12 (38.7)	0.593
Low	1 (3.2)	0 (0.0)	
Leukocytes			
High	1 (3.2)	3 (9.7)	0.244
Low	4 (12.9)	1 (3.2)	
Lymphopenia	1 (3.2)	0 (0.0)	0.313
Monocytosis	0 (0.0)	1 (3.2)	0.313

	Type of exposure		*p*-value
	Direct (n=31)	**Indirect** (n=31)	
Neutrophilia	1 (3.2)	1 (3.2)	1.000
Eosinophilia	0 (0.0)	2 (6.5)	0.151
Thrombocytosis	1 (3.2)	0 (0.0)	0.313
High triglycerides	13 (41.9)	15 (48.4)	0.610
High total cholesterol	18 (58.1)	19 (61.3)	0.796
Low HDL	13 (41.9)	9 (30.0)	0.332
High LDL	12 (38.7)	9 (29.0)	0.421
Cholinesterase			
High	4 (12.9)	2 (6.5)	0.044*
Low	11 (35.5)	4 (12.9)	
High AST	14 (45.2)	3 (9.7)	0.002*
High ALT	12 (38.7)	3 (9.7)	0.008*
High GGT	15 (48.4)	7 (22.6)	0.034*
Hyperglycemia	6 (19.4)	5 (16.1)	0.740

The value expressed as absolute frequency (relative frequency)
AST: aspartate aminotransferase. ALT: alanine aminotransferase. GGT: gamma-glutamyl transferase. HDL: high-density lipoproteins. LDL: low-density lipoproteins. *p<0.05, significant in relation to the indirectly exposed group.
Source: Research Data.

Direct exposure to pesticides leads to a higher frequency of women with altered cholinesterase and higher AST, ALT, and GGT, as shown in Figures 1, 2 and 3, respectively. It is important to mention that these changes were not associated with sex, the presence of comorbidities, drug use, and alcohol consumption ($p > 0.05$).

4 DISCUSSION

Despite the several studies reporting haematological and biochemical negative effect of direct pesticide exposure to pesticides, this study was pioneer since it aimed to compare the effects of both direct and indirect pesticide-exposure towards haematological and biochemical parameters to identify if the different ways of pesticide exposure implies in significant distinct patterns of alterations. So, this study sought to establish if individuals indirectly exposed to pesticides by living in agricultural areas very close to crops, also suffer significant haematological and biochemical alterations since they are also exposed to a high load of pesticides. Besides, this is the only study developed in Central Brazil that characterizes the hematological and biochemical changes associated with exposure to a complex mixture of pesticides, specially focusing on the comparison between the impact of direct exposure and the environmental (indirect) exposure.

Regarding the profile of this complex mixture of pesticides, the exposed individuals evaluated in this study were more exposed to herbicides, followed by insecticides and fungicides, being these mainly applied in soybean and corn crops. The used pesticides, from the most used to the less used by our study population, were: glyphosate, 2,4-D, cypermethrin, deltamethrin, and atrazine, as described previously by our study group (RAMOS *et al*., 2021).

Both occupationally (direct) and environmentally (indirect) pesticide-exposed groups presented a greater number of men. This finding is in accordance with Moreira *et al*. (2015) and Silva *et al*. (2022), as well as our previous studies (GODOY *et al*., 2019; RAMOS *et al*., 2021), which showed a higher amount of men in both directly and indirectly exposed groups, probably because in Brazil men usually perform most agricultural activities and seem to be responsible for purchasing and spraying pesticides.

On the other hand, woman have an important exposure to pesticides considering the environmental (indirect) exposition, as presented in the population studied, considering that most of indirectly exposed individuals were women.

Considering the hematological parameters, previous studies (GARCÍA-GARCÍA *et al*., 2016; CORTÉS-IZA *et al*., 2017; NEJATIFAR *et al*., 2022) indicated that pesticides exposure led to a pro-inflammatory state with an increase of white blood cells, mainly neutrophils and basophils. The pesticide-exposed workers evaluated in these studies also presented an increase in platelet count, red blood cells, and RDW. In addition, it is also important to highlight the study which showed that even low doses of pesticides promoted changes in human bone marrow cells (FOUCAULT *et al*., 2021).

However, in the present study, no significant haematological alterations were observed in both evaluated groups. The only observation that could be cited, although not statistically significant, was that the directly pesticide-exposed group presented more cases of leukopenia, while the indirectly pesticide-exposed group presented more cases of leukocytosis. In this regard, these discrepancies between direct and indirect pesticide-exposed groups as well as the haematological alterations related in the literature may be influenced by the route of contact with the pesticide, mainly the dermal, oral, and respiratory epithelium, the physical state of the products, the presence of solvents, the place of use, the quantity of use and the potential of toxicity of the compound (SHARMA *et al*., 2020; LARI *et al*., 2022).

Pesticides, mainly organophosphates, lead to the inhibition of cholinesterase activity and reduce the levels of the enzyme in the blood, so it is used as a biomarker of exposure to pesticides (NIGG; KNAAK, 2000). Corroborating this, the directly-exposed individuals presented lower levels of cholinesterase and these low levels were more frequent when compared to indirectly-exposed individuals. This result highlights that directly pesticide-exposed individuals are submitted to higher levels of pesticides, especially when they did not use the recommended PPE properly (RAMÍREZ-SANTANA *et al*., 2018; SHENTEMA *et al*., 2020), as in this study.

Additionally, pesticide exposure may lead to several hepatic changes in animal models, such as liver necrosis, fatty deposition, inflammation, and loss of function, often in a dose-dependent manner (BANAEE *et al*., 2013; EZZI *et al*., 2016; KARAMI-MOHAJERI *et al*., 2017). The significantly increased frequency of high levels of AST, ALT, and GGT found in the directly-exposed group showed that hepatic changes are also more evident in individuals exposed to higher levels of pesticides. In the same way, Hérnandez *et al*. (2006), reported an association between pesticide exposure and increased AST activity (as well as decreased LDH and amino-oxidase activity, accompanied by lower levels of serum creatinine and higher levels of phosphorus).

The increased serum liver enzymes due to direct exposure was found in agricultural workers from different regions worlwide, as reported by studies performed in Pakistani tobacco farmers (KHAN *et al*., 2008); in agricultural workers in India (PATIL *et al*., 2003); in farm workers in Gadap Karachi, Pakistan (AZMI *et al*., 2006); and in Israel (HERNÁNDEZ *et al*., 2006).

Specially considering the female agricultural workers, Ibrahim *et al*. (2011) also found increased AST and ALT levels in a population of 35 pesticide exposed women, as compared to a control group of 50 unexposed women. Their findings along with our findings of indicate that pesticide exposure may lead to hepatic impairment in women. Still, Ibrahim *et al*. (2011) found that participation of females in agricultural activities exposed to pesticides have not only a negative impact in their liver functions, but in their reproductive health. In this regard, exposure to pesticides have shown to affect the levels of female hormones.

Pesticides have demonstrated to cause impairment of the reproductive system through disruption of the hormonal balance necessary for proper functioning. In turn, such disruption of hormonal function may be linked, between other possible ways, to pesticide effects on liver since this organ, if healthy, is directly involved in the sexual hormones biotransformation and indirectly involved in the biodisponibilization and transportation of sexual hormones through sex hormone binding globulin (SHBG) production (BRETVELD *et al*., 2006).

Still, since our results showed significantly increased levels of these hepatic enzymes only in women directly exposed to pesticides as compared to the indirectly exposed ones, we analyzed the use of PPE and history of poisonings in the applied questionnaires and no episodes of poisoning were reported in women with high levels of ALT, AST and GGT. However, according to Boedeker *et al*. (2020), the existence of so many underreported cases of acute poisoning by farmers is largely due to the difficulty in accessing emergency services for these individuals, whether due to financial or mobility difficulties, or even confusing the symptoms with other causes, in addition to of mistaken medical diagnosis, inadequate investigation and difficulty in tracking chronic effects.

Therefore, it may be that poisoning occurred, but this was not mentioned by the directly exposed research participants, as well as other study participants, due to the failure to identify the episode of pesticide poisoning. The PPE most used by these women were gloves, so, often without the concomitant use of other PPE such as masks and protective glasses, this may contribute to the increased levels of liver enzymes found in our study.

It is also important to consider that previous studies indicated that women usually have less knowledge about pesticide use and a higher risk of incorrect use of pesticides and acute intoxication (JØRS *et al*., 2013; WANG *et al*., 2017) and also indicated illiteracy and unawareness of the health hazards of pesticide exposure on their reproductive health (IBRAHIM *et al*., 2011). In this regard, it is plausible to consider it as a factor related to our findings of high ALT, AST and GGT found in women occupationally exposed to pesticides since women evaluated in our study reported less use of PPE which reflects unawareness of the health hazards of pesticide exposure.

Finally, a high frequency of elevated total cholesterol cases in both evaluated groups was observed, both presenting mean above the reference levels, which is in accordance with a study investigating the relationship between pesticide exposure and lipid metabolism which reported herbicide exposure as a cause of the change of lipid metabolism, especially on women (YAN *et al*., 2022). It is necessary to ponder that more than a half of the individuals of both studied groups were overweight, so it is more likely to afford for the high cholesterol levels observed.

5 CONCLUSION

Conjunction overall data, this study showed that individuals indirectly exposed to pesticides, although considerably exposed to large amounts of pesticides daily, suffer fewer biochemical changes than directly exposed workers. However, these results do not exclude the need for monitoring these individuals, nor the possibility of significant biochemical impairment due to indirect exposure to pesticides. More studies involving comparison between rural populations directly and indirectly exposed to pesticides should be carried out in other populations, since the exposure profile may differ from each location due to differences in the pesticide load and application frequencies used

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1 Médico. Programa de Pós-Graduação em Oncologia Molecular, Hospital do Câncer de Barretos, Barretos, SP. Brasil. ORCID 0000-0002-1656-6928
E-mail: vinicius.gs2110@gmail.com

2 Bióloga. Doutora em Genética e Biologia Molecular. Universidade Federal de Goiás-UFG, Goiânia, GO. Brasil. ORCID 0000-0002-4669-7603
E-mail: jheneffer.ramos@gmail.com

3 Biomédica. Doutora em Genética e Biologia Molecular. Universidade Federal de Goiás-UFG, Goiânia, GO. Brasil. ORCID 0000-0003-1224-7054
E-mail: thays.millena04@gmail.com

4 Biólogo. Doutor em Genética e Biologia Molecular. Universidade Federal de Goiás-UFG, Goiânia, GO. Brasil. ORCID 0000-0002-8199-8962
E-mail: fnascimentopt@gmail.com

5 Biomédica, Doutora em Biologia Animal. Docente e pesquisadora, Programa de Pós-Graduação em Genética e Biologia Molecular e Programa de Pós-Graduação em Ciências Ambientais. Universidade Federal de Goiás-UFG, Goiânia, GO. Brasil. ORCID 0000-0003-0362-0988
E-mail: danielamelosilva@ufg.br

6 Farmacêutica – Bioquímica, Doutora em Farmacologia. Docente e pesquisadora. Programa de Pós-Graduação em genética e biologia molecular. Universidade Federal de Goiás-UFG, Goiânia, GO. ORCID 0000-0003-1150-3693 Brasil. E-mail: microcha123@ufj.edu.br

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