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Comparison of Buccal Mucosal Epithelial Cell Analysis of Communities Around and Outside the Medan City Waste Landfill

T.Kemala Intan[1*] Universitas Sumatera Utara, Indonesia
Zulham Yamamoto[2] Universitas Sumatera Utara, Indonesia
Causa Trisna Mariedina[3] Universitas Sumatera Utara, Indonesia
Indryani Rachman[4] University of Kitakyushu, Japan
Matsumoto Toru[5] University of Kitakyushu, Japan

***Corresponding author:**
T.Kemala Intan, Universitas Sumatera Utara, Indonesia. ✉t.kemala@usu.ac.id

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Abstract

Background: People living around waste final processing sites (*TPAs*) have the potential to experience chronic exposure to environmental pollution that can have an impact on health. Exposure to various pollutants from waste processing activities has the potential to cause cellular damage, including in buccal mucosal epithelial cells that can be used as biomarkers of cytogenetic damage.
Objective: This study aims to examine the damage to buccal mucosal epithelial cells in people living around the Medan City Landfill and compare it with people who do not live in the area.
Methods: This study used a cross-sectional design involving 100 respondents divided into two groups: the community living around the landfill (50 subjects) and the control group not living near the landfill (50 subjects). Buccal mucosal epithelial cells were collected using the cytobrush technique and stained using the Papanicolaou method. Analysis was carried out on the frequency of pyknosis, karyorrhexis, and karyolysis as cytogenetic biomarkers, with adjustment for potential confounders including sex, age, and duration of residence, then compared between the two groups.
Results: The results showed that the frequency of buccal mucosal epithelial cells exhibiting pyknosis, karyorrhexis, and karyolysis in the community living around the landfill did not show a statistically significant difference compared to the control group ($p > 0.05$ for all cytogenetic endpoints).
Conclusion: There was no significant difference in the frequency of cytogenetic endpoints (pyknosis, karyorrhexis, and karyolysis) of buccal mucosal epithelial cells between people living around the Medan City Landfill and those who did not. These findings indicate no evidence of significant genotoxicity based on the analyzed cytogenetic parameters in the studied population. However, continued environmental monitoring and longitudinal studies with larger sample sizes are recommended to detect subclinical cellular changes at earlier stages.

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INTRODUCTION

According to the Environmental Protection Agency, particulate matter and gas emissions come in a variety of sizes, shapes, compositions, and origins. The size of these particles causes deposits in the respiratory tract and results in constriction of the respiratory tract, increased asthma, and malignant diseases (Gurjar et al., 2010; Manisalidis et al., 2020; Wallbanks et al., 2024). The World Health Organization estimates that there were 865,000 premature infant deaths due to ambient air pollution in China and India. A case of landfill contamination involving one hundred people living 100–500 meters from the Thohoyandou Landfill, Limpopo Province, South Africa, proves that 78% of the community is disturbed by bad smell accompanied by eye irritation, shortness of breath, and fatigue (Njoku et al., 2019).

High emissions of SO2, NO2, O3, CO, and NH3 gases cause increased airway abnormalities and abnormalities in the oral cavity. Likewise, researchers in Lagos, Nigeria, studying adolescent workers at open electronic waste recycling sites showed an increase in heavy metals Pb, Cd, Cr, and Ni and a positive correlation with a decrease in buccal cell micronuclei, the number of buccal cells that are karyolytic and pyknotic, and DNA damage. In Indonesia, especially the Final Processing Site (TPA) of waste in Ward I, Terjun Village, Medan Marelan District, Medan City, it was found that the ambient air level at the landfill site had exceeded the threshold limit values that interfere with health, including the emission of SO2, NO2, CO, O3, and HC gases, and this situation causes an impact on the environmental health of the community at landfill locations (Alabi et al., 2020; Berame et al., 2020). Gas emission components and particulate matter due to landfill activity generate carcinogenic compounds that increase the transformation of buccal mucosal epithelial cells to dysplastic forms, with a large number of black dots in the nuclei, especially in recycling workers at the landfill (Intan et al., 2024). Particulate polycyclic aromatic hydrocarbons (PAHs) from industrial and waste combustion emissions have also been associated with genotoxic damage in buccal mucosal epithelial cells, as demonstrated in children living near petrochemical industries (Sopian et al., 2021). It is possible that people living adjacent to the landfill area are also groups exposed to landfill activities. Exposure to TPA conditions that have an impact on lung disorders, airway damage, and an abnormal increase in micronuclei of epithelial cells is a biological sign that can be observed in a person or a population that is exposed due to a suspected mutagenic, genotoxic, or teratogenic event. Nuclear abnormalities indicate chromosomal damage, which can be detected through degenerative analysis of the nucleus that indicates cell death, namely pyknosis, karyolysis, and karyorrhexis. Exposure to air pollutants emanating from industrial sites has been associated with genotoxic damage in buccal mucosal cells, including elevated nuclear anomalies observed in school children exposed to oil field emissions (Sabah, 2021). Damage analysis was carried out by examining cells that underwent pyknosis, namely cells with a shrinking cell nucleus size and chromatin with a dense and shapeless mass; karyolysis cells were identified with cytoplasmic areas which are the size of terminal cells differentiated by the dissolution of the nucleus, a ghost-like image of the nucleus, and enlargement of chromatin basophilia; while karyorrhexis is characterized by fragmentation and irregular condensation of chromatin within the nucleus, where the nuclear perimeter appears smooth and distinct. This study aims to analyze cytogenetic damage in the form of pyknosis, karyolysis, and karyorrhexis in buccal mucosal epithelial cells of people living around the Final Processing Site of Medan City.

METHOD

Design, Subject, and Location of the Research

The research population was an adult community living around the Waste Disposal Site in Terjun Sub-district, Medan Marelan District, Medan City, selected based on an area map determined after mapping house locations and completing questionnaire instruments. Respondents did not consume alcohol and had no oral abnormalities or systemic diseases (diabetes, heart disease, or cancer). Data were collected using purposive random sampling. Respondents were enrolled after providing approval and signing consent for all research procedures, which were approved by the Ethics Commission of the University of North Sumatra No. 1048/KPEK/USU/2024.

The sampling location was around the Medan City waste landfill, which is affected by particulate matter pollutants (Intan et al., 2023). The control location was Medan Labuhan District, outside the Medan City waste landfill, which is not exposed to pollution; ambient air samples were tested at sampling points near Salsabila School using the Air Quality Detector method for PM2.5, and the results were within normal limits, as documented in Test Result Certificate No. 205/Dis.LHKSU-UPTD.LL/U/XI/2025, dated November 21, 2025, issued by the North Sumatra Provincial Environment and Forestry Service UPTD Environmental Laboratory, Jalan H.M. Said No. 25, Medan.

Buccal mucosal epithelial cells were collected using the cytobrush swab technique at the time of meeting at the location around the landfill. Subjects first rinsed their mouths with water to clear the sampling site before collection. Prior to sampling, the cytobrush was moistened with 0.9% NaCl solution (Poluan & Marlina, 2021).

The cytobrush is used to collect cells from the oral buccal mucosa for deep sample collection. This method employs non-invasive tools, at very little cost, is time-efficient for evaluating cytological changes, and is relatively easy to interpret. After sweeping, the cells adhering to the cytobrush were transferred onto a glass slide by rotating it at least 360° in the opposite direction. Fixation was then carried out by applying a solution of 95% ethanol and glacial acetic acid (3:1) onto the glass slide. Staining was performed using the *Papanicolaou* staining technique (Tolbert et al., 1992).

Cytological analysis was carried out by examining cells undergoing pyknosis, karyorrhexis, and karyolysis (Tolbert et al., 1992). The criteria for assessing cells undergoing pyknosis are: cells with a shrinking nucleus and chromatin condensed into a dense, shapeless mass. Cells undergoing karyolysis are identified by dissolution of the nucleus producing a ghost-like nuclear image, accompanied by loss of basophilic chromatin staining. The assessment of karyorrhexis was based on nuclear fragmentation, intact cytoplasm, and a relatively flat cell position, with smooth and distinct nuclear margins. Pyknotic, karyorrhectic, and karyolytic cells were quantified per 1,000 cells using a light microscope at 1,000× magnification. Cytological analysis of buccal mucosal epithelial cells was carried out independently by two examiners using a blinded method (Tolbert et al., 1992). The assessment criteria for cytological features of buccal epithelial cells were further validated with reference to established parameters for evaluating individual functional status and cellular integrity (Shokabayeva et al., 2025).

Data Analysis

Data were analyzed using SPSS version 20. Normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Continuous data were presented as mean ± standard deviation (SD) or median (IQR) depending on data distribution, and categorical data as n (%). For normally distributed continuous variables, an independent t-test was applied; for non-normally distributed variables, the Mann-Whitney U test was used. Categorical variables were compared using the Chi-square test. The significance level was set at $p < 0.05$. The frequencies of pyknotic, karyorrhectic, and karyolytic cells were compared between groups.

RESULTS AND DISCUSSION

Results

One hundred respondents were divided into two groups (control group, community groups around the landfill) with the same proportion. Most respondents were female, 66 (66%) followed by 34 (34%) males. The age range of respondents was 18–65 years old with a mean of 39.2 ± 10.3 years. Generally, people have lived in each location for 5–20 years. Table 1 shows that, among all subjects, an average of 57.2±12.8 pyknotic cells was found, followed by 55.3±12.9 karyorrhectic cells and 52±14.2 karyolytic cells, while the average number of negative findings i.e., subjects in whom pyknotic, karyorrhectic, and karyolytic cells were not detected per 1,000 cells was 8.5±9.1.

Table 1. Respondent Characteristics

No.	Characteristics	Value/Frequency
1	Gender; n (%)	
	a. Male	34 (34)
	b. Women	66 (66)
2.	Age; average ± elementary school (years)	39.2 ± 10.3
	a. 18–65 years old (full range)	10 (10)
3.	Long Residency; n (%)	
	a. <5 years old	2 (2)
	b. 5 – 10 years	13 (13)
	c. 10 – 15 years old	18 (18)
	d. 15 – 20 years	14 (14)
	e. > 20 years old	53 (53)
4.	Picnics	
	Average ± Elementary School	57.2 ± 12.8
	Median (IQR)	56 (46 – 69,5)
5.	Karyolysis	
	Average ± Elementary School	52 ± 14.2
	Median (IQR)	52 (44 – 57)
6.	Karyorexis	
	Average ± Elementary School	55.3 ± 12.9
	Median (IQR)	53 (44,5 – 62)
7.	Negatives	
	Average ± Elementary School	8.5 ± 9.1
	Median (IQR)	8 (0 – 14,5)

S.D. : A Normal Deviation
n (%): number of respondents and percentage per group

Painting observation results *Papanicolau* (Figure 1) there is a change in the nucleus. Figure 1(A) of the epithelial cells of the buccal mucosa one field of view with a total of 10 cells is found to be characterized by nucleus fragmentation(B)..... (C)....

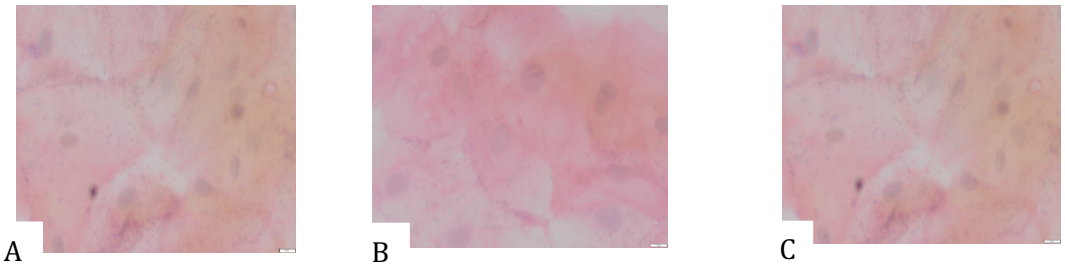

Figure 1. Micrograph photo (A). Epithelial cells of the carioxious buccal mucosa, nucleus fragmentation(1000x), (B)keriotic epithelial mucosa cells of the keriolizes, cytoplasm without nucleus(1000x) (C). Epithelial cells of the buccal mucosa pynosis (1000x)

Table 2 shows a comparison of the respondent characteristics and the number of damaged cells. The proportion of male respondents at the landfill site was 44.1% and female 13.6%, while at the location outside the landfill, male respondents accounted for 55.9% and female 86.4%. This difference indicated that the community surrounding the landfill in this study was predominantly male ($P<0.05$), as they also worked around the landfill site. The age range of the respondents showed a non-significant difference between the two locations ($p = 0.627$; $P>0.05$). The length of residence between the two groups of respondents at the two different locations also showed a non-significant difference ($p = 0.362$; $P>0.05$). Pyknotic cell damage in respondents living around the landfill compared to those living outside showed no significant difference ($p = 0.456$; $P>0.05$).

Cells undergoing karyolysis between the two locations were likewise non-significant (p = 0.087; P>0.05), and karyorrhexis cells between the two sites yielded p = 0.726 (P>0.05). Similarly, observations of cells with no nuclear abnormalities—that is, without pyknosis, karyolysis, or karyorrhexis—were non-significant (p = 0.342; P>0.05). Therefore, no significant difference was found in the number of cells exhibiting pyknosis, karyorrhexis, karyolysis, or no nuclear abnormalities between individuals living around the landfill and those living outside it.

Table 2. Comparison of Respondent Characteristics and Frequency of Cytogenetic Biomarkers (pyknosis, karyorrhexis, and karyolysis) Between Groups

No.	Characteristics	Place to Stay				
		Around the Landfill		Non-TPA		
		n	%	n	%	
1	Gender; n (%)					
	a. Male	15	44,1	19	55,9	0,001*
	b. Women	9	13,6	57	86,4	
2.	Age; average ± elementary school (years)	40.1 ± 10.8		38.9 ± 10.1		0.627⁂
3.	Long Residency; n (%)					
	a. <5 years old	1	4,2	1	1,3	0.362⁂
	b. 5 – 10 years	2	8,3	11	14,5	
	c. 10 – 15 years old	6	25,0	12	14,8	
	d. 15 – 20 years	5	20,8	9	11,8	
	e. > 20 years old	10	41,7	43	56,6	
4.	Picnics					
	Median (IQR)	59 (50 – 66)		53,5 (45 – 72)		0.456⁂
5.	Karyolysis					
	Median (IQR)	47 (36,5 – 54,5)		52 (45 – 57,5)		0.087⁂
6.	Karyorexis					
	Median (IQR)	53 (50,5 – 61,5)		53,5 (43 – 62,5)		0.726⁂
7.	Negatives					
	Median (IQR)	12 (0 – 21)		6,5 (0 – 13)		0.342⁂

* Chi Kudarat test; ** Independent t-test; ⁂ Mann-Whitney U Test

Discussion

The Medan City Waste Final Processing Site (TPA) is a dense area with waste transportation activities from 21 sub-districts of Medan City, causing pollution that results in the surrounding community being chronically exposed. Several studies have proven that changes in buccal mucosal cells in recycling workers show that mutagenic and genotoxic events occur in recycling workers, with the discovery of black dots in the nucleus of buccal mucosal epithelial cells via AgNOR staining. Studies of communities living around landfill areas that are exposed to landfill activity through analysis of buccal epithelial cells can provide an initial picture of nuclear abnormalities before the change towards dysplasia, where karyolysis and karyorrhexis are indicators of apoptosis (Burns & Bernicker, 2023). Supporting this, studies of residents near the Medan City Landfill demonstrated significant alterations in HSP70 expression and Bethesda classification categories in buccal mucosal cells, reflecting adaptive cellular responses to genotoxic pollutant exposure (Ramadansyah et al., 2026).

Pollution particles are inhaled in two ways, namely through the airways and oral cavity, and induce direct and indirect DNA damage and modify epigenetics. Direct effects are generally related to electrophilic agents that interact with nucleophilic DNA and result in DNA damage. DNA genotoxicity is mainly caused by the oxidative stress mechanism and the activation of metabolites due to the absorption of organic components from particulate matter. Indirect genotoxicity is mainly related to intracellular systems such as the DNA repair system, telomerase, and mitosis,

epigenetic DNA methylation processes, modification of histones, nucleosomes, and non-coding RNAs, especially the expression of microRNA. The term "oxidative stress" refers to a disturbance of the oxidant/antioxidant balance that has the potential to cause damage. Oxidative stress depends on the increase in the availability of reactive oxygen/nitrogen species (ROS/RNS).

Reactive oxygen species (ROS) are species with unpaired electrons on their outer shell that readily react with adjacent molecules. Some are free radicals, such as the superoxide anion ($O2•^-$) and hydroxyl radical (•OH), and non-radical species such as hydrogen peroxide (H2O2). ROS play a role in various biological processes such as aging, inflammation, carcinogenesis, and atherosclerosis. Excessive reactive O2 metabolites cause activation of various signaling pathways, namely the Nrf2 pathway, NF-κB, and AP-1, and promote programmed cell death via apoptosis. Buccal cells are early tissues that can serve in the evaluation of cytotoxic and genotoxic effects and are impacted by changes in the epithelial cells of the buccal mucosa upon chronic exposure to pollution. The genotoxic potential of exogenous agents acting on oral mucosal epithelium has further been demonstrated through in vitro studies, wherein nanoparticles such as food-grade titanium dioxide were found to translocate across buccal mucosa and induce DNA damage in oral epithelial cells (Vignard et al., 2023).

The results of the study show that the existence of landfills that are not managed properly causes disturbances to the environment and public health (Ferronato & Torretta, 2019). In addition, the community around the landfill is a community that is exposed to particulate matter and other pollutants, so it requires special attention because it is related to the level of public health (Arumdani et al., 2021). Several studies on children under the age of 3 years have also found respiratory tract disorders and abnormalities in buccal epithelial cells due to PM10 or other particulate matter pollutants, including among recycling workers at several landfills in New Delhi. Similarly, genomic instability assessed in buccal mucosal cells of children living in environmentally degraded areas revealed elevated frequencies of nuclear anomalies, underscoring the vulnerability of populations in proximity to industrial pollution sources (Alpire et al., 2021).

According to research conducted by Chu et al. (2019), the oral cavity is theoretically one of the pathways for PM2.5 inhalation into the lungs, where it settles in the alveoli; the study found that PM2.5 and ozone are significantly associated with an increased incidence of oral cancer in the Taiwanese population, even after accounting for known risk factors including smoking and betel nut chewing, both of which also have significant associations with oral cancer. Several studies have shown the impact of pollution on changes in buccal mucosal epithelial cells. Although in this study the number of pyknosis, karyorrhexis, and karyolysis cells did not show statistically significant differences between groups, the absence of significant differences may be attributed to several factors: (1) the relatively limited sample size (n=50 per group), which may lack statistical power to detect small effect sizes; (2) individual variability in cellular response and antioxidant capacity; (3) potential confounding effects of other exposures such as diet, smoking, and occupational hazards not fully controlled in this study; and (4) the cross-sectional nature of the study, which limits causal inference.

Changes in the nucleus of buccal mucosal epithelial cells in the form of karyorrhexis and karyopyknosis due to smoking have also been found (Harissa, 2025). Nuclear morphological alterations including pyknosis and karyorrhexis have additionally been observed in oral mucosal epithelial cells exposed to electromagnetic radiation from mobile devices, indicating that diverse environmental agents can produce quantifiable cytogenetic changes in buccal cells (Pakpahan & Hartono, 2025). Likewise, pyknotic cells were found in the preparations of the buccal mucosa of betel nut chewers (Triani et al., 2022). Research on HSP70 expression changes in residents around the Medan City Landfill was also found to be significant; the low HSP70 expression pattern indicates that people exposed to genotoxic agents at the landfill site exhibit an adaptive response to surrounding pollutants (Ramadansyah et al., 2026).

In this study, several respondents' cells showed a frequency of normal (negative) cell images, indicating that each cell has a different resistance to exposure to environmental toxic agents. The existence of evidence of damage in these two groups indicates that awareness of exposure to toxic agents is necessary, and cytology examination of the oral cavity can serve as a preliminary examination for the early detection of cell changes due to exposure to genotoxic

agents (Ramadansyah et al., 2026). Other genotoxic materials can come from e-waste, where the accumulation of heavy metals impacts the formation of micronuclei in buccal mucosal epithelial cells of waste recycling workers (Alabi et al., 2020; Berame et al., 2020).

CONCLUSION

The impact of exposure to genotoxic agents with various exposure risks, especially in people living around the Medan City landfill location on buccal mucosal epithelial cells has a risk of cytogenetic endpoints in buccal mucosal epithelial cells. The frequency of pyknosis, karyorrhexis, and karyolysis did not differ significantly between the exposed and control populations, indicating no evidence of significant genotoxicity from landfill exposure based on the parameters analyzed. Future studies should employ larger sample sizes, longitudinal designs, and multivariate analysis to account for confounding variables. Routine cytological surveillance of populations near waste disposal sites is recommended as part of integrated environmental health monitoring programs.

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AUTHOR CONTRIBUTION STATEMENT

T. Kemala Intan plays a role in conceptualizing research, designing methodologies, collecting data, analyzing cytogenetics, and preparing an initial draft of the manuscript. Zulham Yamamoto contributed to the histological analysis and validation of the research methodology. Causa Trisna Mariedina plays a role in the analysis of anatomical pathology as well as the interpretation of research results. Indryani Rachman contributes to environmental analysis and research data processing. Matsumoto Toru provides academic supervision, scientific validation, and critical review of the manuscript until the final version is ready for publication.

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