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GENOTOXIC BIOMONITORING AND EXPOSURE TO PESTICIDES IN WOMEN LABORERS AT MANEADERO VALLEY IN BAJA CALIFORNIA, MEXICO

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ABSTRACT

Objective: Assess the effects to genetic material (DNA) caused by occupational exposure to pesticides in women from Maneadero Valley, an important agroindustrial region in Baja California, Mexico, using genotoxic bio monitoring. Methodology: 48 women signed their informed consent. Twenty-six placed in the exposed group and 22 in the control group. Socio-demographic information was collected by questionnaire and DNA damage was assessed using cytokinesis block micronuclei assay. The nuclear index and three biomarkers of damage were identified: micronuclei, nuclear buds and chromatin bridges. Cluster analysis was used to explore the relationship between variables and the Mann-Whitney U Test enabled the analysis of differences between the groups. Results: The Mann-Whitney U Test revealed that women exposed to agricultural chemicals have significantly greater frequencies of micronuclei ($p < 0.05$) compared to the control group. Nuclear index and chromatin bridges differences were not statistically significant. The cluster analysis showed a strong relationship between micronuclei and exposure. Discussion: These results suggest that genotoxicity is associated with occupational exposure to agrochemicals. Environmental exposure can be considered a modifying variable in the risk of genotoxicity from exposure to pesticides.

KEYWORDS: Genetic Damage, Genotoxicity, Micronuclei, Occupational Exposure, Pesticide, Women Farm Workers

INTRODUCTION

There is a close relationship between intensive agricultural activity and the use of pesticides and fertilizers to improve crop yield. The cost of these consumables sometimes surpasses 80% of the production investment [1]. Besides employee salaries, these investment costs neither include nor specify the current and future health effects on farm laborers, despite diverse studies that reveal the potential harmful effects on humans caused by agrochemicals [2].

In the realm of public health, human exposure to agrochemicals constitutes a worrisome problem. Research into this issue assesses the health effects on those persons who are exposed on the job at different levels, degrees and types of exposure. However, in Mexico, just as in other countries around the world, the majority of studies carried out to determine health effects from exposure to agrochemicals have focused on acute effects, with limited research on the chronic effects such as a decrease in the stability of genetic material (DNA) that can trigger illnesses such as diabetes mellitus, heart diseases, respiratory diseases, leukemia and other types of cancer such as congenital malformations, and neuropathy, among other ailments [3-5].

The effect of these substances on human health, in particular damage to DNA, has been measured through the use of techniques such as the cytokinesis block micronuclei assay (CBMN). This technique is validated internationally, is widely used and is considered an effective biomarker for measuring DNA damage due to exposure to toxic substances [6].

The risk to human health from exposure to agrochemicals is generalized and their effects can be found in the public at large. Nevertheless, this risk is magnified among people who are exposed on the job, such as agricultural workers.

In Mexico, the growing demand for cheap labor for an agroindustry aimed at exports and the deterioration of the traditional agriculture of self-sufficiency have prompted the migration of Amerindian peoples from southern Mexico to northwest states. The majority face social exclusion, poverty, marginalization, unstable working conditions, scarce or no access to healthcare and low wages that entail dietary and educational deficiencies. One must add an exponential risk to exposure to agrochemicals which are used with little or at times no technical consulting, translating into harm to men and women farm workers' health, both in the short and long term [7].

Among women, the above outlook is further complicated because it is not limited to just occupational exposure to agrochemicals. One must look at the physical wear and tear involved in what could be called a double shift, since once her fieldwork is finished, she must engage in household chores that her cultural role obligates to complete. Thus, the incorporation of women in agricultural jobs in Baja California entails greater current and future health risks for women farm workers [8].

METHODOLOGY

A transversal descriptive monitoring study was carried out on 48 women residents of a town located 5 km from Maneadero Valley, which is considered an important agroindustrial zone found in Baja California State in northern Mexico. After informed consent was given, these women responded to a survey and provided a blood sample which was analyzed to determine their level of genotoxicity.

The women were assigned to two groups based on the presence or absence of occupational exposure to agrochemicals. There were 26 women in the exposed group and 22 in the control group. The criteria for inclusion in the exposed group were that the women resided in the study area when the sample was taken, worked (or had worked) in the fields in the last five years for at least one year straight, gave informed consent to participate in the study which included giving a blood sample and answering a survey, and had not had chemotherapy or radiation therapy in the six months before blood was taken. The criteria to be included in the control group were the same with the exception of working in the fields in the last five years for at least one year straight. Those women who did not fulfill the criteria to be included in the study and those who did not complete the survey were excluded.

The level of genotoxicity of exposed and control group members was assessed using the cytokinesis block micronucleus technique in peripheral blood lymphocytes [9], a test standardized between 2007-2008 by the Autonomous University of Baja California's environmental toxicology laboratory. The biomarkers for damage were: nuclear index, micronuclei, chromatin bridges and nuclear buds [6].

Using heparinized Vacutainer® tubes, 5 ml of blood was drawn from each donor and samples were transported in a cooler that contained cold gel packs to the laboratory, where cultures of each sample were carried out.

The culture was done in 15 ml conical tubes into which 6.3 mL of supplemented medium (RPM1-1640 SIGMA) was added at 37 ºC with 0.2 mL of phytohemagglutinin(PHAM de SIGMA L-8902) and 0.5 mL of blood. The tubes were incubated at 37 ºC for 48 hours. Then the cytokinesis block was carried out adding 3 µL/mL of cytochalasin-B (SIGMA C-6762) to each sample. After an additional 24-hour period at 37 ºC, we harvested lymphocytes, beginning with the resuspension of the sample having preset it with 1 ml of pure methanol and glacial acetic acid setting solution (3:1). Using this same solution, we then eliminated organelles and impurities from the sample until obtaining a brown, spongy cellular bud from which we obtained microscope slides previously labeled with the sample details (donor code, place and date).Staining was done using eosin and methylene blue and we then counted cellular proliferation and the frequency of biomarkers of damage. The cellular proliferation index and frequency the number of micronuclei, chromatin bridges and nuclear buds in a sample of 1000 bi-nuclear cells was obtained by tallying [6]

To obtain descriptors such as frequency and measures of central tendency, a basic statistical analysis of the variables obtained from the survey was carried out. The laboratory results were analyzed using a Mann-Whitney U test which uncovered significant differences among biomarker frequency tallied in 1000 bi-nuclear cells.

Using the information from the surveys, six indicators were constructed to more homogenously group information: workplace safety, self-care at work, state of health, dietary quality, habits and reproductive health. To establish the level of association among indicators and genotoxic biomarkers, using Statistics 7.0® we carried out a cluster analysis using the Ward method and Pearson's correlation coefficient 1-r as an indicator of distance among variables.

RESULTS AND DISCUSSIONS

The mean age of the participants in the study was 40.8 years old. The majority are either married (48%) or in a common-law union (27%) with their partner. Among exposed women, the average time working in the fields was 10.8 years. The majority were born in Oaxaca (31%) and Sinaloa (23%).Only 13% were originally from Baja California. The remaining participants come from other Mexican states such as Nayarit, Jalisco, Guanajuato, Guerrero, etc. When considering language spoken in addition to Spanish, while only two women in the control group spoke an Amerindian language, nearly half of the women in the exposed group spoke Mixtec.

Using the information from the surveys, six indicators were constructed to more homogenously group information on: workplace safety, self-care at work, state of health, dietary quality, habits and reproductive health.

The biomarkers for exposure were: the presence of micronuclei (MN), chromatin bridges (CB), nuclear buds and mean cellular proliferation or nuclear index (NI).The first are genotoxicity indicators and the last is a cytotoxicity indicator [6].

The exposed group had a mean of 10 MN, two CP and eight buds, while these means were eight, three and six for the control group, respectively, as shown in Figure 1.

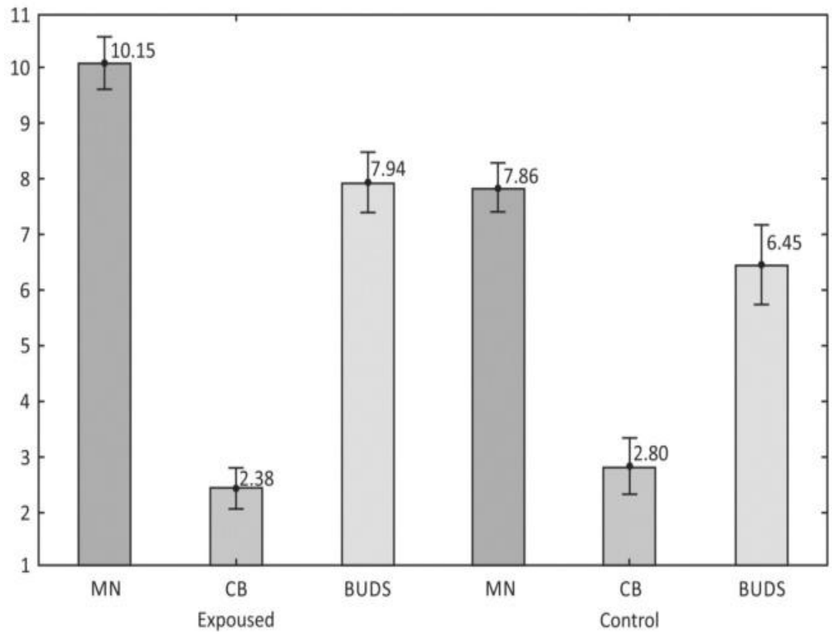

Figure 1: Biomarkers of Damage for Exposed and Control Groups

The Mann-Whitney U test revealed statistically significant differences between the exposed and control groups for buds and micronuclei, a biomarker that more conclusively detects genotoxicity because it reflects the loss of genetic information during cell division. Nuclear index and chromatin bridges had no statistically significant differences (Table 1).

Table 1: Values of Significance for Cellular Proliferation and Biomarkers of Damage

Biomarker	Mean	Standard Deviation	Standard Error	Mean	Standard Deviation	Standard Error	P*
	Experimental Group			Control Group			
NI	1.71	0.12	0.02	1.74	0.13	0.03	0.42
MN	10.15	2.43	0.48	7.86	2.10	0.45	8.08E-5
CB	2.38	1.92	0.38	2.80	2.40	0.51	0.67
BUDS	7.94	2.82	0.55	6.45	3.46	0.74	0.03

*$p \leq 0.05$ show significant differences between experimental and control group

Relationship among Variables

A cluster analysis using the Ward method and Pearson’s correlation coefficient 1-r as a measure between variables was carried out to establish a relationship between genotoxicity biomarkers and the indicators workplace safety, workplace self-care, state of health, quality of diet, reproductive health, habits and lifestyle, which had been grouped based on survey responses. This analysis revealed two main groups of related variables (Figure 2). Group A, related at a distance of 1.4 units, clustered the following variables: state of health, which is related to the cellular proliferation index, both of which in turn are related to diet as well as workplace safety and self-care. The second group of variables (Group B), related at a distance of 1.5 units, showed a strong relationship between micronuclei and exposure while habits and lifestyle associated with reproductive health, which in turn were related to chromatin bridges and buds.

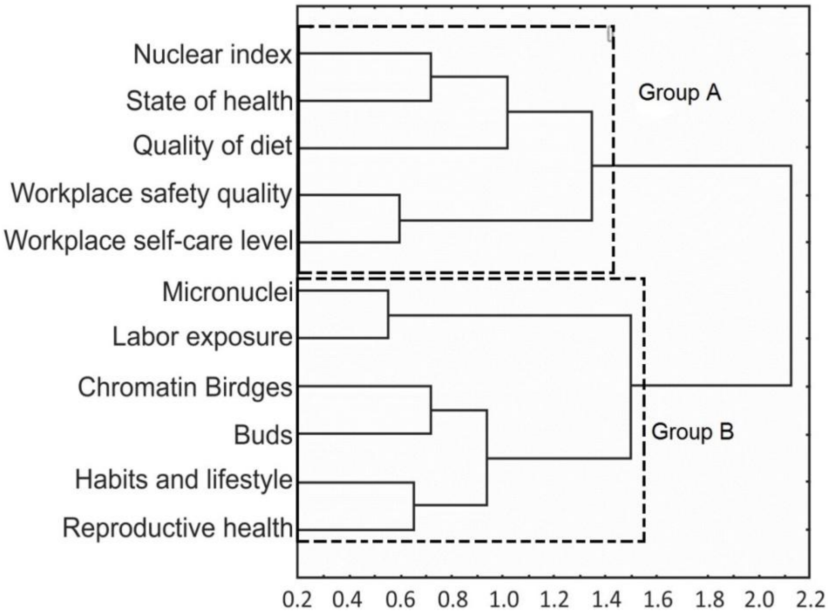

Figure 2: Dendrogram Showing the Relationship between Social, Work, Dietary and Health Factors and Genotoxicity Biomarkers

The cytogenetic analysis shows that the group who are exposed to pesticides at work have significantly greater genotoxicity than that of the control group, even with similar socioeconomics, health and diet. This finding coincides in part with that of other study that found association among genotoxicity with both occupational and environmental exposure to pesticides among San Quintin Valley farmworkers [10]. To confirm this, we did a cluster analysis of the different socio-environmental variables and genotoxicity biomarkers. We discovered a strong relationship between the variable of occupational exposure and micronuclei, which allows us to point to occupational exposure to pesticides as the likely determiner of differences between groups.

Just as in the present research where occupational exposure to agrochemicals showed a significant increase in the number of micronuclei as compared to the control group, in various other studies a relationship between occupational exposure to different agrochemicals and an increase in the number of micronuclei is documented. According to the literature review of Martinez-Valenzuela, et al [3], of the 50 papers they reviewed, 72% found a statistically significant relationship between biomarkers for damage and agrochemical exposure.

Other variables such as age and sex are demographic factors that can be considered important confounding factors and can interfere in the frequency of micronuclei. In this study, we did not observe a significant relationship between age and an increase in the number of micronuclei. Since this study involved only women, sex did not constitute a confounding factor [11].

The women in this biomonitoring study report keeping a balanced diet including all the food groups, thus guaranteeing the daily allowance of folic acid found in green vegetables and vitamin B12 found mainly in animal proteins. Certain studies show the importance of nutrition for metabolism and DNA repair [12-14] as well as the importance of consuming folic acid and vitamin B12, nutrients that play a role in preventing chromosome breakage and in DNA repair. In this study, it is likely that the dietary quality of the individuals in the exposed and control groups keeps the frequency of micronuclei from surpassing a mean of 8.8. ± 5.2 MN/1000 bi-nuclear cells for a healthy person, as referenced by

Fenech [15]. Some of the women also reported taking dietary supplements, thus possibly modifying their daily intake of vitamins. This result is consistent with other studies wherein taking vitamins, including folic acid, has been associated with preventing an increase in cytogenetic alterations [12, 16].One noteworthy point is that the population has high accessibility to a wide variety of foods because the area under study is in proximity to supermarkets and local markets, where one can find affordably priced fruits and vegetables, a factor that contributes to a good diet.

The women from Maneadero rarely engage in tobacco and alcohol use as none smoke and only two occasionally consume alcohol. For this reason, these were not considered confounding factors in the relationship between micronuclei frequency and pesticide exposure [17].

Workplace safety measures taken by employers are insufficient and at times very deficient even though the employer must provide protective equipment and training pursuant to rules and regulations. The reality that these women face differs greatly from the conditions as set forth by rules and regulations, a reality that prevails not just for the women in this study but also for farmworkers across Mexico and the world [18, 19].Although the women have reported that certain measures to reduce exposure have been implemented, such as availability of drinking water, disposable cups and hand washing stations, these are only baby steps when compared to the lack of protective equipment and the poor or non-existent training in pesticide use [20]. This and the insufficient self-care measures taken by the women combine to increase the exposure to genotoxic agents and the risk of suffering damage to genetic material.

In terms of reproductive health, the majority are in good health. However, 12 of the 48 women in the study have had a miscarriage. Of these 12, seven were in the exposed group and five in the control group. Of the exposed group, six reported having gone to work while pregnant, and of the control group, two reported working while pregnant, in other words, eight of the 12 women who miscarried had worked the fields while pregnant. Some studies indicate that there is an important relationship between agricultural work and miscarriage [21], where the frequency of miscarriages among occupationally exposed women could be significantly higher than that of women who are not exposed. Other studies indicate that physical stress and wear and tear that agricultural work entails could also be factors associated with miscarriage [22] that may well be associated with the miscarriage rates among the women in this study since the majority of them worked intense shifts of eight hours or more, six or seven days a week. In addition to what has already been laid out, we must add that of the 12 women with a history of miscarriage, three are second-hand smokers and one an ex-smoker, another variable that could potentially be linked to miscarriage among this group, since studies have reported that smoking and second-hand smoke are factors that could significantly raise the risk of miscarriage [23].

CONCLUSIONS

We conclude that farm worker women who are exposed to pesticides at work has significantly greater genotoxic damage than the ones in the control group, even with similar socioeconomics, health and diet. The strong relationship between the variable of occupational exposure to pesticides and micronuclei, allows to point that occupational exposure to pesticides is a factor to determinate the differences between groups.

The employer's efforts to reduce exposure are only little steps when compared to the lack of protective equipment and the poor or non-existent training in pesticide use for those women. In addition, the insufficient self-care measures taken by the women combine to increase the exposure to genotoxic agents and the risk of damage to genetic material with possible trans-generational effects.

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