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Differential Gene Expression Analysis on Workers Exposed to Pesticides: Genetic Damage and Cancer Susceptibility

Fábio Morato de Oliveira[1*], Caio Fernando de Oliveira Pereira Passos[1], Hellen Miranda Campos[1], Gabriella Leite Sampaio[1], Leandro Hirata Mendes[1], Wagner Gouvêa dos Santos[1], Aline Monezi Montel[1], Tamara Rodrigues Lima Zanuzzi[1], Edlaine Faria de Moura Villela[2]

[1]Laboratory of Genetics, Federal University of Jataí, Jataí, Brazil
[2]Secretary of Health, Government of the State of São Paulo, São Paulo, Brazil
Email: *fabiomorato@ufj.edu.br

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Abstract

We performed aurora kinase gene expression analysis, in combination with FISH and micronucleus test, in buccal mucosa cells isolated from agricultural workers to better understand the dynamics of genomic instability in these groups of professionals. Two out of a total of 97 agricultural workers who consented to participate in the study (39 men and 58 women) reported a history of cancer based on a previously applied questionnaire. The participants were 28.4 years on average and were categorized into three distinct groups based on their habits, as the "not exposed" group included 24 samples analyzed. The group classified as "indirectly exposed" consisted of 21 samples and those classified as "directly exposed" regarding their activities involved in pesticide preparation/application included 52 samples. Our results showed significant differences in the expression of *AURKA* and *AURKB*, among the groups ($p < 0.0001$). The differences found were also confirmed by FISH analysis, evidencing the amplification of the studied genes. To support our data, we also performed micronucleus analysis and found significant differences when comparing the three distinct groups of participants, even if some individuals not directly exposed to pesticides showed cell abnormalities. The results suggest possible ongoing DNA damage, which on a temporal scale could promote genomic instability, and ultimately, tumor development. We believe that biomonitoring strategies, including the analysis of the expression levels of the *AURKA* and *AURKB* genes, may provide important insights into the evolution of genomic instability in the cells of agricultural workers exposed to pesticides.

Subject Areas

Biotechnology, Cell Biology, Genetics, Genomics, Molecular Biology

Keywords

Pesticides, Genomic Instability, Gene Expression, Aurora Kinase, FISH, Micronucleus

1. Introduction

To increase food production to attend the growing demand, the use of pesticides has grown worldwide. Over the years, there have been increased production demands for the effective extermination of pests (undesirable plants, animals, or microorganisms) that may compromise production [1]. On the other hand, studies on workers exposed to pesticide [2] [3] [4], along with animal models of pesticide toxicity showed how these chemicals can be responsible for detrimental effects on health [4]. Considering that for an increased number of hereditary and multifactorial diseases, congenital pathologies, malformations, and carcinogenesis are often seen in ecologically unfavorable regions [5], the chronic effects caused by continuous long-term exposure to pesticides have not been characterized properly [6].

Genotoxic damage caused by pesticides in human DNA has been the target of various studies [7]. Pesticides are reactive substances that can cause changes in genome, being recognized as carcinogenic [8]. The study of the molecular mechanisms able to mediate the effects of pesticides over genome is of great importance to better understand how cells respond to toxicity and try to neutralize the action of certain chemicals. An important approach aimed to investigate different mechanisms by which pesticides could have an impact on human genome, altering gene regulation has been developed [9]. Some molecular functions, including DNA methylation, histone modifications, and microRNA (miRNA) expression, can change genome function under exogenous influence, such as environmental pollutants. Epigenetic changes may mediate specific mechanisms of toxicity and responses to certain chemicals [10].

Although many investigations reporting the relationship between pesticides and cancer have been published so far, [11]-[15] to date some results obtained from epidemiological studies have been inconsistent [16]. According to epidemiological evidence, for agricultural workers, supposed to be more exposed to pesticides than other workers subgroups, the cancer risk seems to be lower than expected [17]; the mortality is lower for esophagus, lung, bladder, and colon cancer. On the other hand, for some specific cancers is possible to find a higher incidence than expected [17]. A strong epidemiological association has been found between hematological malignancies and pesticide exposure. In addition, some types of cancer have been associated with various degrees of pesticides ex-

posure (*i.e.*, soft tissue sarcoma, Hodgkin's and non-Hodgkin's lymphoma, stomach, brain, prostate, pancreatic, breast and ovarian cancer [18] [19] [20].

Under the perspective of the carcinogenesis induced by pesticides, aurora kinase genes (*AURKA* and *AURKB*) play a critical role in mitosis by regulating centrosome duplication, bipolar spindle formation, alignment of chromosomes on the mitotic spindle, and the mitotic checkpoint [21]. High expression of aurora kinase genes has been implicated in many types of neoplasia including breast, gastric, colon, ovarian, liver, non-small cell lung, uterine, esophageal and leukemias [22] [23]. In this context, the measurement of aurora kinase mRNA may be better than protein as a potential carcinogenic "biomarker" since it provides benefits considering efficient utilization of small tissue amount and simplified sampling procedure for field conditions [24]. Further, several biomarkers can be measured at an individual level within the same isolated RNA sample. This strategy may represent an important tool for evaluating the initiation and maintenance of a neoplastic process [24].

Biomonitoring studies, focusing on genomic abnormalities, have been performed in potentially exposed agricultural workers to better elucidate the risk associated with exposure to specific compounds, and the eventual consequences of it [25]. Nevertheless, this exposure usually involves complex mixtures of pesticides belonging to different chemical classes varying with the type of crop. To the best of our knowledge, this is the first report where gene expression analysis of *AURKA* and *AURKB* are performed in parallel with FISH and micronucleus test using buccal mucosa cells from agricultural workers.

2. Material and Methods

2.1. Samples

This study was conducted using samples from buccal epithelium cells extracted from agricultural workers in a city located in the Midwest of Brazil. All samples were collected in rural schools, between July 2017 and November 2019, and all agricultural workers signed a consent form to participate in the study. All participants completed a questionnaire consisting of demographic information and general habits, such as alcohol consumption, smoking habits, family history for cancer, use of personal protective equipment (PPE), and time working in agriculture settings. This study was previously submitted to the *Research Ethics Committee* of the Federal University of Jataí, Goiás. The study was subsequently approved by the *Brazilian National Committee on Research Ethics* (proc. n.: 51493215.3.0000.5083).

For the investigation, ninety-seven samples were collected and divided into three groups before analysis as follow: Group *directly exposed to pesticides*, Group *indirectly exposed to pesticides*, and Group *not exposed*. The criteria used to group the samples considered the daily habits related to the handling of pesticides in the field, based on the answers of the questionnaire. Thus, the agricultural workers classified as *directly exposed* were responsible for handling and

applying pesticides in the field. The second category, *indirectly exposed*, included the relatives of the agricultural workers, who were exposed to pesticides through the handling of clothes and other equipment used in the field. Finally, the classification of *not exposed* was for those individuals who lived in the countryside areas, but without contact with pesticides.

2.2. Gene Expression Profile Analysis

Genomic RNA was isolated from buccal epithelium exfoliated cells using TRIzol reagent (Thermo Fisher Scientific, Waltham-Massachusetts, EUA) according to the manufacturer's recommendations. Complementary DNA (cDNA) was synthesized from ~1 µg of total RNA using a High-Capacity cDNA reverse transcription Kit (Thermo Fisher Scientific, Waltham-Massachusetts, EUA), according to Oliveira *et al*., 2013 [26]. For analysis of aurora kinase genes, primers and probes developed by Assay on Demand were used (*AURKA*: Hs00269212_m1 and *AURKB*: Hs00177782_m1; Thermo Fisher Scientific). The *AURKA* and *AURKB* genes and *GAPDH* mRNA, used as endogenous internal control for each sample, were analyzed in duplicate on the same MicroAmp optical 96-well plates using a 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham-Massachusetts, EUA).

Real-time quantitative polymerase chain reaction (RQ-PCR) assays were performed in a final reaction volume of 20 µl. The comparative cycle threshold (Ct) method was used to determine the relative expression level of *AURKA* and *AURKB* genes. For comparative analysis of exposed and non-exposed agriculture workers samples, *AURKA* and *AURKB* gene expression was calculated as a relative quantification to the *GAPDH* housekeeping gene. The gene expression *AURKA* and *AURKB* from agricultural workers' samples were calculated as relative quantification to normal controls ($\Delta\Delta Ct = \Delta Ct_{\text{agricultural workers}} - \Delta Ct_{\text{controls+}}$) and expressed as $2^{-\Delta\Delta Ct}$. Comparisons between different groups were made using the student *t* test and the two-sided exact Fisher test (dichotomous variables) (GraphPad Prism 8). $p < 0.05$ was considered significant.

2.3. Fluorescence *in Situ* Hybridization (FISH)

Buccal epithelium exfoliated cells were fixed and prepared for hybridization according to the manufacturer's instructions. The hybridization spots were evaluated using an AxioImager M1 microscope (Carl Zeiss, Jena, Germany) equipped with a set of filters and software for capture and documentation analysis. A commercial set of probes (AURKA: ON AURKA (20q13)/20q11 and AURKB: AURKB (17/p13)/SE17; Kreatech Diagnostics, Amsterdam, The Netherlands) were used to confirm the elevated copy number of *AURKA* and *AURKB* genes. The probes were designed as a dual-color assay to detect amplification at 20q13 and 17p13, respectively. Amplification involving these genes regions will show multiple red signals, while the controls (MPARE1 for AURKA and SE17 for AURKB), both located in the centromeric region of their chromosomes, will

provide 2 green signals. The criteria used for *AURKA* and *AURKB* genes amplifications were based on the number of spots presented during analysis.

2.4. Micronucleus Assay

Micronucleus test (MN) was performed according to Salaija and co-workers (2006). Briefly, the buccal cells were collected, fixed, and prepared onto a slide and air-dried at room temperature. The slides were then stained with *Giemsa* solution, rinsed in distilled water, and air-dried. The nuclear abnormalities were evaluated as described by Holland and co-workers (2008). Biomarkers of DNA damage (micronuclei), cytokinetic defects (binucleated cells) and cell death (karyolytic cells) were analyzed and quantified for each agricultural worker.

3. Results

Currently, many studies aiming to assess the genotoxic effects of pesticides in human health have been done. However, herein, we combined for the first time, the use of3D nuclear telomeric profiles based on telomere numbers, telomeric aggregates, telomere signal intensities, nuclear volumes, and nuclear telomere distribution, and gene expression analysis of two important genes, closely related to cancer development. In addition, we performed micronucleus assay to better understand the relationship between cancer and pesticides exposure. According to a questionnaire previously applied to the research participants, only two of them reported a history of cancer. Table 1 shows the profile of the individuals enrolled in this study.

To better understand the potential genomic instability on agricultural workers exposed to pesticides, we compared the expression levels of two important genes related to carcinogenesis (*AURKA* and *AURKB*). As previously mentioned, the participants were categorized into three distinct groups based on their habits. The "not exposed" group comprised of 24 samples of buccal epithelium cells. The "indirectly exposed" comprised of 21 samples and finally, the "directly exposed" group consisting of workers involved in activities directly related to preparation/application of pesticides included 52 samples. Based on these distributions we performed the gene expression analysis. According to our results, we observed significant differences in the expression of *AURKA* and *AURKB* (Figure 1).

The levels of expression of both genes were markedly higher in the "directly exposed" group, when compared to "not exposed" or "indirectly exposed" (Figure 1(a) and Figure 1(b)). The values obtained were as follow: (*AURKA* [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.487 ± 0.4032 *versus* 1.083 ± 0.05337, $p < 0.0001$****, in "directly exposed" group *versus* "not exposed"); (*AURKA* [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.283 ± 0.1997 *versus* 1.083 ± 0.0369, $p < 0.0001$**, in "indirectly" exposed group *versus* "not exposed"). For *AURKB*, we found the following results: (*AURKB* [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.294 ± 0.2159 *versus* 1.078 ± 0.02733, $p < 0.0001$**, in "directly exposed" group *versus* "not exposed"); (*AURKB* [mean

Table 1. Participants profile, history of cancer, age, sex, and sample size of the studied group.

Characteristic	N
Number of participants	97
Gender	
Male (n/%)	39 (40.2%)
Female (n/%)	58 (59.8%)
Age average (years)	28.5
0 to 20 years (n/%)	44 (45.3%)
20 to 40 years (n/%)	34 (35.0%)
>40 years (n/%)	19 (19.7%)
History of cancer (n/%)	2 (2.06%)
Years of exposure (average)	8.65
Use of PPE (n/%)	
Always	19 (26.1%)
Some times	15 (20.5%)
Never	39 (53.4%)
Pesticides Exposure (n/%)	
Directly exposed	52 (53.5%)
Indirectly exposed	21 (21.6%)
Not exposed	24 (24.7%)
Sex (pesticides exposure) (n/%)	
Male	
Directly exposed	27 (69.2%)
Indirectly exposed	3 (7.08%)
Not exposed	9 (23.0%)
Female	
Directly exposed	25 (43.1%)
Indirectly exposed	18 (31.0%)
Not exposed	15 (25.9%)

value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.157 ± 0.0786 *versus* 1.078 ± 0.0308, p = 0.0021, in "indirectly" exposed group *versus* "not exposed"). Our results also indicated that the *AURKA* expression levels were later than the *AURKB* gene (Figure 1(a) and Figure 1(b)). A fact that caught our attention was the degree of heterogeneity of expressions observed in the group of individuals categorized as directly exposed (Figure 1(a) and Figure 1(b)). This heterogeneity is likely to reflect several factors, such as age, time of exposure, type of the pesticide, sex, etc. The differential levels of expression of the *AURKA* and *AURKB* genes, on "directly exposed group"

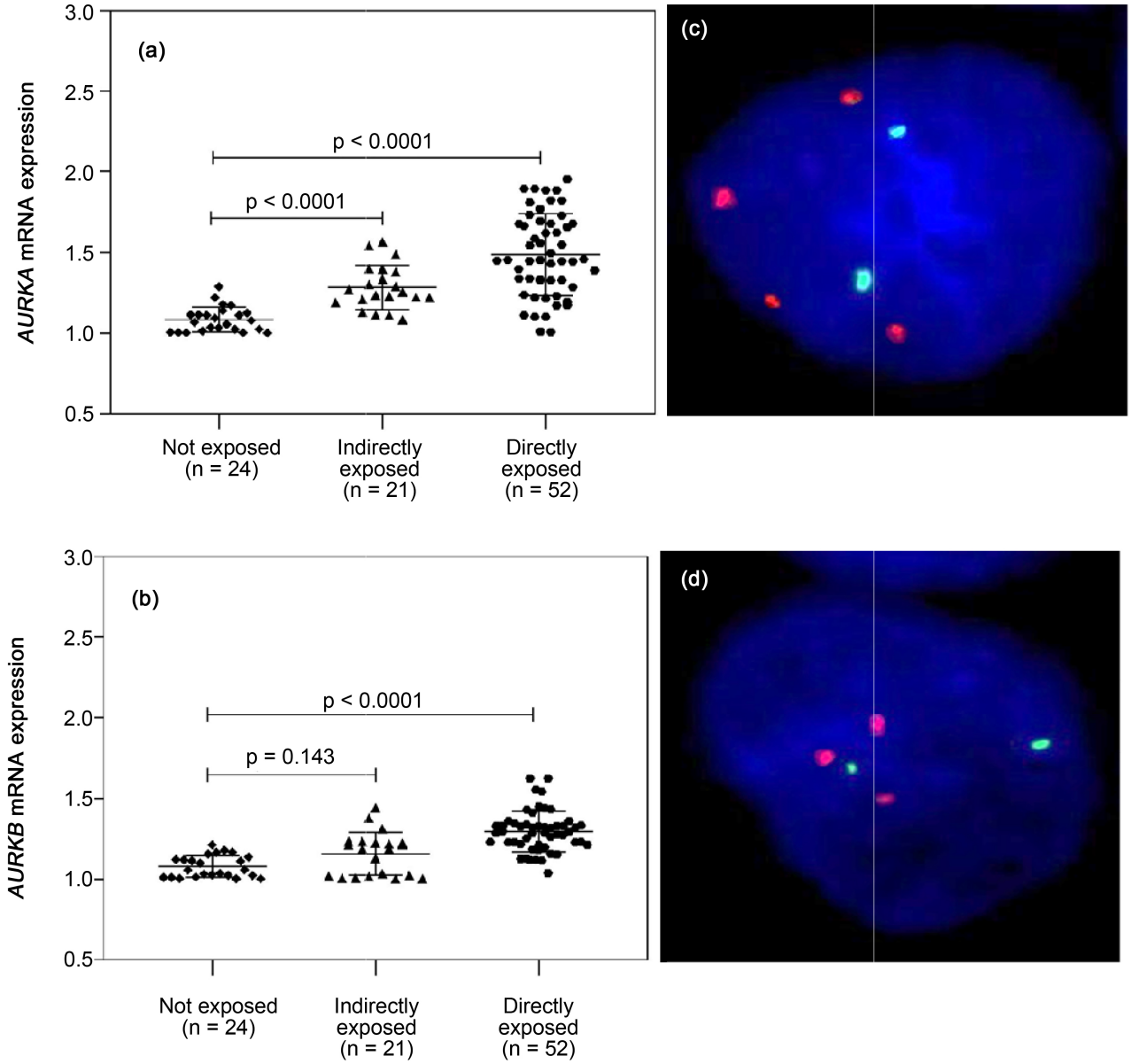

Figure 1. qPCR analysis of *AURKA* and *AURKB* mRNA expression, and interphase FISH analysis in agricultural workers exposed and not exposed to pesticides. ((a)/(b)) The graph represents the mean SD of three independent experiments. Significant differences between groups ($p < 0.0001$) are shown in the graph; ANOVA test and Bonferroni post-test were performed. (c) Interphase FISH analysis of *AURKA* gene demonstrating elevated DNA copy number in directly exposed group (spots in red, control in green). (d) Interphase FISH analysis of *AURKB* gene demonstrating elevated DNA copy number in directly exposed group (spots in red, control in green).

were confirmed using the FISH technique, with commercial probes for both genes. The results allowed to confirm the amplification of the studied genes (Figure 1(c) and Figure 1(d)).

The participants were sub-stratified by gender, to verify whether the expression profile of both studied genes would be influenced by this variable. In fact, we found that in men, the expression levels of *AURKA* and *AURKB* were higher in relation to women (Figure 2). However, in both groups the results were still

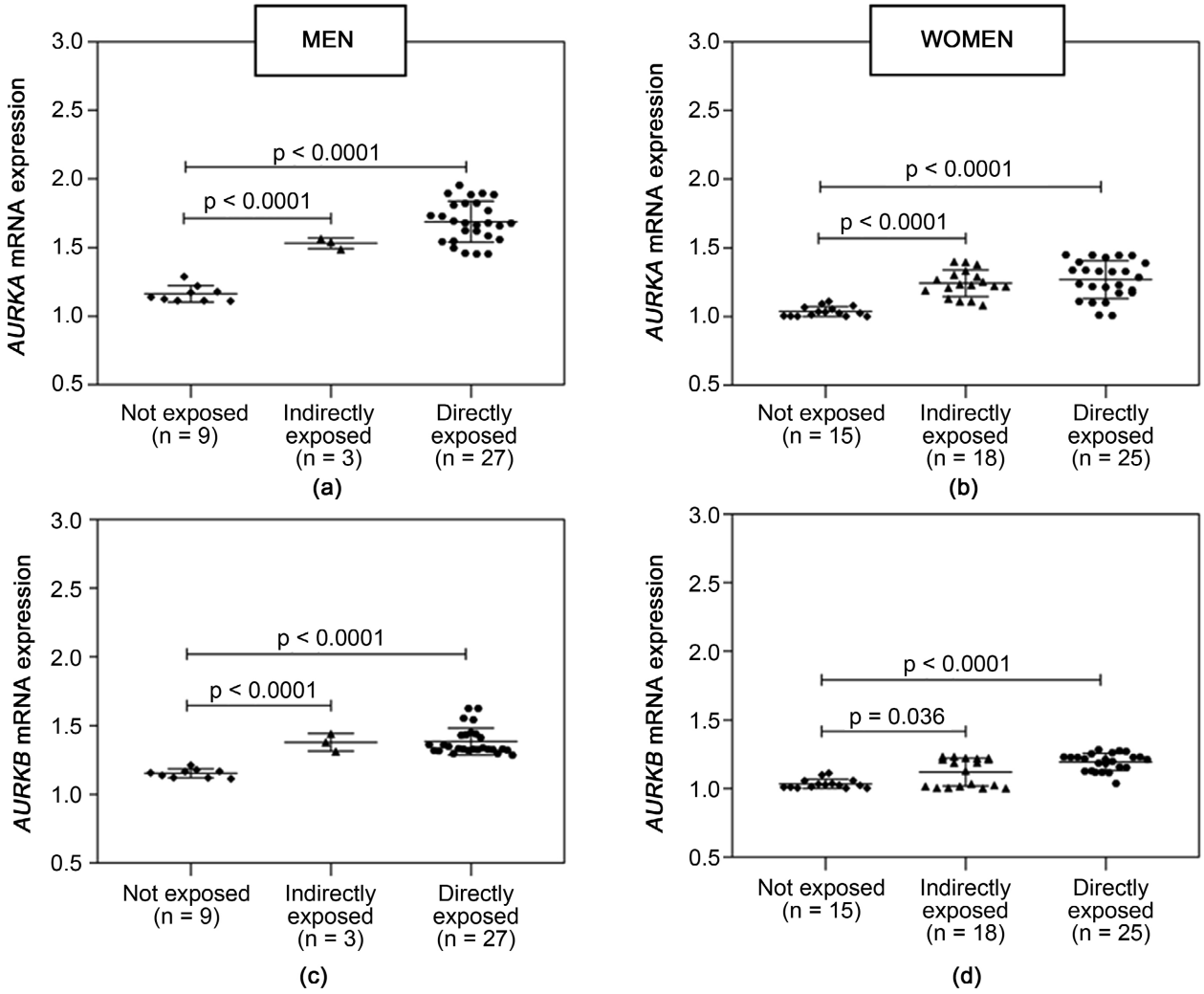

Figure 2. qPCR analysis of *AURKA* and *AURKB* mRNA expression in agricultural workers exposed and not exposed to pesticides, subdivided by gender. ((a)/(c)) Expressions of *AURKA* and *AURKB* genes, respectively, defined for the male group. The graph represents the mean SD of three independent experiments. ((b)/(d)) Expressions of *AURKA* and *AURKB* genes, respectively, defined for the female group. The graph represents the mean SD of three independent experiments. The p values are indicated in the graphs; $p < 0.0001$ statistically significant; ANOVA test and Bonferroni post-test were performed.

significant. In the case of male individuals, the low number of "indirectly exposed" is justified, because this group is represented mostly by women, who are engaged in domestic activities. In this sense, according to the responses to the questionnaires, the contact with pesticides occurred through handling contaminated clothes or other instruments used for the application of pesticides by their respective husbands. The values obtained by the gene expressions were (*AURKA* ♂ [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.689 ± 0.5269 versus 1.162 ± 0.05136, $p < 0.0001$****, in "directly exposed" group versus "not exposed"); (*AURKA* ♂ [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.531 ± 0.3688 versus 1.162 ± 0.03789, $p < 0.0001$****, in "indirectly" exposed group versus "not exposed") (**Figure 2(a)**). For *AURKB*, we found the following results: (*AURKB* ♂ [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.384 ± 0.2318 versus 1.152 ± 0.03362, $p < 0.0001$**, in "directly exposed"

Table 2. Frequencies of MN and Nuclear abnormalities.

Participants	Pyknotic cells	Karyorrhectic cells	Karyolytic cells
Not exposed	4.12 ± 1.44	3.46 ± 2.07	2.06 ± 1.32
Indirectly exposed	7.65 ± 2.65*	12.54 ± 3.66*	10.76 ± 3.12*
Directly exposed	34.54 ± 4.66*	27.76 ± 6.77*	19.76 ± 3.79*

*$p < 0.005$.

group versus "not exposed"); (*AURKB*♂ [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.377 ± 0.2251 versus 1.152 ± 0.02726, $p < 0.0001$****, in "indirectly" exposed group versus "not exposed") (Figure 2(c)). For female participants, we found the following results: (*AURKA*♀ [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.268 ± 0.2320 versus 1.036 ± 0.03637, $p < 0.0001$****, in "directly exposed" group versus "not exposed"); (*AURKA*♀ [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.242 ± 0.2055 versus 1.036 ± 0.02655, $p < 0.0001$****, in "indirectly" exposed group versus "not exposed") (Figure 2(b)). For *AURKB*, we found the following results: (*AURKB*♀ [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.193 ± 0.1596 versus 1.034 ± 0.01765, $p < 0.0001$****, in "directly exposed" group versus "not exposed"); (*AURKB*♀ [mean value of $2^{-\Delta\Delta Ct}$ ± SD]: 1.120 ± 0.08641 versus 1.036 ± 0.02747, $p = 0.0036$**, in "indirectly" exposed group versus "not exposed") (Figure 2(d)). Regardless of gender, the heterogeneity of expression for *AURKA* gene was again observed (Figure 2(a) and Figure 2(b)).

The buccal MN test is considered a minimally invasive and reliable biomarker to determine the effect of mutagenic compounds. It evaluates DNA damage, chromosomal instability, and cell death. Thus, to support our data regarding gene expression profile, we performed MN analysis and, found significant differences when comparing the three distinct groups of participants (Table 2). Buccal epithelium exfoliated cells from agricultural workers "directly exposed" to pesticides presented a high frequency of cells with micronucleus, compared to "not exposed" or "indirectly exposed" ($p < 0.005$). Even when comparing those "indirectly exposed" with "not exposed" individuals, the results also proved to be significant ($p < 0.005$). The MN frequencies from "directly exposed" and "indirectly exposed" were compared with "not exposed" using the *t* student test.

Although only two participants reported a history of cancer, the results obtained, through different methods, allow us to show the presence of genomic instability in agricultural workers using pesticides.

4. Discussion

Biomonitoring studies focusing on the detection of genomic abnormalities have been carried out in pesticide exposed populations aiming to elucidate the risk associated to the exposure to specific compounds or classes of compounds [25] [27] [28] [29] [30]. Agricultural workers exposed to pesticides were found to present a greater risk to some malignancies such as leukemia, neuroblastoma,

Wilm's tumor, non-Hodgkin lymphoma, ovarian cancer, cancers of lung, and stomach cancer [31]-[35]. The overexpression of important genes linked to the cell cycle has been identified in several neoplasms. Aurora kinase gene family (*AURKA* and *AURKB*) is an example of these genes associated to malignancies. In this sense, this study sought to correlate the relationship between minimum levels of expression of aurora kinase genes in rural workers exposed to pesticides. From information obtained through interviews with rural workers associated with the results of gene expression, we demonstrated significant differences in the expression of the studied genes among the group of individuals categorized as "directly" or "indirectly" "exposed" and "not exposed" group, although, the levels of gene expression obtained here are not at the same degree as those found in biopsies of neoplastic tissues. However, our results suggest that possible damage to DNA may be occurring, which on a temporal scale could promote genomic instability, and ultimately, tumor development.

DNA damage and subsequent genomic instability promote gene mutations that help to generate the hallmarks of cancer [36] [37] [38]. Additionally, extensive, or unrepaired DNA damage is toxic to cells. DNA damage may cause cell cycle arrest and/or, lead to apoptosis or necrotic cell death [39]. Pesticides cause Reactive Oxygen Species (ROS)-mediated stress and DNA damage, leading to premature cellular senescence and programmed cell death [40]. Generation of ROS is associated with the risk of cancer. It can influence the expression of many genes involved in inflammation; cell transformation; and tumor cell death or survival, proliferation, invasion, angiogenesis, and metastasis [40]. In 1999, Infante-Rivera and colleagues (1999) [41] demonstrated that mothers who got exposed to pesticides during pregnancies and if the child presents a *CYP1A1m1* or a *CYP1A1m2* mutation had an increased risk to develop acute myeloid leukemia, or AML. Of note, *CYP1A1*, *CYP2D6*, *GSTT1*, and *GSTM1* are genes that encode enzymes responsible for metabolizing carcinogenic substances. Cytochrome P-450 family is involved in the transformation of pro-carcinogenic compounds to reactive species which have genotoxic and cytotoxic effects [41]. These events may also render the repair machinery less efficient resulting in premature aging and apoptosis. ROS imbalances may also recruit aberrant proteins which may result in the imbalance of the signaling pathway leading to tumorigenic processes [42]. ROS generation may in turn result in polymorphisms that may change the expression of important genes, for example, aurora kinase genes [43] [44].

Some studies have reported different "degrees" of genomic instability in agricultural workers exposed, and not exposed to pesticide with significant difference between the groups [45]. Follow up of previously exposed agricultural workers who had not been exposed to pesticides for a period of six months showed that they continued presenting significant genotoxic damage, leading to the conclusion that pesticides can cause changes in the mechanisms that repair mutations [46]. In this context, recent research also shows that exogenous or

endogenous ROS caused by pesticides exposure promoted mitotic arrest. Delayed formation and abnormal function of the mitotic spindles directly impeded mitosis, promoted abnormal chromosome separation and was responsible for ROS-induced mitotic arrest [47]. It is important to keep in mind that abnormal expression of aurora kinase genes play roles in centriole duplication, an important process during cell mitosis. An important study demonstrated that overexpression of *AURKA* and *Plk4* was associated with emergence of amplified centrosomes [48], with production of aneuploid cells as result. Although, we have not performed a cytogenetic study on cells obtained from the exposed, and not exposed agricultural workers, we believe that structural chromosomal abnormalities, for example, chromosome gaps and breaks could be found in the samples studied, based on *AURKA*/*AURB* expression profile, and in accordance with the results of the micronucleus test.

Benedetti and co-workers (2013) [49] and, Kvitko and co-workers (2012) [50] independently, observed significant differences for the quantification of DNA damage in agricultural workers subjected to the comet assay, corroborating with our results obtained from the micronucleus test. These studies were conducted among workers exposed to different formulations of pesticides in their work. In this context, the exposure to a mix of chemical formulations may be responsible to produce cross-link DNA-DNA and DNA-protein, rupture of the two chains of DNA, formation of DNA adducts, all affecting the gene expression profile of important genes, closed related to cell cycle [45]. This scenario may present itself worsened by the continual exposure to pesticides without proper protection with adequate equipment (PPE). According to our results, 53.4% of the agricultural workers reported have never used PPE. Thus, the persistent cytological damage can lead to a higher level of cytogenetic changes [51].

In addition, our results showed a clear correlation among individuals categorized as "directly" and "indirectly" "exposed" to pesticides and the damage rate, either by the micronucleus test or aurora kinase expression profile. This fact raises some concerns about possible risk for cancer development. Merhi and co-workers (2007) [52] concluded a study that followed a group of agricultural workers through a long period of exposure to pesticides (more than 10 years). They observed an increased risk for the development of hematopoietic tumors and non-Hodgkin's lymphoma. Ferraz and co-workers (2016) [53], observed that among healthy subjects distributed in two groups (19 - 29 years old × over 60 years old), the frequency of micronuclei and nuclear degenerative changes was significantly higher among the older group. On the other hand, they showed that avoiding the use of pesticides or at least reducing their exposure, it could minimize the effects of aging, reducing the risk of developing degenerative diseases.

Epigenetic modifications have also been noted as consequence of pesticides exposure. Li and co-workers (2011) [54] evaluated the epigenetic effects of pesticides, in a porcine kidney epithelial cell line (PK15) in order to achieve a better

understanding of its non-neuronal cytotoxicity. Microarray analyses showed an altered miRNA and mRNA expression profile, showing that the epigenetic mechanisms involving miRNA expression modifications play a pivotal role in cytotoxicity. In addition, Collotta and co-workers (2013) [9] evaluated the effect of a mix of pesticides on miRNA expression in zebrafish. The expression profile of some miRNA was altered after treatment with these chemicals, suggesting their role in the toxicity mechanisms of these compounds and representing a possible novel toxicological biomarker. In general, the exposure to environmental factors can alter DNA methylation patterns, inducing destabilizing changes in gene expression patterns potentially leading to cell transformation and tumorigenesis [9]. Thus, alteration on genome hypomethylation and/or hypermethylation of CpG islands of specific genes, including aurora kinase genes, and have been increasingly found in different types of tumors [55] [56].

5. Conclusion

The consequences of exposure to pesticides in humans have been reported in the literature. The use of pesticides around the world has reached unimaginable proportions, even though some countries have banned the use of pesticides and gradually are adopting strategies that offer fewer potential risks to human health, for example, the advent of transgenic foods. Our results showed, in an unprecedented way, alterations in the expression patterns of important genes of the aurora kinase family, often associated with neoplastic development. We note that even individuals not directly exposed to pesticides can present cell abnormalities and these abnormalities increase with exposure. The development of this work proposal progressed with several campaigns about the awareness of the risks caused by exposure to pesticides, as well as the importance of using PPE during pesticide management. As previously mentioned, changes in habits are the main attitudes aimed at avoiding health problems resulting from exposure to pesticides. From a genetic point of view, our results may be interpreted as "a warning sign" regarding the negative effect of pesticide exposure. We believe that biomonitoring strategies, based on the expression levels of the *AURKA* and *AURKB* genes may provide important indicators on the evolution of genomic instability in the cells of agricultural workers exposed to pesticides.

Authors' Contribution

FMO: Conceptualization, manuscript writing and critical review of the work, with the addition of important observations; CFOPP: Substantial contributions to data acquisition, analysis, and interpretation of data; HMC: Substantial contributions to data acquisition, analysis, and interpretation of data; GLS: Substantial contributions to data acquisition, analysis, and interpretation of data; LHM: Substantial contributions to data acquisition, analysis, and interpretation of data; LARS: Substantial contributions to data acquisition, analysis, and interpretation of data; TOC: Substantial contributions to data acquisition, analysis, and inter-

pretation of data; FLSN: Critical review of the manuscript, with the addition of important observations; AMM: Important technical support offered; EFMV: Critical review for important intellectual content, and final approval of the version to be published.

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Conflicts of Interest

The authors declare no conflicts of interest.

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