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HEALTH SCIENCES

Genetic damage among children living in agricultural areas in the North of Colombia

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Abstract: In recent years, the use of pesticides has increased considerably for pest control and to improve agricultural production. The rural areas of several municipalities of department of Cordoba, north of Colombia, are highly dependent on agriculture. In this study, a questionnaire and field observations about pesticide use and genotoxic damage through the comet assay in peripheral blood lymphocytes of children who live near crop fields was evaluated. Damage Index for Comet Assay (DICA) of five children populations exposed to pesticides (mean of 94.73±53.95 for the municipality of Monteria, the higher damage in this study) were significantly Higher than control children population (mean of 7.56±7.39). Results showed the damage index in children exposed group was higher than in the control group. An inadequate management of pesticides, as well as incorrect disposal of toxic wastes was observed in the study zone.

Key words: Pesticides, comet assay, DNA damage, public health.

INTRODUCTION

Pesticides are among the chemical substances most frequently released into the environment due to their generalized use in agricultural and livestock activities. Many pesticides are used to improve food security, the active ingredient can be a single or a mixture of chemical products destined to controlling pests, including vectors of human and animal diseases, as well as undesired species that damage to or interfere with agricultural and forestry Production (Sharma et al. 2019). Exposure to these xenobiotics continues being a substantial human health and environmental problem (Hassaan & El Nemr 2020).

Exposure to pesticides and their adverse consequences to human health is a theme of increasing complexity when considering children populations, who should not be exposed to substances of recognized hazard (Butler-Dawson et al. 2016, Bahrami et al. 2022). The children population is more susceptible to toxic components from the environment, compared to adults, due to physiological and behavioral factors. This includes the fact that their organs and systems, still under development, are more sensitive (especially the nervous and digestive systems), they inhale two times more air than adults; have lower capacity to detoxify and excrete chemical products and are at higher risk of contact due to their behavior and playing habits (Holme et al. 2016). Intoxications through pesticides during childhood and adolescence are cause of intense concern for public health, given that special challenges must be confronted for their treatment, follow up, and prevention (Wang et al. 2018). In addition, organic contaminants, like pesticides can cause long-term adverse effects on the child population, causing cancer (among them possibly, renal), motor, cognitive, and behavioral disorders (Liu & Schelar 2012, Roberts et al. 2012, Martos et al. 2013, García-Pérez et al. 2016). Pesticides have been considered potential chemical mutagens. Experimental results reveal that various ingredients of agricultural chemicals induce mutations, chromosome alterations and DNA damage (Benítez-Leite et al. 2012). The problems mentioned are enhanced in the poorest and most vulnerable sectors in urban and rural settings, including children from families dedicated to subsistence agriculture or who live near pesticide application zones (Tolosana et al. 2009, Rodríguez 2012, Ramírez-Jiménez et al. 2014).

Monitoring of genotoxic damage in human populations is a useful tool to estimate genetic risk from exposure integrated to complex mixtures of chemical substances (Bolognesi 2003, Aiassa et al. 2014). Early detection of genetic damage is important because it permits implementing the necessary measures to diminish or suppress exposure to the deleterious agent, when it is still reversible, thus, diminishing the risk of developing diseases or other alterations of the organism (Aiassa et al. 2012). Specifically, the comet assay has proven to be an excellent tool in detecting the risks to the health of children who live in zones of high levels of chemical contamination (Jasso-Pineda et al. 2015).

Colombia is a country dependent on the agricultural and livestock sector, in 2018 6.3% of GDP was represented by both sectors, these activities are carried out mainly in rural areas with low development (OECD 2020). A considerable amount of extremely, highly, and moderately toxic products are used in this country; around three quarters of these pesticides are classified as slightly toxic (III), while the amount of highly hazardous products accounted for 20–25% (Valbuena et al. 2021). The institution that regulates the use of pesticides in Colombia is the Instituto Colombiano Agropecuario - ICA, they categorize the products based on acute toxicity for humans. In the Cordoba department, pesticide was monitoring and detected in soils, water, fish, and sediments (Lans et al. 2011, Marrugo-Negrete et al. 2014, Cruz-Esquivel et al. 2017, Arteaga Palomo et al. 2018, Lans-Ceballos et al. 2018) and recently in urine samples of farm (Ruiz-Guzman et al. 2017). Pesticides may affect all stages of aquatic life by direct toxic action, or indirectly by contamination of species that serve as food for others, or by producing physical-chemical changes in the aquatic environment (Navis et al. 2013).

The children population in the agricultural zones of the Department of Córdoba, Colombia has always been exposed to pesticides. It is further known that the hygienic conditions in the homes and places where the agrochemicals are applied are deficient, thereby, permitting incorporation of the chemical compounds present not only through inhalation, but also in foods and in the drinking water (Amador et al. 2017). Studies available in the scientific literature have fundamentally focused on the final cytogenetic points to assess the potential genotoxicity of pesticides in populations occupationally exposed, including workers from pesticide factories, pesticide applicators, and agricultural workers. However, few studies have been conducted in children populations residing in zones close to croplands. This research evaluates genotoxic damage through a biomarker of effect (comet assay) to establish the potential risk to public health generated by exposure to pesticides in children's populations (between 5 and 15 years) living close to agricultural crop areas in the department of Córdoba, Colombia.

Experimental part

Study area

The department of Córdoba is located to the north of Colombia, it is crossed by the Sinú River, whose waters are used by the irrigation districts to supply different crops and allows the development of intensive farming where crops of corn and rice predominate (Feria et al. 2010). According to MINAGRICULTURA reports in 2020 the production of corn and rice in the middle and lower valley of the Sinú river exceeded 23.000 and 5.700 ha cultivated, respectively (MINAGRICULTURA 2021). This study was conducted at five rural areas of San Carlos (P1), San Pelayo (P2) Monteria (P3), Cotorra (P4) and Lorica (P5) municipalities denoted as exposed groups; P1, P2 and P3 were in the Middle Valley of the Sinú river, meanwhile P4 and P5 municipalities include the lower valley of the Sinú river. A control group was located in the urban area of the municipality of Monteria were denoted as non-exposed group. The sampling sites are represented in figure 1. Aerial spraying of pesticides were observed in P2 and P4 municipalities, on the other hand, the presence of schools near farmland was common in all municipalities. A previously report from our research group evidenced the exposure of children from this area to Atrazine pesticide by biomonitoring in urine samples (Ruiz-Guzmán et al. 2017).

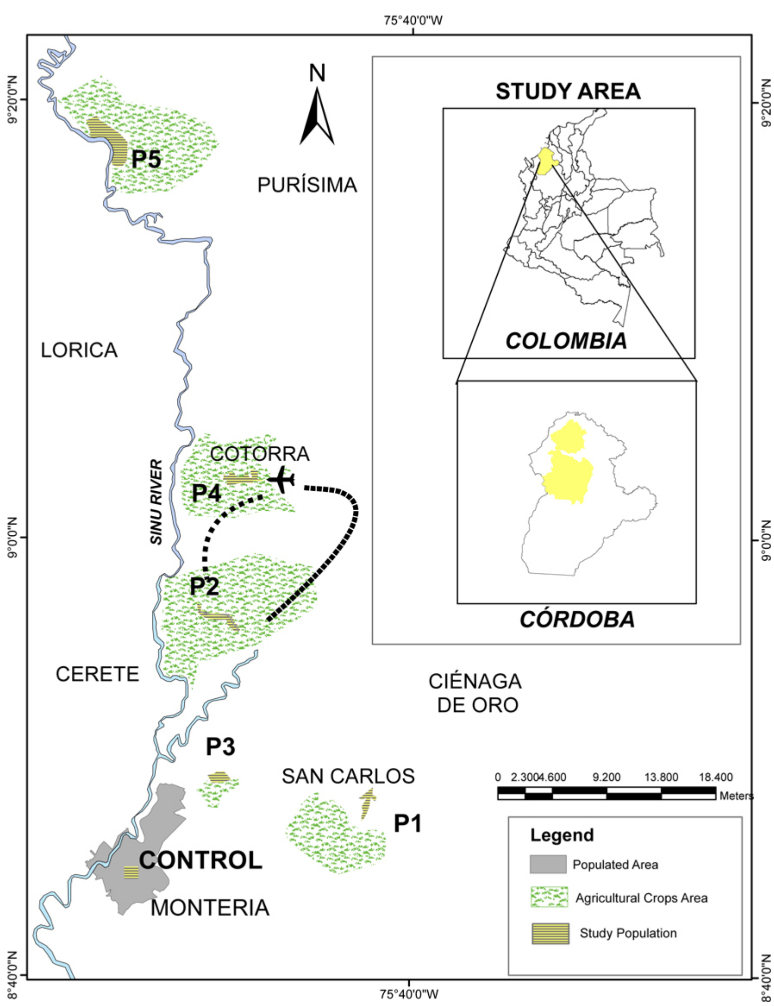

Figure 1. Study area and municipalities where the research was carried out.

Study population

All the procedures were approved by Ethical Committee for Research of the Cordoba University of Colombia, it was accord to Resolution 008430 of 04 October 1993 by the National Health Council, articles 15 and 16, with prior informed consent involving the participants and their parents at the beginning of the study. All the parents and legal guardian of children signed an informed consent declaring your willingness to participate, after trained personal of research explaining the study protocol.

The population selected corresponded to children between 5 and 15 years, without hereditary family history of genetic alterations, chronic diseases, medical or pharmacological treatments. The parents or legal guardians, members of the household answered the questionnaire suggested by the International Commission for protection against carcinogens and environmental mutagens (Da Silva 2012). These included demographic aspects (age, gender, weight), occupational (type of labor activity of the parents, occupational history, closeness to agricultural zones), scholarship of the parents, smoking habits of the parents, pesticide application frequency and storage.

Collection of blood samples

A total of 143 samples of which 119 samples corresponded to exposed group and 24 samples were non-exposed group. Total blood samples for the Comet Assay were taken from all study participants through extraction via venipuncture with vacutainer tubes added with Heparin (Singh et al. 1988). The samples were covered with aluminum paper, refrigerated between 18 and 23 °C, and stored for a maximum time of 4 h until their analysis.

Comet alkaline assay

This test was done according to the original methodology (alkaline version) described by Singh et al. (1988), with some modifications (Da Silva et al. 2008, Cruz-Esquivel et al. 2019). For the preparation of the samples, 10 μL of isolated lymphocytes was taken with the Ficoll-1077 gradient technique (Histopaque-1077™), following the manufacturer instructions (Singh et al. 1988), and mixed with 75 μL of low melting point agarose. This mixture was placed on a slide that was previously covered with the first normal melting point agarose layer; the slides were immersed in a lysis solution; at this point, lysis of the cell membranes occurred and the released DNA was subjected to electrophoresis under controlled conditions. Subsequently, the slides were placed in an electrophoresis chamber containing an alkaline solution for 30 min. They were electrophoresed for 30 min at 25 V and 300 mA to evaluate the migration (tail of the comet). The slides were washed with a 0.4 M Tris neutralizing solution. Finally, the samples were stained with an ethidium bromide solution (2 μg/mL) and observed in the dark with a fluorescence microscope equipped with a 505–560-nm excitation filter and ×40 magnification.

We quantified 100 cells per sample of each individual, 50 in each replication. The number of cells at each damage level was determined, with the following levels: level 0, absence of damage (without migration of fragments, without tail); level 1, with slight damage (tail length less than the diameter of the nucleoid); level 2, with moderate damage (length of the tail greater than the diameter of one nucleoid, but less than the diameter of two); level 3, with a high degree of damage (tail length greater than the diameter of two nucleoids and less than that of three); and level 4, with an extremely high degree of damage (tail length greater than the diameter of three nucleoids).

This classification was determined according to the visual classification system (Collins et al. 2008, León-Mejía et al. 2011) and was expressed as the damage index for Comet Assay (DICA), taking into account the following formula:

$$DICA = n1 + 2 \times n2 + 3 \times n3 + 4 \times n4,$$

where n is the number of cells classified in each category of damage. The length parameters of the tail (μm) and its percentage of DNA in the tail were analyzed by an image analysis program, Comet Score.

Statistical analysis

The STATISTICA 7.0 statistical package was used for the statistical analysis. After the normality analysis and homogeneity of variances of original and transformed data (Shapiro-Wilk test and Bartlett's test negative in some cases), data were treated through non-parametric tests. The Mann-Whitney U test was applied to compare the results of the comet assay in the study groups with respect to the control. For all cases, $p < 0.05$ was taken as significance criterion.

RESULTS

Study population

The socio-demographic characterization of the studied populations is presented in the Table I, the overall average age of all children was 9.8 years, 70 children were male and 73 females. Only 11% of the parents were smokers, most of them presented a low level of education (78.5% of fathers and 68.5% of mothers did not finish high school). On the other hands, 89% of the mothers were dedicated to housework, while 47% of the fathers carried out agricultural activities, in the

Table I. Socio-demographic characteristics of the study population.

Groups		P1	P2	P3	P4	P5	Control	All Groups
		Mean (Range)						
Age (years)		10.8 (7-13)	10.1 (5-15)	9 (5-15)	10 (8-14)	10.6 (6-14)	8.2 (6-12)	9.8 (5-15)
Height (cm)		131.7 (90-152)	127.4 (99-170)	141.4 (110-170)	125 (115-178)	130.5 (110-160)	124 (110-150)	130 (90-178)
Weight (kg)		31.6 (20-42)	28 (15-56)	36.7 (22-63)	34.5 (19-58)	30.7 (19-57)	28.9 (23-35)	31.7 (15-63)
		n (%)						
Genre	Masculine	11 (52.4)	14 (46.7)	18 (56.3)	1 (14.3)	14 (48.3)	12 (50)	70 (49)
	Femenine	10 (47.6)	16 (53.3)	14 (43.7)	6 (85.7)	15 (51.7)	12 (50)	73 (51)
Mother's occupation	Howswife	21 (100)	30 (100)	31 (96.9)	7 (100)	29 (100)	9 (37.5)	127 (88.8)
	Other	0(0)	0 (0)	1 (3.1)	0 (0)	0 (0)	15 (62.5)	16 (11.2)
Father's occupation	Agriculture	3 (13.6)	26 (86.7)	8 (25)	7 (100)	23 (71.9)	0 (0)	67 (46.9)
	Other	18 (86.4)	4 (13.3)	24 (75)	0 (0)	6 (28.1)	24 (100)	76 (53.1)
Mother's education	Incomplete secundary or below	11 (52.4)	27 (90)	23 (71.9)	7 (100)	29 (100)	1 (4.2)	98 (68.5)
	Complete secundary or above	10 (47.6)	3 (10)	9 (28.1)	0 (0)	0 (0)	23 (95.8)	45 (31.5)
Father's education	Incomplete secundary or below	9 (42.9)	21 (70)	28 (87.5)	7 (100)	29 (100)	11 (45.8)	105 (73.4)
	Complete secundary or above	12 (57.1)	9 (30)	4 (12.5)	0 (0)	0 (0)	13 (54.2)	38 (26.6)
Parents' smoking status	yes	1 (4.8)	2 (6.7)	2 (6.3)	3 (42.9)	5 (20.8)	3 (12.5)	16 (11.2)
	no	20 (95.2)	28 (92.3)	30 (92.7)	4 (57.1)	25 (79.2)	21 (87.5)	127 (88.8)

control group no father or mother performed agricultural activities. Although not shown in Table I, all the children were natives of the study site, and their length of stay was determined by their age.

Field observations

One of the most important observations reported by the interviewers was the reuse of pesticide containers in households. On the other hand, the presence of schools near farmland was common in all municipalities. When asked the name of commercial pesticide products, parents who worked in agricultural activities did not associate them with any, with some exceptions, where the answers were "baygon" and "clorpirifos", however, in all cases they reported that they could perceive the smell of the "poison", although they did not recognize which product is being applied, they are aware of the presence of the pesticide. Some interviewers from San Pelayo (P2) and Cotorra (P4) municipalities reported aerial spraying in crop fields.

Genotoxic damage

Table II report the values of Damage Index for Comet Assay (DICA), tail length and %DNA in tail of exposed and control groups. The DNA damage was evident, showing a statistically significant difference between the exposed and control groups ($p < 0.05$) for DICA and %DNA in tail. We observed for tail length that P3 and control group was similar. The cell viability evaluated was in all cases above 85%, meaning that the samples were suitable for analysis. The highest values of DICA were registered from participants of P2 municipalities with 94.73±53.95 followed by P4 with 83.33±26.93; between exposed groups P3 registered the lowest DICA values with 25.94±31.34. % DNA in tail was higher in children blood samples of P5 municipality with 19.5±3.4%, being two-fold than in non-exposed group which reported 10.4±3.0 %.

DISCUSSION

The comet assay has proven to be a sensitive method to detect the genotoxicity of chemical products and complex mixtures, as well as biomarkers in the biomonitoring of human populations exposed to environmental pollutants (Ansoar-Rodríguez et al. 2015) and in the population exposed to pesticides (Valencia-Quintana et al. 2021). The results from this study

Table II. DICA, tail length and %DNA in tail values in peripheral blood lymphocytes of children who live near crop fields in different municipalities from the department of Cordoba, Colombia. Different letters indicate significant differences between groups (Mann-Whitney U-test, p < 0.05).

	Mean ± Standard Deviations (Minimum–Maximum)					
	control group (n=24)	Exposed groups				
Parameter		P1 (n=21)	P2 (n=30)	P3 (n=32)	P4 (n=7)	P5 (n=29)
DICA	7.56±7.39 b (0.00-29.00)	55.78±46.16 a (10.00-223.00)	94.73±53.95 a (8.00-229.00)	25.94±31.34 a (0.00-168.00)	83.33±26.93 a (51.00-125.00)	44.52±23.36 a (0.00-94.00)
Tail lenght (μm)	28.9±17.6 b (6.7-67.0)	69.5±50.3 a (20.4-234.9)	99.9±68.6 a (30.7-350.8)	40.7±22.8 b (10.5-99.9)	136.9±42.9 a (81.0-196.2)	59.4±21.1 a (18.3-102.7)
% DNA in tail	10.4±3.0 b (7.1-20.3)	18.8±7.2 a (11.6-41.1)	18.9±5.9 a (10.3-27.9)	16.9±5.1 a (8.0-28.2)	16.9±3.5 a (11.08-21.46)	19.5±3.4a (13.7-27.4)

reveal that populations of children living near agricultural zones present alterations in their DNA, with these being statistically higher ($p < 0.05$), compared to the control group accordingly of DICA and %DNA in tail.

This damage may be due to the exposure to pesticides used to control pests in crop zones. In addition, the hygienic conditions in the homes and sites where the agro-chemicals are applied are deficient, thereby, permitting the incorporation of the chemical compounds present not only through inhalation, but also adhered to foods (Amador et al. 2017). Several pesticide components produce alterations in the genetic material in adults and children, with this being one of the principal problems in agricultural zones (IARC 2002), given that pesticide concentration in the environment is higher in agricultural communities and near the fields treated with pesticides (Teske et al. 2002, Weppner et al. 2006).

In Latin America, farming families work with their children in the fields, apply pesticides in their presence, store these toxic substances in their homes, and children even help adults in these tasks (DNP 2008). The study population in these sites lives very close to crop areas (Approx. 1-100 m) where herbicides are applied to control weeds in corn, cotton, and rice crops prevalent in those zones. Additionally, the information consigned in the questionnaires on the study population revealed that many of the pesticides used in the crop zones are stored in the homes where the children live. In addition, unawareness of the effects of these chemical substances in human beings and the poor disposal of residues spreads practices, like storage of drinking water in discarded pesticide containers, which increases the degree of exposure. In most cases, the children's parents are pesticide applicators and the elements used for this activity, besides not being adequate for this work, are brought to their homes with pesticide residues to be washed.

We observed that families visited in this study do not carry out an adequate management of pesticides, the reuse of pesticide containers, lack of knowledge of trade names could suggest that they do not read the labels and the hazard warnings on them, on the other hand, the low level of education in the parents could be a factor that increases the risk of children.

This is reflected on their bad practices with respect to the use, management, storage, and final disposal of agro-chemical containers, like abandoning the containers in vacant lots or in the backyards of the homes, storing them in the homes of the applicators, and not using protection equipment during their workday. It should be stressed that this study also detected reuse of empty containers to store water, food, and other products of human and animal consumption.

Different studies have demonstrated that the pesticide levels in the dust in the houses are associated to the proximity of the dwellings to agricultural fields treated with agricultural chemicals and with the para-occupational paths, given that agricultural workers inadvertently bring pesticide residues into the home in their garments, boots, skin, and hair (Butler-Dawson et al. 2016).

Additionally, many applications of agro-chemicals are aerial applications, implying greater dispersion of the contaminant that can be inhaled, or easily reach water and food sources, which are then consumed by the population.

Several studies conducted in countries like Brazil (Ristow et al. 2020), Cuba (Dávila et al. 2019), Costa Rica (Barraza et al. 2011) and Mexico (Polanco Rodríguez et al. 2015) about practices, knowledges, and risk perception on pesticide handling of farm families evidenced similar cultural, social, and educational troubleshooting's found in this study.

With the parameters analyzed in the comet assay, DNA damage of the population exposed was evident. Diverse epidemiological and bio-monitoring studies have evidenced DNA damage generated by exposure to these compounds in agricultural zones (Bernieri et al. 2020, Ramos et al. 2021). Garaj-Vrhovac & Zeljezic (2000), in a study conducted in a population exposed to pesticides, showed the degree of damage to DNA through the comet assay, manifesting an increase de length of tail and moment of the tail compared to the control group. This suggests that long-term exposure can cause significant increase in DNA damage. Similarly, Laborde et al. (2006) conducted a clinical evaluation and of genotoxicity biomarkers in a population of children and adults exposed to multiple pesticides, revealing high prevalence of respiratory and cutaneous diseases in children, obtaining a high tendency to genetic damage in children and women, with increased DI with respect to the control group. These results would be similar to the DI reported in our study, which was also high in children exposed that in the control; these would also agree with the concept of higher infant vulnerability. Furthermore, children have greater exposure to pesticides due to behaviors, like dragging themselves to play on the floor and putting their hands in their mouths more frequently (Butler-Dawson et al. 2016).

In addition, in the province of Córdoba, Argentina, Peralta et al. (2011) evaluated genetic damage in inhabitants of the city of Marcos Juárez exposed to pesticides via labor or environmental means. To obtain an evaluation of the potential effect of the exposure a battery of biomarkers (genotoxicity assays) was used, finding significant differences in DNA damage, through the Comet Assay, for inhabitants environmentally exposed with respect to the control group. Likewise, Bernardi et al. (2015) report genotoxicity in children who live in the same city at different distances from the pesticide zones compared to children from the city of Río Cuarto (Córdoba, Argentina), which are not considered exposed to pesticides. The authors found significant differences in genotoxicity between the children exposed who live less than 500 meters from the zones subjected to fumigation and those who were not exposed. Forty percent of the children exposed endure some type of persistent condition, which could be associated to chronic exposure to pesticides, these results indicate the genotoxicity of the group exposed compared to the control group.

The findings in the present study evidence the importance of the tests used for the early detection of an increased risk of developing diverse pathologies, being able to attribute such to the pesticide exposure reported by the study's participants.

CONCLUSIONS

The children population residing in zones near agricultural crops in the department of Córdoba, Colombia is exposed to pesticides used in the study area, likely causing genotoxic damage. Probably, the DNA damage is associated to bad practices in the use of these substances during their application and final disposal of the residues increases exposure to these pesticides and genetic damage at an early age. This is of concern because it can affect infant development and generate possible diseases in the short and long term, causing a public health problem.

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