



Assessment of Cytogenetic Biomarkers in Populations Living in High-Traffic and Industrial Areas of Baghdad

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Article Info	ABSTRACT
Article type: Research Article	Environmental pollution remains a major public health concern in rapidly urbanizing cities because of its potential to induce oxidative stress, DNA damage, and cytogenetic abnormalities that puts human health at grave risk for toxicity and genotoxicity. This study investigated cytogenetic damage associated with environmental pollution exposure among residents of Baghdad City using the Buccal Micronucleus Cytome (BMCyt) assay as a biomonitoring tool. Nuclear abnormalities in buccal epithelial cells, such as pyknosis, karyolysis, karyorrhexis, and micronucleus formation, are well-known indicators of cell death and genotoxicity. A validated technique for identifying chromosomal instability and exposure to environmental mutagens is the micronucleus assay. In comparison with the control group, this study examined cytogenetic damage in people exposed to occupational and environmental pollutants in Baghdad, Iraq. Seventy participants (35 controls and 35 exposed; mean age 42.2 ± 7.6 years) had buccal cell samples taken. Chi-square tests were used to evaluate nuclear abnormalities and determine statistical significance. Nuclear abnormalities were significantly more common in the exposed group ($\geq 94\%$) than in the control group ($\leq 14.3\%$). Pyknosis, karyolysis, karyorrhexis, and micronucleus formation were all markedly increased ($p < 0.001$), suggesting severe genotoxic stress linked to environmental exposure.
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INTRODUCTION

Environmental pollution remains one of the most pressing public health challenges worldwide due to its widespread distribution and potential to induce oxidative stress, cellular injury, and genetic damage. Rapid urbanization, industrial expansion, increased traffic density, and the continuous release of chemical contaminants into the environment have contributed to elevated human exposure to a complex mixture of pollutants, including heavy metals, particulate matter (PM), volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbons (PAHs). These pollutants persist in environmental matrices such as air, water, and soil and may accumulate in biological systems, resulting in adverse health effects ranging from respiratory and cardiovascular disorders to carcinogenesis and genetic instability (Olympio et al., 2023).

Environmental contaminants exert their toxic effects through multiple biological

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mechanisms, including the generation of reactive oxygen species (ROS), induction of oxidative stress, disruption of cellular signaling pathways, and direct interaction with DNA molecules. Numerous studies have demonstrated that exposure to airborne pollutants and toxic chemicals can promote chromosomal instability, DNA strand breaks, micronucleus formation, and other cytogenetic alterations associated with increased disease risk (Flora et al., 2012; Pinto et al., 2024). Such genotoxic effects are of particular concern because they may contribute to long-term health consequences and serve as early indicators of environmentally induced cellular damage.

Baghdad, the capital of Iraq, represents an important urban environment for studying pollution-related health effects. During recent decades, the city has experienced substantial industrial growth, increasing traffic congestion, population expansion, and extensive use of private electricity generators. These activities have contributed to elevated levels of environmental pollution, including particulate matter, heavy metals, and combustion-derived pollutants (IASJ, 2025). Consequently, residents living in highly trafficked and industrialized districts may experience greater exposure to environmental contaminants than populations residing in suburban or less polluted areas.

Environmental pollution has been widely recognized as a major contributor to genetic and cellular damage in exposed populations. Several studies have demonstrated that chemical contaminants and airborne particles can exert toxic and genotoxic effects on biological systems (Pinto et al., 2024; Nimr et al., 2023). In human biomonitoring studies, cytogenetic biomarkers have become valuable tools for assessing DNA damage associated with environmental exposure. Among these methods, the Buccal Micronucleus Cytome (BMCyt) assay has gained considerable attention because of its sensitivity, reproducibility, non-invasive nature, and suitability for large-scale population studies (Holland et al., 2008; Thomas et al., 2009).

Buccal epithelial cells are particularly suitable for biomonitoring because they are continuously exposed to inhaled and ingested pollutants and can reflect both local and systemic genotoxic effects. The BMCyt assay enables the detection of micronuclei, which originate from chromosome fragments or whole chromosomes that fail to be incorporated into daughter nuclei during cell division. In addition, the assay identifies other nuclear abnormalities such as pyknosis, karyorrhexis, and karyolysis, which are recognized indicators of cytotoxicity, apoptosis, and cellular degeneration (Tolbert et al., 1992; Fenech et al., 2025). Recent methodological advances have further improved the reliability and applicability of this assay in environmental health investigations (Fenech et al., 2025).

Numerous studies have validated the use of the BMCyt assay for environmental biomonitoring. Abdul-Ameer et al. (2020) reported increased cytogenetic damage among traffic policemen in Baghdad, while Khan et al. (2010) observed elevated micronucleus frequencies among occupationally exposed workers. Similarly, Baptista et al. (2023) demonstrated significant cytogenetic alterations among healthcare professionals exposed to volatile organic compounds. Additional investigations have confirmed the usefulness of micronucleus-based biomarkers for evaluating environmental and occupational genotoxicity across diverse exposure scenarios (Farabaugh et al., 2023; Harte et al., 2024).

Given the increasing environmental burden in Baghdad and the need for effective biomonitoring approaches, the present study aimed to assess cytogenetic damage associated with environmental pollution exposure among residents living in high-traffic and industrial areas of Baghdad using the Buccal Micronucleus Cytome (BMCyt) assay. The frequencies of micronuclei, pyknosis, karyorrhexis, and karyolysis were evaluated as biomarkers of genotoxicity and cytotoxicity and compared with those observed in a control population residing in less polluted areas.

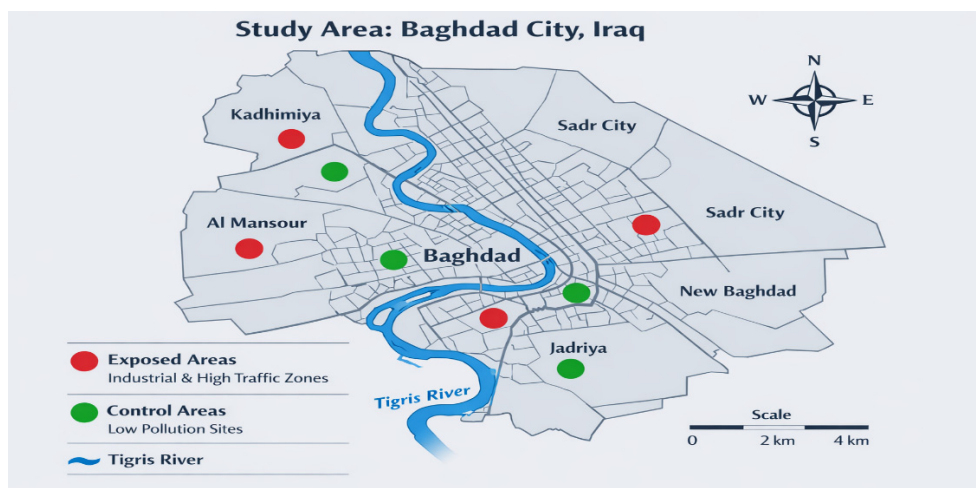


Fig. 1. Map of Baghdad city showing the study area where exposed and control populations were recruited for cytogenetic biomonitoring.

MATERIALS AND METHODS

Research Population

To enable comparative analysis, the study population was divided into two separate groups. The first group consisted of people who lived in Baghdad City's industrial and highly trafficked areas, which are known to have higher concentrations of environmental contaminants, including traffic-related and industrial pollutants. These people were chosen because they had lived in these areas for a long time, guaranteeing that they had been exposed to pollutants on a regular basis. Information about demographic variables including age, gender, smoking status, and medical history (such as diabetes) was collected from all participants to evaluate potential confounding factors. Residents from Baghdad's suburbs with less industrial activity and less traffic density made up the second group, which acted as a control. A clear comparison between populations with high and low environmental exposure was made possible by this design (Springer, 2025). To reduce confounding variables, participants had to be free from chronic illnesses and those not be exposed to other known genotoxic agents at work. The study was conducted in Baghdad city, Iraq. Participants were recruited from urban districts characterized by high traffic density and industrial activities, representing the exposed group, while the control group was selected from suburban regions with relatively lower pollution levels (Figure 1).

Sample Collection

Using sterile cytobrushes, buccal epithelial cells were extracted from each participant. To guarantee consistency and reproducibility, the collection process adhered to established protocols (Thomas et al., 2009). Before sampling, each participant was told to rinse their mouth with distilled water to get rid of debris and exfoliated cells. The inner cheek mucosa was gently scraped with the cytobrush to gather epithelial cells, which were then promptly placed into tubes filled with a fixative solution (methanol-acetic acid). To minimize variability and avoid contamination, stringent sample handling protocols were implemented. Standardized collection and fixation are crucial for increasing assay reliability, according to recent methodological recommendations (Fenech et al., 2025).

Microscopy and Staining

Giemsa stain, a popular cytological stain that makes nuclear structures easily visible, was

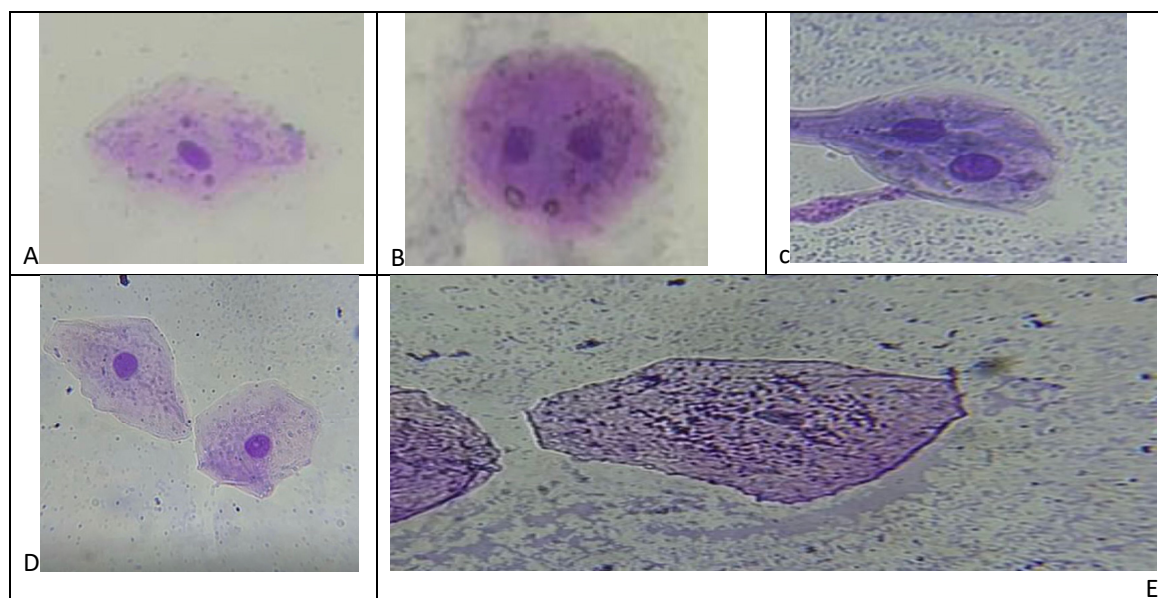


Fig. 2. Representative microscopic images of buccal epithelial cells observed in the present study using Giemsa staining under light microscopy (1000× magnification). (A) Early differentiated cell with micronucleus. (B) Binucleated cell. (C) Binucleated cell. (D) Basal cell. (E) Condensed chromatin cell.

used to stain the collected buccal cells. The fixed cells were smeared onto pristine glass slides, allowed to air dry, and then stained in accordance with standard procedures. Micronuclei, karyorrhexis, karyolysis, and pyknosis are examples of nuclear abnormalities that were scored at 1000× magnification using a light microscope. While karyorrhexis, karyolysis, and pyknosis were scored according to morphological criteria indicating nuclear fragmentation, dissolution, and condensation, respectively, micronuclei were recognized as small, round extranuclear bodies with staining characteristics similar to the main nucleus (Tolbert et al., 1992). To ensure statistical robustness, at least 2000 cells per participant were examined, and the scoring was carried out by qualified cytogeneticists. According to recent research, these endpoints are reliable for environmental biomonitoring (Baptista et al., 2023).

Quality Control

Two independent analysts blindly coded and scored the slides to reduce observer bias. Variability between observers was evaluated, and disagreements were settled by consensus. To verify staining and scoring methods, positive controls (cells treated with known genotoxic agents in pilot assays) and negative controls (unstained slides) were used.

Statistical Analysis

Chi-square tests were used to evaluate nuclear abnormalities and determine statistical significance.

RESULTS AND DISCUSSION

Representative microscopic images of buccal epithelial cells showing nuclear abnormalities are presented in Figure 2.

The demographic characteristics of the study participants are summarized in Table 1.

A major limitation of the present study is the difference in smoking status between exposed and control groups. Cigarette smoking is a well-established source of oxidative stress, DNA damage, and micronucleus formation. Therefore, the observed increase in nuclear abnormalities

Table 1. Demographic Characteristics of the Study Participants

Parameter	Control (n=35)	Exposed (n=35)
Age (years)	41.3 ± 7.2	43.1 ± 8.0
Weight (kg)	80.5 ± 10.6	83.2 ± 11.4
Male	20 (57%)	22 (63%)
Female	15 (43%)	13 (37%)
Smoking	0 (0%)	35 (100%)
Diabetes	3 (8.6%)	4 (11.4%)

Table 2. Frequency of Nuclear Biomarkers in Control and Exposed Groups

Nuclear biomarker	Control group (n = 35)	Exposed group (n = 35)	Chi-square (χ^2)	p-value
Micronucleus	1 (2.8%)	33 (94.3%)	54.2	<0.001
Karyolysis	3 (8.6%)	33 (94.3%)	45.7	<0.001
Karyorrhexis	5 (14.3%)	34 (97.1%)	42.9	<0.001
Pyknosis	2 (5.7%)	34 (97.1%)	50.6	<0.001

Table 3. Characteristics and Geographic Coordinates of the Sampling Locations

Sampling Site	Area Type	Latitude (N)	Longitude (E)	Group
Al-Kadhimiya	High traffic / Urban	33.3780	44.3386	Exposed
Al-Mansour	High traffic / Commercial	33.3119	44.3543	Exposed
Al-Jadriya	Urban / Traffic area	33.2775	44.3853	Exposed
Sadr City	High population density	33.3816	44.4652	Exposed
Al-Dora	Residential suburb	33.2306	44.3674	Control
Al-Ghazaliya	Residential area	33.3242	44.2738	Control
Al-Amiriya	Residential area	33.3075	44.2994	Control

may reflect the combined effects of environmental pollution and tobacco smoke exposure. Future studies should include matched smoking and non-smoking groups to better distinguish the contribution of each exposure source.

The nuclear abnormalities in buccal epithelial cells between a control group and an exposed group in Baghdad are summarized in Table 2. Micronucleus formation, karyolysis, karyorrhexis, and pyknosis were significantly more common in the exposed group than in the control group. As shown in Table 2, statistical analysis using chi-square tests verified that these differences are highly significant ($p < 0.001$) across all biomarkers, indicating strong evidence of genotoxic stress in the exposed population.

Micronucleus formation, karyolysis, karyorrhexis, and pyknosis are examples of genotoxicity biomarkers that are commonly used to indicate DNA damage and cell death. In particular, the micronucleus assay is a validated technique for identifying exposure to environmental mutagens and chromosomal instability. In this study, a control group and an exposed group of adult residents of Baghdad, Iraq, were compared for cytogenetic damage. The geographical characteristics of the study locations are presented in Table 3. Sampling sites were selected to represent areas with different levels of environmental pollution, including highly trafficked and industrialized urban districts (exposed group) and relatively less polluted residential areas (control group).

Interpretation of the data reveals notable distinctions between the two groups. The exposed group had very high frequencies ($\geq 94\%$), whereas the control group had very few nuclear abnormalities ($\leq 14.3\%$). These differences strongly imply that severe genotoxic stress is linked to environmental exposure in Baghdad. 94.3% of exposed individuals had an increased micronucleus frequency, which is consistent with chromosomal mis-segregation and DNA strand breaks. Pyknosis and karyolysis, both recognized indicators of necrotic cell death, were observed in more than 94% of exposed individuals, indicating extensive nuclear deterioration and

Table 4. Comparison of Cytogenetic Biomarkers in Buccal Cells Between Control and Pollutant-Exposed Individuals in Baghdad

Result	Biomarker	Population	Study
Increased	Micronucleus	Painters	Khan et al. 2010
Increased	Micronucleus	Traffic police	Hamzah et al. 2018
Increased	Cytogenetic damage	Healthcare workers	Baptista et al. 2023
Highly increased	Micronucleus	Baghdad residents	Present study

severe cytotoxic stress. Nearly every exposed person had karyorrhexis nuclear fragmentation, which is indicative of necrotic and apoptotic processes.

The current results support earlier studies showing the value of micronucleus assays as sensitive genotoxicity biomarkers. Although their findings showed less noticeable differences between exposed and control groups, Hamzah et al. (2018) reported higher micronucleus frequency among traffic police officers in Baghdad, indicating that exposure duration and intensity may modulate the degree of nuclear damage. The sensitivity of micronucleus assays in identifying DNA damage caused by engineered nanomaterials was highlighted more recently by Farabaugh et al. (2023), supporting the biomarker's resilience in a variety of toxicological contexts. Simultaneously, Harte et al. (2024) developed a multi-biomarker micronucleus assay using imaging flow cytometry, which allowed for the simultaneous detection of multiple nuclear abnormalities and confirmed its applicability in environmental toxicology.

The frequencies of nuclear abnormalities observed in buccal epithelial cells among the control and exposed groups are presented in Table 4. The results indicate a markedly higher occurrence of micronucleus formation, karyolysis, karyorrhexis, and pyknosis in individuals exposed to environmental pollutants compared with the control group. Statistical analysis using the Chi-square test confirmed that these differences were highly significant ($*p < 0.001$), indicating substantial cytogenetic damage associated with environmental exposure.

In toxicological investigations, morphological nuclear alterations like pyknosis, karyolysis, and karyorrhexis have been confirmed as trustworthy markers of necrosis and apoptosis in addition to micronucleus formation (Nimr et al., 2024; Obaid et al., 2026). These changes are commonly known as markers of cell death pathways and reflect basic processes of chromatin condensation, nuclear fading, and nuclear fragmentation, respectively (Elmore, 2007). These nuclear anomalies provide complementary evidence of cytotoxicity and genotoxic stress by reflecting underlying cellular mechanisms of nuclear disintegration and DNA fragmentation (Halder et al., 2024).

The elevated nuclear abnormalities seen in the exposed group could be explained by a number of mechanisms. It has been demonstrated that air pollution, specifically heavy metals, fine particulate matter (PM), and polycyclic aromatic hydrocarbons (PAHs), can cause oxidative stress, DNA adduct formation, and epigenetic changes, all of which can result in chromosomal instability (DeMarini, 2013). Studies relating traffic-related air pollution to elevated DNA damage biomarkers have shown that occupational hazards, such as long-term exposure to emissions from traffic and industrial pollutants, further increase genotoxic risk (Pinto et al., 2024; Salem et al., 2022). Additionally, prolonged oxidative stress produces reactive oxygen species (ROS), which cause DNA fragmentation and nuclear architecture instability, leading to necrosis or apoptosis (Garcia-Llorens et al., 2025; MDPI, 2024; Fiorin et al., 2021).

CONCLUSION

The present findings demonstrate significantly elevated frequencies of micronuclei and other nuclear abnormalities among individuals living in highly polluted urban environments. However, because smoking status differed substantially between study groups, the observed cytogenetic damage may reflect the combined influence of environmental pollution and

tobacco smoke exposure. Further studies incorporating matched smoking status and direct environmental measurements are required to clarify the specific contribution of each factor. Chronic oxidative stress, occupational hazards, and air pollution are all closely associated with elevated nuclear abnormalities in exposed groups. These results highlight the critical need for protective measures and environmental monitoring in high-risk populations. To evaluate long-term cancer risk and protect public health, biomarker-based surveillance is crucial.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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