Original Article

Pesticide-Induced Genotoxicity in Farmers of Southern Punjab as Indicated by Frequency of Micronuclei

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Abstract

Background: Farmers have an increased risk of pesticide-induced DNA damage which can be detected by the presence of micronuclei (MN) in buccal cells. Smoking may further amplify this genotoxic effect, making MN a valuable biomarker for assessing both occupational and lifestyle related DNA damage.

Objective: To determine the frequency of micronuclei as a biomarker of DNA damage in buccal mucosal cells of pesticide exposed farmers, with and without smoking.

Methods: A comparative analytical cross sectional study was conducted from May to November 2021 on farmers in Moza Durana Nigana, Tehsil & District Multan. A total of 135 participants aged 21–60 years were divided into three groups of 45 each: Group I – nonsmokers and non-farmers (control); Group II –pesticide exposed farmers, who were nonsmokers or smoked fewer than five cigarettes per day; Group III – pesticide exposed farmers who smoked 15–20 cigarettes per day. Buccal smears were collected and stained using the Papanicolaou technique. Slide preparation and analysis were performed in the Departments of Anatomy and Pathology, King Edward Medical University, Lahore, to detect micronuclei (MN) as an indicator of DNA damage.

Results: Pesticide exposed farmers showed a significantly higher frequency of micronuclei (MN), averaging 30.79 ± 9.65 per 1000 cells as compared to the control group, which had a frequency of 1.15 ± 0.38 per 1000 cells ($p < 0.05$). Among the exposed groups, Group III exhibited the highest MN frequency, indicating a synergistic genotoxic effect of pesticide exposure and smoking.

Conclusion: Pesticide exposure in farmers demonstrated a significant increase in DNA damage which is further amplified in those who smoke.

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Introduction

The rural sector plays a vital role in the economy of any country by providing food, revenue and employment.[1] Pakistan's economy is predominantly agro-based with more than 60% of the population living in rural areas and depending primarily on agriculture for their

livelihoods.[1] This sector accounts for roughly 19.3% of the national Gross Domestic Product (GDP) and provides direct or indirect employment to about 42% of the country's labor force.[1,2] Despite its global economic significance among all industries around the world, the situation is worsening in developing countries due to inadequate safety measures and weak regulatory standards.[3]

The Food and Agriculture Organization of the United Nations (FAO) defines a pesticide as any chemical or combination of substances designed to prevent, destroy, or control pest.[4] Farmers are exposed to pesticides not only during spraying but also during the preparation, mixing, and disposal.[4] Pesticides are widely used by farmers in all parts of Pakistan for control of various crop pests. Mealybugs attack cotton, beetles damage wheat, stem borers and grasshoppers affect rice and sugarcane and maize are targeted by army worms and maize borers (Chilo partellus Swinhoe).[5] Commonly used chemicals include Lambda spray (cyhalothrin) for wheat, Fipronil for maize, Malathion (Suthion) for cotton and Bifenthrin (pyrethins) for mango cultivation.[6]

The International Agency for Research on Cancer (IARC) has classified certain pesticides, including glyphosate, 2,4-D, and parathion, as probably or possibly carcinogenic to humans.[7,8] Exposure to pesticides such as organophosphates, phenoxy herbicides and insecticides has been associated with increased morbidity and mortality, placing agricultural workers and nearby residents at heightened risk of developing chronic illnesses from both short- and long-term exposure.[9,10] Despite the widespread use of pesticides, most of the farmers in southern Punjab do not use personal protective equipment, with only 10–15% covering their faces with a simple cloth.[11]

Micronuclei (MN) are small extranuclear bodies formed when whole chromosomes or chromosomal fragments fail to incorporate into daughter nuclei during anaphase or telophase.[12,13] They serve as a reliable biomarker to assess genomic damage and mitotic abnormalities and can be evaluated in vivo or ex vivo.[14,15] MN can be detected in erythrocytes, lymphocytes, and exfoliated cells such as oral, urothelial, and nasal epithelium.[15] The MN assay is a rapid, efficient and cost-effective technique.[16] Higher frequencies of micronuclei are seen in individuals exposed to pesticides, natural solvents, antineoplastic and diesel fuel.[17] In particular, buccal cells MN analysis provides a non-invasive biomarker for genetic damage in occupation and environmental settings.[18]

Despite extensive global research on pesticide-induced genotoxicity, data from Southern Punjab, Pakistan remain limited. This region is characterized by rich agricultural practices but with inadequate use of personal protective measures. The lack of local biomonitoring studies highlights a critical gap in understanding pesticide-related cytogenetic damage in this population. Therefore, this study was carried out to determine the micronuclei frequency in buccal mucosal cells of pesticide-exposed farmers compared with non-exposed controls. In addition, the study aimed to evaluate the potential synergistic effect of smoking on pesticide-induced genotoxicity.

Methods

This cross-setional analytical study was conducted in Moza Nigana Durana, Tehsil and District Multan, Southern Punjab, Pakistan from May to November 2021. A purposive sampling technique was used to recruit 135 participants from the study area in accordance with predefined inclusion and exclusion criteria. Both male and female participants aged 21–60 years were included in the study after obtaining an informed consent. All participants had been engaged in agricultural activities for at least one year and had not interrupted farming for more than three months per year. Individuals who had a personal or family history of oral or any other malignancy, active oral ulcers, alcohol or betel nuts users and with a recent history of high-grade fever were excluded from the study.

The study population was divided into three groups of 45 participants each. Group I served as the control group and included healthy individuals with no history of pesticide exposure and residing at least 15–20 km away from agricultural fields. Groups II and III comprised pesticide-exposed farmers who extensively used pesticides and fertilizers in mixed cropping activities. Group II consisted of farmers who were non-smokers or smoked fewer than five cigarettes per day while Group III included farmers who were smokers consuming 15–20 cigarettes per day for at least one year.

The study participants were directed to flush their mouths with 0.9% normal saline water. A sterile wooden tongue depressor was used to obtain the buccal mucosa from the inner cheeks. To guarantee sufficient cellular yield and even distribution, four buccal smears were taken from various intraoral locations of all participants. Fresh smears were immediately prepared on glass slides and fixed with 95% ethanol prior to staining. Papanicolaou (Pap) stain was used to provide optimal nuclear detail for the analysis of micronuclei. All the pre-labelled and stained slides were examined under a light micro-

scope in the Department of Anatomy, King Edward Medical University, Lahore. The zigzag technique was used for micronuclei assay in which 1000 cells were screened across 4-5 high-density regions per smear. High-density regions were areas where cells were densely packed and clearly visible under light microscopy while sparse or overlapping regions were excluded. Micronuclei were identified and scored according to Tolbert's criteria as darkly stained round or oval chromatin bodies having similar texture to the main nucleus and a diameter between one third and one sixth of the main nucleus. Up to four micronuclei per cell were recorded as an indicator of genotoxic damage.

Data was entered and analysed using SPSS version 26.0. A comparison of three groups I, II, and III was done using ANOVA. P-value ≤ 0.05 was considered as statistically significant.

Results

This study included 135 individuals divided into three groups of 45 each. The age of participants across all groups ranged from 21 to 60 years, with a mean age of 43.59 years. Non farmer group (control) included 22 (48.9%) males and 23 (51.1%) females. In the farmer groups (groups II and III), there were 85 (94.4%) males and 5 (5.6%) females.

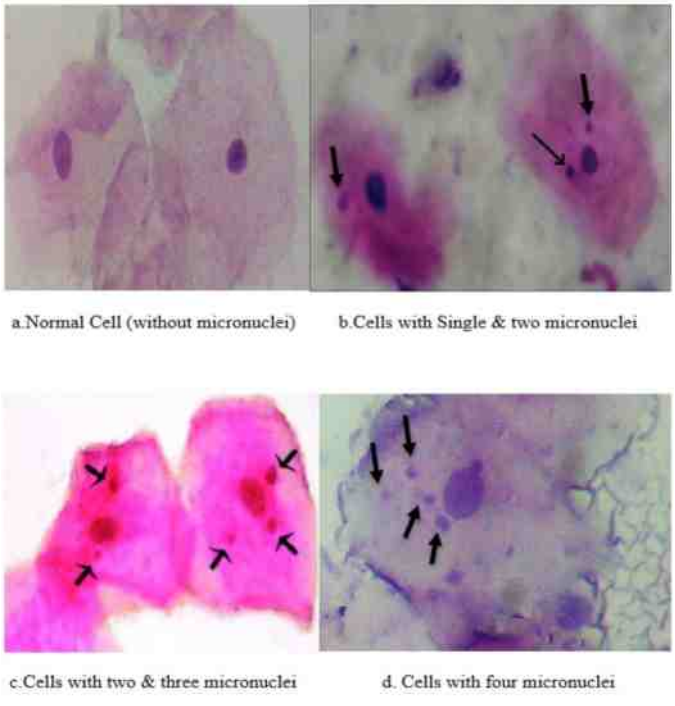


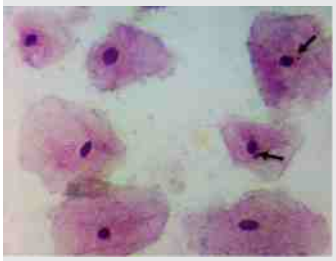
Fig-1a-d: *Photomicrographs illustrate buccal mucosal cells with varying number of micronuclei per cell in group I,II and III (Pap stain, 40X)*

The mean frequency of micronuclei per cell was highest in Group-III (2.8 ± 1.1; range 1–4), followed by Group-II (1.9 ± 0.7; range 1–3) and Group-I (0.8 ± 0.3; range 0–1). A significant association was observed between pesticide exposure and smoking, with smoking farmers (Group-III) showing higher micronuclei frequencies per cell compared to non-smoking farmers (Group-II). (Figure-1a-d) The number of micronuclei in a single cell indicated the degree of DNA damage in that particular cell whereas micronuclei per 1000 cells in hotspot areas reflected the total cytogenetic damage in regions with the highest cell density

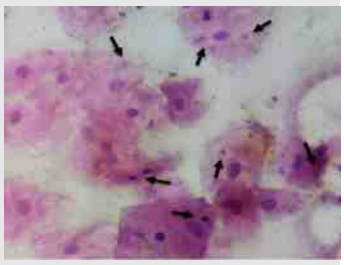
Analysis of hotspot areas (areas with the highest number of micronuclei per 1000 cells) revealed maximum counts of 0–4 in Group-I, 2–29 in Group-II, and 7–60 in Group-III. Smoking farmers (Group-III) exhibited the highest number of micronuclei in these hotspot areas, followed by non-smoking farmers (Group-II) and controls (Group-I). (Table -1 & Figure-2a - c)

Table 1: *Frequency of micronuclei per 1000 cells in hotspot areas of group I,II and III*

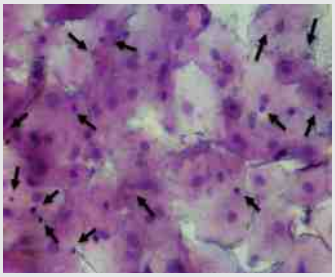
Groups	Mean ± SD	Minimum Micronuclei per 1000 cells	Maximum Micronuclei per 1000 cells
Group-I	1.15 ± 0.38	0	4
Group-II	5.74 ± 3.24	2	29
Group-III	30.79 ± 9.65	7	60



2a: (02 micronuclei in a hotspot area of group-I)



2b: (07 micronuclei in a hotspot area of group-II)



2c: (14 micronuclei in a hotspot area of group-III)

Fig- 2a-c. *Illustrate maximum number of micronuclei in hot spot areas of group-I, II and III. (Pap stain, 40X)*

Discussion

This study investigated the risk of cytogenetic damage among pesticide-exposed farmers from southern Punjab. The findings revealed a significantly higher frequency of micronuclei (MN) in the buccal mucosal cells of pesticide-exposed farmers compared with non-exposed controls, thereby confirming the presence of cytogenetic damage in this population.

In this study participants were selected using clearly defined inclusion and exclusion criteria to ensure a reliable and well documented history of pesticide exposure. This approach aligns with the methodology recommended by Cepeda et al. who emphasized the importance of rigorous participant selection in cytogenetic studies to accurately assess chromosomal instability in pesticide-exposed farmers.[19] Four buccal smears were collected from different intraoral sites of each participant to ensure an adequate number of cells and uniform cell distribution. Various studies highlighted methodological limitations associated with conventional buccal mucosal cell collection as Grover S et al. reported that liquid-based cytology (LBC) provides superior cell preservation and more homogeneous distribution compared with conventional cytology, whereas Nambiar S et al. observed that traditional methods result in lower cell counts, reduced cell size, and uneven distribution.[20,21] Collectively, these findings support the methodology used in the present study and underscore the importance of collecting multiple smears to obtain accurate and reliable micronucleus results.

In the present study, Pap stain was used for its ability to provide clear nuclear detail, facilitating reliable detection and scoring of micronuclei. Several studies have reported that pap stain produces higher micronuclei counts and better nuclear contrast than other conventional stains.[22] According to some studies fluorescence staining is more sensitive but it is time consuming, costly and requires specialized equipment whereas H&E staining often results in excessive eosin obscuring nuclear details and complicate interpretation.[23,24]

Several studies have confirmed the induction of cytogenetic damage, supporting the role of micronuclei frequency as an efficient biomarker for genotoxic screening.[25] In the present study, the average frequency of micronuclei in the buccal mucosa of farmers exposed to pesticides (group-III) was 30.79 ± 9.65 per 1000 cells which is substantially higher than that reported in other exposed populations.[25,26] Farmers of group III in this study also exhibited up to four micronuclei per cell with statistically significant differences compared to group II and control group, highlighting the severity of the observed genotoxic damage. While the number of micronuclei per cell indicates the extent of DNA damage in individual buccal cells, the frequency of micronuclei per 1000 cells in hotspot areas provides a measure of overall cytogenetic burden in regions with the highest cellular density, highlighting both the severity and distribution of genotoxic damage among exposed farmers.

DNA damage is also induced by tobacco smoke which is a well-established genotoxicant. In this study the increased frequency of micronuclei observed in group-III (30.79 ± 9.65) is attributed to both pesticide exposure and concomitant smoking compared to group-II (5.74 ± 3.24). This finding is consistent with the reports of Bolognesi CJ and Pereira CP et al.[27,28] who demonstrated a dose-dependent increase in genotoxic damage associated with smoking. However, Hassan S et al. observed no significant association with tobacco use possibly because their study population primarily included patients undergoing cancer treatment which may have resulted in false-negative findings.[29]

Farmers directly engaged in pesticide handling were therefore at considerable risk, particularly in the absence of appropriate personal protective equipment (PPE). Sherif M et al. highlighted that chromosomal damage may be transient, and that the timing of sample collection plays a critical role in determining the accuracy of cytogenetic findings. Likewise, Mozzoni P emphasized that sampling should ideally be performed within two days of acute exposure or immediately following the end of chronic exposure to minimize the risk of underestimating damage. Santos CF et al. reported a positive association between longer agricultural work duration and increased chromosomal damage, whereas Cobanoglu N et al. found no significant relationship between farming activity and micronucleus (MN) frequency.

In the present study, pesticide exposure was also evaluated according to the farmers' years of agricultural experience. However, no statistically significant association was observed between duration of exposure and cytogenetic damage. This finding suggests that the intensity and frequency of pesticide exposure, along with inadequate use of PPE, may exert a more pronounced influence on genotoxic outcomes than duration of exposure alone. Exposure assessment was based on self-reported data and occupational history, which may be subject to recall bias. Multiple smears were obtained to enhance reliability and ensure adequate cellular representation. Future longitudinal investigations incorporating more precise exposure quantification are recommended to

strengthen the evidence and clarify the temporal relationship.

Conclusion

Farmers exposed to pesticides showed a higher frequency of micronuclei compared to non-farmers, with significantly greater damage observed among smokers, suggesting a synergistic effect of pesticide exposure and tobacco use. Inadequate use of personal protective equipment (PPE) was associated with increased cytogenetic damage, highlighting the urgent need to promote proper protective practices and targeted preventive strategies to safeguard farmers' health in southern Punjab, Pakistan.

Ethical Approval: The Institutional Review Board, KEMU approved this study vide No. 137/ RC/ KEMU.

Conflict of Interest: The authors declare no conflict of interest.

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Authors' Contribution:

HMAAS: Conception & design, acquisition of collection, analysis & interpretation of data, drafting of article

SK: Conception & design, analysis & interpretation of data, final approval of the version to be published

AH: Analysis & interpretation of data, drafting of article

RT: Critical revision for important intellectual content, final approval

SA: Analysis & interpretation of data, final approval

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