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Correlation between Particulate Matter 10 (PM_{10}) Exposure Time and Black Dots in Buccal Cells of Medan Landfill Recycling Workers

T. K. Intan*[1,2], M. Ichwan[3,4], D. Munir[5], N. N. Soeroso[6], A. P. Tarigan[6], Z. Yamamoto[7], N. C. Siregar[8]

[1]Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
[2]Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
[3]Department of Pharmacology & Therapeutic, Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
[4]Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
[5]Department of Ear, Nose and Throat, Head and Neck, Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
[6]Department of Pulmonology and Respiratory, Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
[7]Department of Histology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
[8]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia.
*Corresponding Author.

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Abstract

This research explores the link between particulate matter (PM10) exposure and number of black dots in buccal epithelial cell nuclei in recycling workers (PDU), at the Medan City landfill, Indonesia. The study aims to identify the correlation PM10 levels, pollutant characteristics at the landfill, the appearance of AgNOR-expressed black dots in PDU groups. The measurements of PM10 exposure were carried out in open landfills in Medan city, and the subject of study is recycling workers who worked in landfills. Buccal epithelial cells were collected using an oral cytobrush smear, and the black dots were examined cytologically. Statistical analysis assessed the relationship between prolonged PM10 exposure and the number of black dots. The study found that most recycling workers were older than 55 (36%), with significant pollution exposure effects indicated by a higher average number of black dots in their buccal cells compared to non-recycling workers (BPDU), with the highest numbers in the 36-45 and 46-55 age groups (each 30%). When looking at their work hours, more than half of BPDU workers (52%) worked for 8 hours a day, and the majority of PDU also worked for the same duration of time. This suggests an association between their occupational exposure to environmental pollutants and health impacts, also considering age and work hours. The findings underscore the need to factor in these aspects when addressing worker health in polluted environments.

Keywords: AgNORs, black dots, buccal epithelial cells, early detection, malignancy, Open landfill, recycling workers.

Introduction

Indonesia, with a population of 251 million people, plays a substantial role in waste production in Asia, contributing 0.7 kg/person/day. A study revealed that Medan ranked fourth among Indonesian cities, generating 3,730,953.8 kg/day of urban waste, following Jakarta and Surabaya [1]. Across various

Indonesian cities, a comprehensive analysis detected 15 heavy metals in ambient air, including sodium, calcium, magnesium, and zinc, all of which have associations with morbidity [2]. Open urban waste landfill sites are significant sources of anthropogenic gas emissions, particularly methane and particulate matter (PM_{10}), resulting from incomplete waste combustion [3]. This incomplete combustion leads to environmental pollution, encompassing human, animal, and natural ecosystems. Studies in India, Indonesia, and the Philippines identified carcinogenic substances such as aldrin, asbestos, cadmium, chromium, mercury, and volatile inorganics in landfill environments [4–6]. Therefore, it is necessary to investigate the emissions of hazardous gases and pollutants in Indonesia as these have adverse effects on human health, particularly about respiratory disorders and the potential risk of cancer in those exposed.

Landfill-generated particulate matter 10 (PM_{10}), particles with a diameter of 10 micrometers or less, pose respiratory risks. Exposure dosage depends on PM_{10} concentration, aerodynamic diameter, deposition rate, and respiratory system cleaning mechanisms. PM_{10} exposure has been linked to respiratory problems and changes in cells of the nose and throat, with studies in Nigeria and India showing a connection to nose and throat cancers. Workers at recycling landfill sites experience lung function issues and cell alterations [7,8]. Different types of elements in PM_{10} and combustion emissions are what lead to molecular-level studies of black dots that appear after being exposed to PM_{10} and black carbon in the air [9]. Polycyclic aromatic hydrocarbons in ambient air, considered carcinogenic, exhibit mutagenic and epigenetic effects by binding with DNA, RNA, and cellular proteins [10]. Chemical pollutants in the environment kill over 13 million people every year through epigenetic processes like changes in DNA methylation, histone modification, and microRNA. This causes global hypomethylation, which is linked to many types of cancer [11]. PM_{10} exposure is linked to hypermethylation of genes like p16 (ink4a), APC, SYK, and CCND2 [12]. A previous study suggests using samples from lung or nasal organs as biomarkers for detecting the impact of environmental pollutants, including metals like Cd, As, Co, and Cr, on humans [13]. Some studies have found that environmental pollution causes over 13 million deaths annually, with 24% linked to epigenetics, including DNA methylation, histone modification, and microRNA, playing a crucial role as etiological factors in various diseases [12,14,15]. Scientists are still looking into the molecular mechanisms that make PM_{10} cause cancer. They are focusing on epigenetic changes and the possibility of using biomarkers to find out how environmental pollutants affect people's health.

The oral cavity, as the first entry point for various harmful substances, reflects systemic conditions, and its moist mucosal layer serves as the primary barrier against potential hazards like microorganisms or toxic substances. The oral epithelial cells, observed for early genotoxic events induced by carcinogenic agents through inhalation and ingestion, are crucial targets of karyolysis [16]. Using a cytobrush and Papanicolaou staining to look at the cytogenetics of buccal epithelial cells from fuel station workers in Yogyakarta showed a lot of cytogenetic damage, such as pyknosis, karyorrhexis, and karyolysis [16]. Prasanna and team conducted a study on the proliferation index of buccal epithelial cells, analyzing 90 samples from normal, smoker, and betel chewer groups to demonstrate the utility of Argyrophilic Nucleolar Organizing Regions (AgNORs) cytology staining as a method to screen large populations likely to be exposed to carcinogenic pollutants, such as PM_{10} [17]. The argyrophilic protein selectively binds with silver and two proteins involved in tRNA transcription, resulting in the formation of black dots. Therefore, the expression of black dots via AgNORs in a nucleolus cell could serve as an indicator of the binding of carcinogenic pollutants [18]. Recycling workers, who are at high risk of carcinogenic exposure in landfills, are informal workers reliant on surrounding litter for their livelihood. Consequently, understanding any associations linked to cancer development is crucial, emphasizing the need for effective strategies to protect public health, which remains a major concern.

This study aims to investigate the relationship between PM_{10} and the expression of buccal epithelial cells of waste recycling workers (PDU) in landfill sites, specifically in Indonesia, where such research is lacking. The goal is to identify the correlation and ratio of PM_{10} and pollutant characteristics at the Medan City landfill site concerning the number of black dots in the nuclei of buccal epithelial cells in the recycling workers' group (PDU). The research results will help us to understand the meaning of the black dots found in the nuclei of buccal epithelial cells, as shown by AgNORs staining. This is especially important for the high-risk PDU group in Indonesia, especially in Medan, where the Medan City landfill site raises the risk of cancer.

Materials and Methods

Research Design

This cross-sectional study looks at how exposure to PM_{10} affects the number of black dots found in the nuclei of buccal epithelial cells using AgNORs staining in recycling workers.

Research Population and Sample

The research population comprises recycling workers at the landfill site in Medan, specifically in Desa Terjun, Sub-district Medan Marelan, Medan city, based on predefined geographical maps. Respondents were selected based on PM_{10} concentration mapping, and their willingness to participate served as the sampling criterion. The control group consists of non-recycling community members located in Medan city districts with normal PM_{10} concentrations.

The study sample included recycling workers (PDU) at the landfill site in Desa Terjun, Kecamatan Medan Marelan, Kota Medan, meeting inclusion and exclusion criteria. The control group consisted of non-recycling community members (BPDU) outside the landfill site with normal PM_{10} concentrations, meeting the following inclusion criteria: (1) Working at the final waste disposal site in Kelurahan Terjun, (2) Employment status as recycling workers, whether permanent or temporary, (3) Age between 18 and 55 or more, (4) Work experience of 5 years or more, (5) Both male and female, (6) Providing written consent after receiving detailed explanations about the research (informed consent). Exclusion criteria include recycling workers with a history of or currently experiencing diseases such as tuberculosis, cancer, during pregnancy, and/or lactation.

Sample size calculations were performed for each variable under examination. For the first variable, investigating the relationship between PM_{10} exposure and black dots occurrence in recycling workers at the landfill, the sample size was calculated using the following equation based on a previous study[19] to satisfy statistical analysis for unmatched categorical data analysis:

$$n1 = n2 = 2\left(\frac{(Z_\alpha + Z_\beta)S}{x_1 - x_2}\right)^2$$

With n as the sample size needed; Z_α is the critical value from the standard normal distribution corresponding to the desired significance level α, equal to 1.96; Z_β is the critical value corresponding to the probability of a Type II error; S is the pooled standard deviation of the outcomes; x expected means. Thus, the required sample size is 47 individuals.

PM_{10} Data Collection

The data collection methods for PM_{10} in this study involve both primary and secondary data. Secondary data include the quantity and types of waste transportation equipment, wind speed and direction, and surrounding data around the landfill site in Desa Terjun, TPA Terjun. Primary data encompass the concentration of pollutant parameters such as SO_2, CO, NO_2, O_3, HC, and PM_{10} at the landfill location. The table 1 below outlines the methods used for collecting primary data:

Tabel 1. Methods for collecting primary data on air sampling

No	Parameter	Metode
1	PM_{10}	Air Quality Detector
2	Carbon Monoxide (CO)	Air Quality Detector
3	Nitrogen Dioxide (NO_2)	*SNI 7119-2:2017
4	Sulfur Dioxide (SO_2)	*SNI 7119-7:2017
5	Ozone (O_3)	*SNI 7119-8:2017
6	Hydrocarbons (HC)	Air Quality Detector

*SNI = Indonesian National Standard

The parameters for PM_{10} were assessed in accordance with the Indonesian Standard Air Quality Ambient guidelines measured by the local staffs of the Local Environmental Division Services, as outlined in Appendix VII of Government Regulation No. 21 of 2021. The pollutants were quantified using the equation:

$$C_2 = C_1 \left[\frac{t_1}{t_2}\right]^p$$

In this equation, C_1 represents the pollutant parameter concentration per hour (μg/m³), t_1 is equivalent to one hour, t_2 is 24 hours, and p is a constant with a value of 0.18.

The concentrations of CO, NO_2, SO_2, O_3, and HC were determined according to the air pollutant standard index issued by the Indonesian Ministry of Environment and Forestry, No. 14 of 2020. This is calculated as follows:

$$I = \left(\frac{I_a - I_b}{X_a - X_b}\right).(X_x - X_b) + I_b$$

In this equation, APSI is an integer; I_a and I_b are the upper and lower limits, respectively. X_a is the upper limit of ambient concentration (μg/m³), X_b is the lower limit of ambient concentration (μg/m³), and X_x is the real-time measurement of ambient concentration (μg/m³).

Procedure for buccal epithelial cells and AgNORs staining

The procedure for collecting cytological specimens of buccal epithelial cells and AgNORs staining involves the removal of specimens by healthcare professionals, both at the landfill site and in the community. The AgNORs staining examination is conducted at the Pathological Anatomy Laboratory, Faculty of Medicine, University of Sumatera Utara (FK USU). The interpretation of staining results and cytology specimen evaluation were evaluated, assisted by a pathology expert.

Before buccal epithelial cell samples are taken, research subjects go through a full oral hygiene routine. This includes gargling to get rid of extra saliva and oral debris. Subsequently, subjects follow up with gargling using betadine mouthwash after the sample collection. The necessary tools for buccal epithelial cell smear collection, such as a cytobrush, glass slides, fixation alcohol solution, gloves, and a storage box for microscopic specimens, are then prepared. The cytobrush procedure is conducted by placing the brush in the left and right buccal cavities, rotating it 360° several times, and gently applying the brush to the glass slide in a circular motion. Following the procedure, the glass slide is immersed in an ethanol solution for 15 minutes, and the specimen is air-dried before storage for subsequent staining.

Following the collection of cytological smears from the oral cavity, the specimens undergo a meticulous staining process for AgNOR analysis. The specimens are immersed in a silver staining solution, comprising 1 part 2% gelatin in 1% formic acid and 2 parts 25% silver nitrate solution, maintained at 37°C for precisely 11 minutes. The staining reaction is carefully halted by washing the slides with distilled water to eliminate any non-specific silver precipitate. Following that, there is a quick counterstaining step using light green for one minute and then a running water rinse. Gradual dehydration of the specimens is achieved through a series of ethanol concentrations (50%, 70%, and 95%). Subsequently, the slides are air-dried to completion before being mounted for microscopic examination. The final step involves scrutinizing the specimens under a light microscope at a powerful 1000x magnification. The evaluation focuses on quantifying AgNOR parameters, with particular attention to AgNOR, requiring the enumeration of cells exhibiting more than 5 black dots in every 100 observed cells.

Statistical Analysis

The gathered data will be subjected to analysis using the statistical program SPSS v20. Univariate analysis will be conducted to obtain mean values, standard deviation, median, minimum, and maximum values. Prior to this, all data will undergo a normality test using the Shapiro-Wilk test, where a p-value > 0.05 indicates a normal distribution. To examine the relationship between PM_{10} and AgNORs, multivariate analysis will be carried out using multiple linear regressions. The goal of this test is to find out what factors affect the amount of AgNORs expressed in cells in the buccal cavity and what factors are different between people who were exposed to PM10 and people who were not exposed to PM_{10}. A significance level of $p < 0.05$ means that the results are statistically significant.

Research Ethics

This research was conducted after obtaining approval from the Research Ethics Committee of the University of Sumatera Utara with the given number No:1092/KEP/USU/2021. Following approval, the subsequent steps were as follows: (1) Acquire a location permit letter from the Department of Cleanliness and Greenery of Medan City; (2) Conduct a briefing for the respondents who have signed the informed consent form provided by the authors.

Results and Discussion

The Open-Landfil Terjun comprises an active zone and an inactive zone. The active zone serves as the area actively utilized for waste stacking and accumulation processes, while the inactive zone refers to the area not involved in waste stacking and accumulation processes. Points 1 through 3 (UA-1, UA-2, and UA-3) represent the active zone in this research location and point 4 (UA-4) represents one inactive zone for comparison purposes. The division of these zones introduces variations in the activities taking place within them. Waste management activities at TPA Terjun primarily occur in the active zone, involving tasks such as registering and weighing waste transport trucks, as well as levelling and compacting waste using heavy machinery like excavators and bulldozers. As a comparison of the air quality in ambient conditions, Fig. 1 below shows the air quality in TPA Terjun and the National Ambient Air Quality Standards (NAAQS) issued by the US Agency.

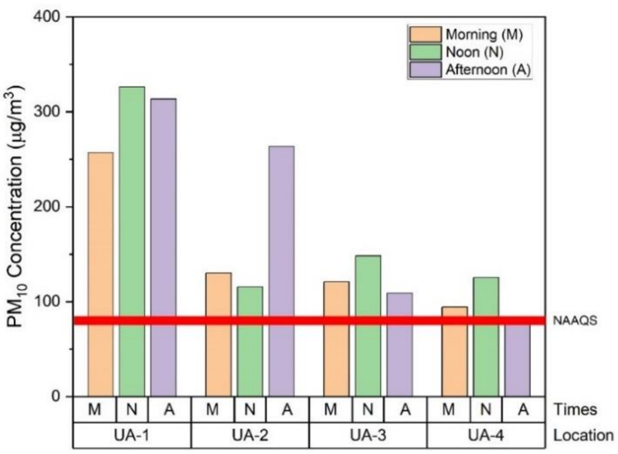

Figure 1. The concentration of PM_{10} at the TPA Terjun and its comparison to the NAAQS standard

The elevated levels of PM_{10} in this study emanate from various activities within the landfill, including waste loading and unloading, transportation, waste covering, and accumulation, as well as the chaotic actions of waste pickers, leading to airborne particulate matter. Besides PM_{10}, the sampling results also detected emissions of several gases, such as sulfur dioxide (SO_2) with <11.7-<11.7 µg/m^3 (SNI 7119-7:2017), nitrogen dioxide (NO_2) with 32,01 – 73.80 µg/m^3 (SNI 7119-2:2017), CO with 238 – 212,6 µg/m^3 (Air Quality Detector), O_3 with 19.74 – 24,85 µg/m3 (SNI 7119-8:2017) and HC with 25,5 – 30,6 µg/m3 (Air Quality Detector). A significant negative consequence of the open dumping system at the landfill is the deterioration of air quality. The waste disposal method in Medan, specifically at the open dumping site located in Kelurahan/Desa Terjun, Kecamatan Medan Marelan, has been operating since 1993, contributing to air quality degradation as the population increases. Approximately 66% of waste management practices in Indonesia terminate in open dumping sites, as reported by the Ministry of Forestry and Environment in 2021 [20]. Ambient air pollutant parameters around the landfill can be categorized as conventional pollutants and greenhouse gas pollutants. Conventional pollutants include particulate matter, SO_2, NO_2, H_2S, CO, HC, and O_3, while the predominant greenhouse gas emitted at the open dumping site is methane (CH_4). Other frequently found parameters at the landfill include odors, H_2S, Pb, Polychlorinated dibenzo-p-dioxins dibenzofurans (PDDs/Fs), and particulate matter [21]. The research outcomes emphasize that poorly managed landfills pose environmental and public health disturbances.

Recyclers and the local community near the landfill, particularly recycling workers, are exposed to particulate matter and pollutants, necessitating attention due to their direct impact on public health

[22]. This is associated with recycling workers' activities in waste sorting, waste transport vehicle operation, and occurrences of fires managed by personnel and recycling workers, affecting ambient air quality. Pollutant parameters around the landfill include both conventional and greenhouse gas pollutants [23–25]. Air pollutant parameters around the landfill can be characterized as both conventional pollutants and greenhouse gas pollutants. The pollutants not only diminish air quality but also lead to secondary impacts, such as public health disturbances. Parameters identified in this study include SO_2, CO, NO_2, Total Suspended Particles (TSP), O_3, HC, PM_{10} and lead, aligning with findings from other studies [6,26–28]. The distribution of research participants based on sexes is outlined in Table 2 for the Recycling Worker Group (PDU) and in Table 3 for the Non-Recycling Worker Group (BPDU).

Table 2. Distribution of Recycling Workers (PDU) groups based on sex

No.	Sexes	Numbers	Percentage (%)
1	Male	28	56
2	Female	22	44
	Total	50	100

Table 3 illustrates the distribution of PDU groups based on gender, where the number of male workers is higher than female workers, with 28 individuals (56%) compared to 22 (44%) females. As a comparison, the distribution of male and female workers in the BPDU group can be seen in Table 3, where 33 females (66%) and 17 males (34%) are identified.

Table 3. Distribution of Non-Recycling Community Members (BPDU) groups based on sex

No.	Sexes	Numbers	Percentage (%)
1	Male	17	34
2	Female	33	66
	Total	50	100

The characteristics of the research respondents based on age are depicted in Table 4 for the PDU group and Table 5 for the BPDU group.

Table 4. Distribution of PDU groups based on age

No	Age (years)	Frequency	Percentage (%)
1	26-35	5	10
2	36-45	15	30
3	46-55	12	24
4	>55	18	36
	Total	50	100

Table 5. Distribution of BPDU groups based on age

No	Age (years)	Frequency	Percentage (%)
1	18-25	2	4
2	26-35	10	20
3	36-45	15	30
4	46-55	15	30
5	>55	8	16
	Total	50	100

Table 4 displays the distribution of the PDU group based on age, revealing that the majority fall in the age group of over 55 years, comprising 18 individuals (36%). Furthermore, Table 5 illustrates the characteristics of the BPDU group, where participants range in age from 18 to over 55 years.

Recycling workers, often referred to as waste pickers or scavengers, play a crucial role in supporting family economic sectors, particularly in lower socio-economic groups. These individuals depend on the informal waste sector for their livelihoods, which involves working without employment contracts (non-permanent workers), lacking regular income, using simple tools without protection, and facing high risks and vulnerability to various diseases. Thirarattanasunthon conducted a study in Thailand to understand the health risks faced by these unprotected workers, their health knowledge, behaviors, and gender aspects. The study was conducted on recycling workers, with a control group provided with protective equipment and a group without any protection. The motivation for this research was the growing and increasing waste problem in Thailand, and the fact that recycling workers from lower socio-economic backgrounds rely on this work to supplement their family income. This situation poses a significant threat to the health of recycling workers [29,30]. The study found that the average age of recycling workers ranged from 14 to 60 years, with an average age of 43.59 years. The most common age group was between 36-45 years

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(30%). Analyzing the subject characteristics based on gender, it is evident that the majority of recycling workers are male, accounting for 56%, while females make up 44%. These findings align with studies conducted by Thirarattanasunthon and other researchers such as Hafiar and Subekti reports [29,30]. The age and gender distribution of recycling workers highlight the demographic challenges and health risks associated with this occupation.

Workers exposed to and at high risk from waste include government employees managing landfill sites, waste collectors, and communities surrounding landfill areas. The risk of pollutant and waste exposure depends on the protection and equipment used, as well as the duration of employment as a recycling worker. Respiratory tract infections and complications from inhaled particulate matter are among the impacts experienced by recycling workers [31,32].

Respondents' characteristics based on working hours in PDU and BPDU groups

The distribution of working hours per day for the PDU group is presented in Table 6, where the majority works 8 hours a day, comprising 46% of the workers. Beyond age and gender characteristics, critical aspects include employment status, duration of work in years, the number of working days per week, and the number of working hours per day, all of which significantly impact health. The entire recycling worker group in this study consists of non-permanent workers (100%). The research indicates that most recycling workers have been employed for more than 10 years (74%) and engage in waste sorting for an average for 9.04±3.69.

Table 6. Distribution of PDU groups based on working hours

No.	Working hours	Frequency	Percentage (%)
1	4	10	20
2	8	23	46
3	12	11	22
4	16	6	12
	Total	50	100

Table 7. Distribution of BPDU groups based on working hours

No.	Working hours	Frequency	Percentage (%)
1	4	13	26
2	6	4	8
3	8	26	52
4	12	4	8
5	16	3	6
	Total	50	100

Table 6 reveals the distribution of the PDU group based on the number of working hours per day. The working hours for the BPDU group are outlined in Table 7, where 52% work for 8 hours. The correlation between the duration of work and the number of working hours for recycling workers at the TPA Terjun site significantly impacts their health, especially when working without protection and having low knowledge of pollutant risks and toxic materials found in the TPA Terjun's area. Apart from particulate matter, the toxicity mechanism due to inhaled pollutants can lead to both local and systemic damage in the body.

In this study, the community group serves as a control, located outside the TPA Terjun in the Medan Marelan District, not working as recycling workers, and situated in the Kota Medan District with low and normal PM_{10} concentrations. The majority of community workers are female (66%), and males comprise 34%, with the highest age group falling between 36-45 years (30%) and 46-55 years (30%). The observed phenomena can be attributed to several factors. Firstly, the community was assembled through a network of individuals in the surrounding district, where the PM_{10} threshold value is within normal limits. After the community was gathered, they were educated about this study. The location being an office area resulted in the presence of working-age participants under 55 years (pre-retirement age). Lastly, the selection of participants was skewed towards women due to discussions related to smoking issues, which led to more women participating as samples compared to men.

Analysis of Differences and Ratios in the Number of Black Dots in the Nuclei of Buccal Epithelial Cells Expressing AgNORs in the PDU and BPDU Groups

A statistical test (t-test) was used to look at the differences and ratios in the number of black dots inside the nuclei of buccal epithelial cells expressing AgNORs in the PDU and BPDU groups. Fig. 2 shows a significant difference with a value of P=0.0001, indicating that the number of black dots in the nuclei of buccal epithelial cells expressing AgNORs is higher in the PDU group compared to the BPDU group.

The analysis using the AgNOR staining technique is commonly utilized to explain the prognosis of malignant lesions. Argyrophilic proteins, which define NORs, show up as black dots inside the nucleus. The number of black dots found is closely related to proliferation and differentiation, serving to differentiate between normal, benign, and malignant lesions [33]. The calculation procedure follows Crocker's method, utilizing an Olympus Binocular Microscope (Model CY21Fs1) with a magnification of 1000x under immersion oil and an LCD HDMI Samsung (Model S22D300HY – Type LS22D300. There are several steps involved in standardization: (1) Count all structures displaying black dot patterns, whether inside or outside cell groupings, (2) Calculate the count per cell grouping, (3) Tally the number of black dots in satellite cells [34]. Once tallied, the mean value for each specimen is computed as the result representing the number of black dots in each specimen.

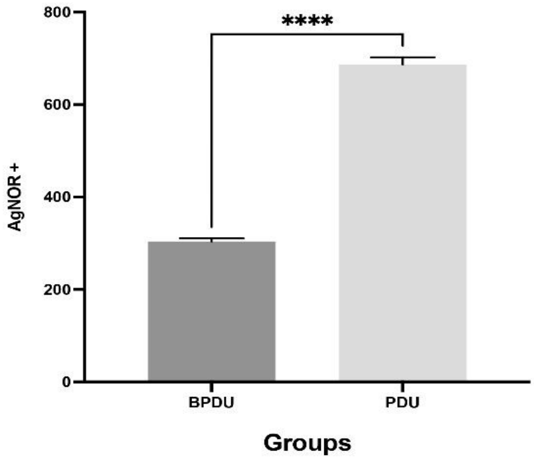

Figure 2. Mean differences and ratios (±SD) of black dots in the nuclei of buccal epithelial cells expressing AgNORs between PDU and BPDU groups

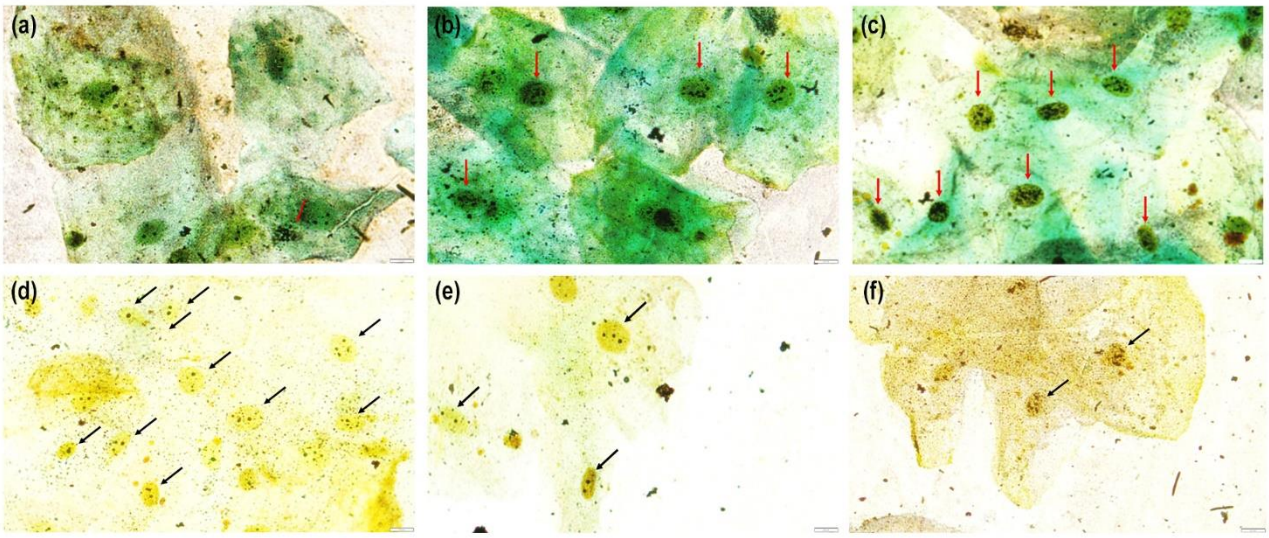

Figure 3. Photomicrographs of buccal epithelial cells in the PDU group expressing AgNORs (1000x) with high proliferation (>5 black dots in the nuclei of buccal epithelial cells, a, b, and c). Photomicrographs of buccal epithelial cells in the BPDU group expressing AgNORs (1000x) with low proliferation (<3 black dots in the nuclei of buccal epithelial cells, d, e, and f).

Particulate Matter (PM_{10}) induces both direct and indirect DNA damage and cellular modification. The interaction of PM_{10} with cell plasma membranes, receptors, and ion channels triggers a biological

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response in cells, leading to the formation of reactive oxidative stress (ROS). ROS alters the cellular redox cycle, initiating a cascade of processes, including inflammation, apoptosis, and damage to protein, lipid, and nucleotide molecules. In fact, every cell type has three options in its cycle: division, non-division, or cell death (apoptosis), as a response to internal and external signals, ultimately resulting in cell dysregulation causing uncontrolled cell proliferation and, consequently, cancer [11,35]. Ultimately, cell dysregulation results in the manifestation of cell proliferation, one of which is seen in the form of black dots, implying cellular changes within the cells. Fig. 2 above shows the differences in the expression of black dots found in the nuclei of buccal epithelial cells in the PDU and BPDU groups.

The comparison of numbers of black dots in epithelial cell between the PDU and BPDU during the exposure of PM_{10} is displayed in Table 8. As the data distribution is not normal, the data was analysed via Mann-Whitney in exploring the association between PM_{10} and the number of black dots in the nuclei of buccal epithelial cells per 100 cells expressing AgNORs.

Table 8. Comparison of number of black dots based on PDU and BPDU workers

Characteristics	PDU (n=50)	BPDU (n=50)	*p-value*
Numbers of black dots in epithelial cell	686,12±104,25	303,56±46.10	0,0001*

*Mann-Whitney U

In the BPDU group, the average value of the number of black dots in Table 8 is a mean of 303.56±46.10 in the nuclei of buccal epithelial cells per 100 buccal epithelial cells expressing AgNORs, while in the PDU group, it is a mean of 686.12±104.25. Therefore, with the Mann-Whitney test, the difference between the two groups is significant (P=0.0001). The difference with a P value of 0.0001 is also obtained after analysis with the t-test, where the PDU group exposed to particulate matter at the TPA location in Medan City shows an increase in the number of black dots in the nuclei of buccal epithelial cells compared to the BPDU group (Fig. 2).

The number of black dots obtained in the PDU group is 686.12±104.25, and in the BPDU group, it is 303.56±46.10 with a significant P value (P=0.0001). AgNORs staining is a simple, easy, and cost-effective staining technique. This staining can identify changes in epithelial cells at the dysplasia level, making it easier to identify benign, premalignant, and malignant epithelial cell lesions as prognostic markers for tumors [36].

The morphological aspects of cell proliferation can also be observed and evaluated using AgNORs histochromic staining. The AgNORs staining method is simple and relatively cost-effective, facilitating the evaluation of morphology in cytology, biopsy, diagnosis, and prognosis [37]. Counting black spots of AgNORs staining in cell nuclei is related to cell proliferation activity, where NORs are chromosomes undergoing reorganization during telophase [34]. The evaluation of black dots is the same, whether it is done histopathologically or cytologically. If more than five black dots are found in the nucleolus of a cell, the cell is considered dysplastic, regardless of whether the assessment is histopathological or cytological. A study noted that AgNORs staining is progressively used in histopathological diagnostic research due to the strong correlation between the number of black dots found and cell transformation into malignancy. Even the shape, number, and distribution of AgNORs in the nucleus can predict the characteristics of breast carcinoma. The S phase is the cell division phase where AgNORs staining is sensitive and easily observable, specifically the number, size, and distribution within the nucleus, which is useful for diagnostic and prognostic purposes. The number, size, and distribution of AgNORs in the nucleus are related to cell proliferation activity and can be parameters for diagnostic and prognostic detection of neoplasia, including oral cavity neoplasia, by determining the number or size of black dots [38] [39]. The potential of AgNORs has also been reported by Tomazeli and Modolo's work who have demonstrated the potential detection of malignant lesions using AgNORs staining by quantitatively analysing AgNOR protein content, with a significant Oneway ANOVA value (P=0.01) [37]. Similarly, research conducted by Malgaonkar who have analyzed buccal mucosa cytology specimens from betel nut chewers using the cytobrush technique with AgNORs staining, revealing a P value of 0.0000 [34]. Proliferation can be observed both cytologically and histopathologically. In the application of histopathology, NORs can be used to observe tumor growth, potential malignancy, differentiate between benign and malignant lesions, and also to predict prognosis and tumor recurrence. The calculation per 100 cells showed both intra-nucleolus and extra-nucleolus, and if the cell nuclei overlapped, the calculation was considered as one cell [40]. This implies that NORs can serve as a significant tool in the clinical diagnosis as shown in this study with PDU group had more black dots in each nucleus cell (Fig. 3).

The results of this study with AgNORs staining on buccal epithelial cells of the PDU and BPDU groups show a significant difference, where the depiction of the number of black dots in the nuclei of buccal epithelial cells in the PDU group indicates a count of >3 per one nucleus of buccal epithelial cell, and in the BPDU group, the number of black dots is below 3 points per one nucleus of buccal epithelial cell. A study was conducted on 30 individuals, comparing normal individuals, smokers, and tobacco/betel chewers aged between 20 and 70 years. Cytology was performed on the buccal epithelial cell mucosa using a scraper or spatula. The results of Prasanna's research showed a comparison of scoring among the three groups: normal, smokers, and tobacco chewers. An ANOVA test revealed the highest value of 10.83 in smokers and 7 in normal individuals. This difference was statistically significant with a t-test p-value of 93.404 (<0.001). However, when comparing smokers and tobacco chewers, the mean difference was 0.6, which was not statistically significant with a p-value of 0.258. In other words, there was no significant difference between the smoker group and the betel chewer group (P=0.258) [17].

Research to discover pollutants and the distribution of ambient air quality at the Final Disposal Site of Desa Terjun, Medan City, may have been extensively conducted. Still, the relationship between PM_{10} pollutants and the number of black dots in the nuclei of buccal epithelial cells in waste recyclers has never been explored. With the significant relationship and ratio obtained on the number of black dots in the nuclei of buccal epithelial cells between the PDU and BPDU groups, the use of the AgNORs staining technique can be considered an efficient and cost-effective method as an evaluation tool for the condition and morphology of buccal epithelial cells in communities vulnerable to working in pollutant conditions, in an effort to prevent and early detect conditions of malignant diseases in the oral cavity.

Conclusion

The relationship between PM_{10} pollutants and the number of black dots in the nuclei of buccal epithelial cells of waste recyclers is a significant area of study, especially for communities at high risk due to their occupations. The AgNOR staining technique on exfoliative smears can serve as an effective early detection method for cancerous lesions, particularly useful for mass screening. This is crucial for recycling workers exposed to dangerous pollutant levels, if the black dots exceed five points in a single cell nucleus, further examination becomes mandatory to prevent malignant diseases. The significant relationship and ratio obtained on the number of black dots in the nuclei of buccal epithelial cells between the PDU and BPDU groups underscore the efficiency and cost-effectiveness of the AgNORs staining technique. This serves as an evaluation tool for the condition and morphology of buccal epithelial cells in communities vulnerable to working in pollutant conditions, emphasizing the importance of early detection and prevention of malignant diseases in oral epithelial cells, particularly among those working in high-risk environments such as waste recycling.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at Universitas Sumatera Utara, Indonesia.

Authors' Contribution Statement

T.K.I, D.M. and M.I. designed the study. T.K.I, and N.N.S. performed the extraction and administration of experiments. T.K.I performed data collection. T.K.I, M.I. and N.N.S. performed cytological experiments and analyzed the data. T.K.I., D.M. and M.I. wrote the paper with input from all authors.

References

1. Nasution EN. Kota Medan Dalam Angka 2019. (Nasution EN, ed.). BPS_Statistics of Medan Municipality; 2019; 203-205.
2. Mukhtar R, Wahyudi H, Hamonangan E, Lahtiani S, Santoso M, Lestiani D D, et al. Kandungan Logam Berat Dalam Udara Ambien Pada Beberapa Kota Di Indonesia. Jurnal Ecolab. 2013; 7(2): 49-59. https://doi.org/10.20886/jklh.2013.7.2.49-59
3. Ali SM, Pervaiz A, Afzal B, Hamid N, Yasmin A. Open dumping of municipal solid waste and its hazardous impacts on soil and vegetation diversity at waste dumping sites of Islamabad city. J King Saud Univ Sci. 2014; 26(1): 59-65. https://doi.org/10.1016/j.jksus.2013.08.003
4. Kimani NG. Environmental Pollution and Impacts on Public Health: Implications of the Dandora Dumping Site Municiap in Nairobi, Kenya. United Nations Environment Programme (UNEP). 2007; 1-14.
5. White SS, Birnbaum LS. An Overview of the Effects of dioxins and Dioxin-like Compounds on Vertebrate, as Documented in Human and Ecological Epidemiology. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2009; 27(4): 197-211. https://doi.org/10.1080/10590500903310047.An
6. Chatham-Stephens K, Caravanos J, Ericson B, Sunga-Amparo J, Susilorini B, Sharma P, et al. Burden of disease from toxic waste sites in India, Indonesia, and the Philippines in 2010. Environ Health Perspect. 2013; 121(7): 791-796. https://doi.org/10.1289/ehp.1206127
7. Ray M, Roychoudhury S, Mukherjee G, Roy S, Lahiri T. Respiratory and General Health Impairments of Workers Employed in a Municipal Solid Waste Disposal at an Open Landfill Site in Delhi. Int J Hyg Environ Health. 2005; 208: 255-262. https://doi.org/10.1016/j.ijheh.2005.02.001
8. Adoga AA, Kokong DD, Nimkur TL, Ma'an ND. Environmental and Life-Style Related Risk Factors for Sinonasal and Nasopharyngeal Malignancies among a Prospective Cohort in Jos, Nigeria. Int J Otolaryngol. 2018; 2018(October): 1-6. https://doi.org/10.1155/2018/8524861
9. Mordukhovich I, Lepeule J, Coull BA, Sparrow D, Vokonas P, Schwartz J. The effect of oxidative stress polymorphisms on the association between long-term black carbon exposure and lung function among elderly men. Thorax. 2015; 70(2): 133-137. https://doi.org/10.1136/thoraxjnl-2014-206179
10. Boström CE, Gerde P, Hanberg A, Jernström B, Johansson C, Kyrklund T, et al. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ Health Perspect. 2002; 110(SUPPL. 3): 451-488. https://doi.org/10.1289/ehp.110-1241197
11. Hou L, Zhang X, Wang D, Baccarelli A. Environmental chemical exposures and human epigenetics. Int J Epidemiol. 2012; 41(1): 79-105. https://doi.org/10.1093/ije/dyr154
12. Ferrari L, Carugno M, Bollati V. Particulate matter exposure shapes DNA methylation through the lifespan. Clin Epigenetics. 2019; 11(1): 1-14. https://doi.org/10.1186/s13148-019-0726-x
13. Alabi OA, Adeoluwa YM, Bakare AA. Elevated Serum Pb, Ni, Cd, and Cr Levels and DNA Damage in Exfoliated Buccal Cells of Teenage Scavengers at a Major Electronic Waste Dumpsite in Lagos, Nigeria. Biol Trace Elem Res. 2020; 194(1): 24-33. https://doi.org/10.1007/s12011-019-01745-z

14. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2009; 31(1): 27-36. https://doi.org/10.1093/carcin/bgp220
15. Stirzaker C, Zotenko E, Song JZ, Qu W, Nair SS, Locke WJ, et al. Methylome sequencing in triple-negative breast cancer reveals distinct methylation clusters with prognostic value. Nat Commun. 2015; 6. https://doi.org/10.1038/ncomms6899
16. Tandelilin RTC, Widita E, Puspita RM, Mun TS. Analisis sitogenetik sel epitel mukosa bukal pekerja stasiun pengisi bahan bakar umum di kota yogyakarta. Jurnal Teknosains. 2021; 10(2): 160. https://doi.org/10.22146/teknosains.43852
17. Prasanna S, Srikant N. Silver dots - A screening and prognostic aid in oral health. Biomed Pharmacol J. 2021; 14(1): 513-517. https://doi.org/10.13005/bpj/2152
18. Abd A, Idris A, Abdelbadie A. The Role of AgNOR in Improving the Efficacy of FNA in the Detection of Breast Lumps. Res Sq.. Published online February 28, 2022. https://doi.org/10.21203/RS.3.RS-1118299/V1
19. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian J Psychol Med. 2013; 35(2): 121-126. https://doi.org/10.4103/0253-7176.116232
20. Bappenas, KLHK. Sipsn Klhk 2021. Published online 2021.
21. Khoiron K, Probandari AN, Setyaningsih W, Kasjono HS, Setyobudi RH, Anne O. A review of environmental health impact from municipal solid waste (MSW) landfill. Ann Trop Med Public Health. 2020; 23(3): 60-67. https://doi.org/10.36295/ASRO.2020.23316
22. Arumdani IS, Puspita AS, Budihardjo MA. MSW handling of top 5 leading waste-producing countries in Southeast Asia. In: IOP Conf Ser: Earth Env Sci; 896. IOP Publishing Ltd; 2021. https://doi.org/10.1088/1755-1315/896/1/012003
23. Huang HC, Tantoh DM, Hsu SY, Nfor ON, Frank CFL, Lung CC, et al. Association between coarse particulate matter (PM 10-2.5) and nasopharyngeal carcinoma among Taiwanese men. J Investig Med. 2020; 68(2): 419-424. https://doi.org/10.1136/jim-2019-001119
24. Chu YH, Kao SW, Tantoh DM, Ko PC, Lan SJ, Liaw YP. Association between fine particulate matter and oral cancer among Taiwanese men. J Investig Med. 2019; 67(1): 34-38. https://doi.org/10.1136/jim-2016-000263
25. García-Cuellar CM, Santibáñez-Andrade M, Chirino YI, Quintana-Belmares R, Morales-Barcenas R, Quezada-Maldonado E, et al. Particulate matter (PM10) promotes cell invasion through epithelial–mesenchymal transition (EMT) by TGF-β activation in A549 lung cells. Int J Mol Sci. 2021; 22(23). https://doi.org/10.3390/ijms222312632
26. Suryati I, Akbar MN, Latifah N. Dampak. Studi Kandungan Logam Berat (As , Cd , Cr , Pb Dan Hg) dalam Particulate Matter 10 Mikron (PM 10) di Beberapa Ruas Jalan Kota Medan. Jurnal Dampak. 2019; 02: 77-85. https://doi.org/10.25077/dampak.16.2.77-85.2019
27. Suyatmi, Subiyantoro P, Indrakila S. Buccal swab, a minimally invasive method for the screening of oral cancer in active smokers. J Phys Conf Ser. 2018; 1022(1): 5-10. https://doi.org/10.1088/1742-6596/1022/1/012049
28. Njoku PO, Edokpayi JN, Odiyo JO. Health and environmental risks of residents living close to a landfill: A case study of thohoyandou landfill, Limpopo province in S Afr Int J Environ Res Public Health. 2019; 16(12): 10-12. https://doi.org/10.3390/ijerph16122125
29. Thirarattanasunthon P, Siriwong W, Robson M, Borjan M. Health risk reduction behaviors model for scavengers exposed to solid waste in municipal dump sites in Nakhon Ratchasima Province, Thailand. Risk Manag Healthc Policy. 2012; 5: 97-104. https://doi.org/10.2147/RMHP.S30707
30. Hafiar H, Subekti P, Komala L, Bakti I, Romli R. Health Search Behavior by The Scavenger in The Landfill Area. Proceedings: Workshop on Environmental Science, Society, and Technology. Westech 2019. https://doi.org/10.4108/eai.8-12-2018.2283935
31. Ziraba AK, Haregu TN, Mberu B. A review and framework for understanding the potential impact of poor solid waste management on health in developing countries. Arch Public Health. Published online 2016: 1-11. https://doi.org/10.1186/s13690-016-0166-4
32. Fazzo L, Minichilli F, Santoro M, Cecarini A, Sea MD, Comba P, et al. Hazardous waste and health impact: A systematic review of the scientific literature. Environ Health. 2017; 16(1). https://doi.org/10.1186/s12940-017-0311-8
33. Tupkari J, Rajput D. Early detection of oral cancer: PAP and AgNOR staining in brush biopsies. J Oral Maxillofac Pathol. 2010; 14(2): 52-58. https://doi.org/10.4103/0973-029x.72501
34. Malgaonkar N, Dagrus K, Vanaki S, Puranik R, Sharanesha M, Tarakji B. Quantitative analysis of agnor counts of buccal mucosal cells of chewers and non chewers of gutkha: A comparative cytologic study. J Cancer Res Ther. 2016; 12(1): 228-231. https://doi.org/10.4103/0973-1482.148713
35. Espitia-Perez L, Jimenez-Vidal L, Espitia-Perez P. Particulate Matter Exposure:Genomic: Instability,

2024, 21(12):3749-3761
https://doi.org/10.21123/bsj.2024.10007
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Disease and Cancer Risk. Env Health – Man Preve Prac. 2020: 13. http://dx.doi.org/10.5772/57353
36. Remmerbach TW, Weidenbach H, Müller C, Hemprich A, Pmjanski N, Buckstegge B, et al. Diagnostic value of nucleolar organizer regions (AgNORs) in brush biopsies of suspicious lesions of the oral cavity. Anal Cell Pathol. 2003; 25(3):139-146. https://doi.org/10.1155/2003/647685
37. Tomazelli KB, Modolo F, Rivero ERC. Evaluation of AgNORs in oral potentially malignant lesions. J Oncol. 2015; 2015. https://doi.org/10.1155/2015/218280
38. Küchler EC, Tannure PN, Falagan-Lotsch P, Lopes TS, Granjeiro JM, de Amorim LMF. Buccal cells DNA extraction to obtain high quality human genomic DNA suitable for polymorphism genotyping by PCR-RFLP and Real-Time PCR. J Appl Oral Sci. 2012; 20(4): 467-471. https://doi.org/10.1590/S1678-77572012000400013
39. Shirani S, Kargahi N, Razavi SM, Homayoni S. Epithelial dysplasia in oral cavity. Iran J Med Sci. 2014; 39(5): 406-417.
40. Singh S, Bhargava D, Ali S, Mishra R, Chandavarkar V. Prognostic Potential of Argyrophillic Nucleolar Organizer Regions (Agnors) in Oral Lesions: A Systematic Review. Biosci Biotechnol Res Asia. 2022; 19(4): 857-866. https://doi.org/10.13005/bbra/3036

العلاقة بين وقت تعرض الجسيمات 10 (PM_{10}) والنقاط السوداء في الخلايا الشدقية لعمال إعادة تدوير مدافن النفايات في ميدان Landfill

إنتان ت.ك. [1،2]، إشوان م. [3، 4]، منير د. [5]، سويروسو ن.ن. [6]، تاريجان أ. ب. [6]، زولهام ز. [7]، سيرجار ن. سي. [8]

[1]قسم علم الأمراض التشريحي، كلية الطب، جامعة سومطرة أوتارا، إندونيسيا.
[2]كلية الطب، جامعة سومطرة أوتارا، إندونيسيا.
[3]قسم الصيدلة والعلاجية كلية الطب جامعة سومطرة أوتارا، إندونيسيا.
[4]كلية الطب، جامعة سومطرة أوتارا، إندونيسيا.
[5]قسم الأذن والأنف والحنجرة والرأس والرقبة كلية الطب جامعة سومطرة أوتارا، إندونيسيا.
[6]قسم أمراض الرئة والجهاز التنفسي كلية الطب جامعة سومطرة أوتارا، إندونيسيا.
[7]قسم الأنسجة، كلية الطب، جامعة سومطرة أوتارا، إندونيسيا.
[8]قسم علم الأمراض التشريحي، كلية الطب، جامعة إندونيسيا، إندونيسيا.

الخلاصة

يتناول هذا البحث العلاقة غير المدروسة بين التعرض للمواد الجسيمية (PM_{10}) وعدد النقاط السوداء في نوى الخلايا الظهارية الشدقية لدى عمال إعادة التدوير، والمختصرين بـ PDU، في مكب نفايات مدينة ميدان، وتحديدًا في إندونيسيا. تهدف الدراسة إلى تحديد العلاقة والنسبة بين PM_{10} وخصائص الملوثات في تأثير مكب النفايات على عدد النقاط السوداء التي يعبر عنها AgNOR في نوى الخلايا الظهارية الشدقية بين مجموعات PDU. تم إجراء قياسات التعرض لـ PM_{10} في مدافن النفايات المفتوحة في مدينة ميدان، وموضوع الدراسة هو عمال إعادة التدوير الذين عملوا في مدافن النفايات. تم جمع الخلايا الظهارية عن طريق مسحة فرشاة خلوية عن طريق الفم، في حين تم إجراء فحص النقاط السوداء من خلال الملاحظات الخلوية. تم إجراء تحليل إحصائي لقياس العلاقة بين التعرض طويل الأمد للجسيمات PM_{10} وعدد النقاط السوداء. كان عمر معظم عمال إعادة التدوير في دراستنا أكبر من 55 عامًا (36%)، وكانت المجموعة الأكبر التالية تتراوح أعمارهم بين 36-45 عامًا (30%). 24٪ فقط تتراوح أعمارهم بين 26 و 35 عامًا. كمجموعة مراقبة، كان لدى العاملين غير العاملين في مجال إعادة التدوير (المختصرين بـ BPDU) مزيج من الأعمار، مع أعلى الأعداد في الفئات العمرية 36-45 و46-55 (كل 30٪). عند النظر إلى ساعات عملهم، فإن أكثر من نصف عمال BPDU (52٪) عملوا لمدة 8 ساعات يوميًا، وعملت غالبية PDU أيضًا لنفس المدة الزمنية. كان متوسط عدد النقاط السوداء في الخلايا الشدقية، مما يشير إلى التأثيرات المحتملة من التلوث، أعلى بكثير في PDU مقارنة بـ BPDU (ع = 0.0001). تظهر دراستنا أن عمال إعادة التدوير، وخاصة أولئك الذين تزيد أعمارهم عن 55 عامًا، لديهم المزيد من النقاط السوداء في خلاياهم الشدقية، مما يشير إلى وجود صلة بين عملهم والتعرض للتلوث البيئي. وتتأثر هذه النتائج أيضًا بالعمر وعدد ساعات العمل يوميًا. وهذا يؤكد أهمية أخذ هذه العوامل بعين الاعتبار في حماية صحة العاملين في بيئات دفن النفايات.

الكلمات المفتاحية: AgNORs، النقاط السوداء، الخلايا الظهارية الشدقية، الكشف المبكر، الأورام الخبيثة، المكب المفتوح، عمال إعادة التدوير.