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Cytogenetic Bio monitoring of Waste Pickers in Onitsha Municipal Dumpsites, with Micronuclei Test in Buccal Epithelia Cells

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Abstract

The genotoxic effects, using the micronucleus (MN) test of buccal cells was evaluated in waste pickers working on two major dumpsites (Fegge and Obosi dumpsites) in Onitsha municipal. Sixty waste pickers (30 from each dumpsite) and 30 control individuals of either sex, in the age range of 18 to 54 years participated in the study after giving informed consent. Buccal cells were collected from the buccal mucosa of each participant using sterile wooden spatula. Frequencies of MN and nuclear abnormalities (NA) in 2000 cell were scored for each individual. Significant ($P< 0.05$) increase in the frequencies of MN and NA were observed in buccal epithelial cells of waste pickers from the two studied dumpsites compared with the control. Significant correlation between sex, age, working hours and years was established with the formation of MN and NA in waste pickers. This study revealed that waste pickers might possibly be at risk of cytogenetic damage from exposure to mixture of pollutants in the dumpsites.

Keywords: Buccal mucosa cells; Genotoxicity; Occupational health; Waste picking; Waste dumpsites

Introduction

Urbanization and population growth in developing countries such as Nigeria have attendant consequences of high solid waste generation and a large population of under-employed residents. Solid wastes have become a major source of environmental pollution, threatening the health of both humans and other biotas [1]. Waste management in the country, however, remains a grave problem to the relevant agencies. Open dumping and burning of waste has remained the usual unhygienic method of waste management in Nigeria [2]. Uncontrolled dumping of waste in dumpsites account for a significant proportion of total waste collection [3]. Dispersion of toxic pollutants has been a major problem arising from this uncontrolled waste dumping with serious public health concern [4]. These wastes constitute a wide range of xenobiotic organic compounds, macro-inorganic compounds, heavy metals and microorganisms, which has been associated to cause severe human health problems [5]. Studies have shown that pollutants present in these wastes have damaging effects on living cells [1,2,6]. It has also been associated with increases in cancer incidence, diseases outbreaks, reproduction and developmental problems among human populations residing close to landfill sites [7]. In addition, microbial decomposition of solid waste through anaerobic process significantly increases the production and release of methane gas, hydrogen sulphide and carbon dioxide into the environment, contributing greatly to global warming and climate change [8]. The population growth and economic decline presented both a challenge, in terms of poor waste management and un employment, and at the same time an employment opportunity, which resulted to the emergence and growth of an informal sector, such as waste picking. Waste pickers, therefore, play an important role in the waste management system in most urban centers [9].

Waste picking is an informal and important economic activity that depends on the amount and value of waste generated by the urban population [10].Waste pickers collect plastics, paper, rubber materials, glass, metals and electronic materials from dump sites, add value to them through sorting, cleaning, and reshaping to make them commercially viable products [9]. They achieve significant recycling rates and are often the major suppliers of secondary materials to industry. Waste picking provides revenue for more than 15 million individuals globally, with a monetary value of several billions of US dollars yearly. More than half of the plastic wastes all over the globe is recycled by waste pickers. The United Nations Environmental Programme, therefore, refers to waste pickers as "the invisible environmentalists of the world". In Nigeria, wastepickers contribute to the growth of the local economy and indirectly ensure public health safety and environmental sustainability through their activities.

Despite the economic significance of waste picking as an efficient livelihood strategy and their contribution to recycling policies, limited concern has been shown to the occupational health risks related with waste picking. Waste pickers work in unhygienic conditions, along with the lack of proper protective wears. They are, thus, exposed to various toxic compounds and pathogenic bio-aerosols that may cause sickness and spread of various diseases [11]. The inhalation of offensive odours and dusts, bites from disease-transmitting fleas and occasional accidental injuries or burn smakes the working conditions of waste pickers even more dangerous. Common health symptoms experienced by waste pickers include; respiratory symptoms, sneezing and coughing, frequent headache, skin rashes, vomiting, diarrhea and musculoskeletal symptom like tiredness, backache and body pain [12,13].

Onitsha, one of the major cities in Anambra State of eastern Nigeria, is one of the most important commercial centers in sub-Sahara

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Africa with a significant human population. Majority of the solid waste generated in Onitsha metropolis is discarded in open dumpsites without any primary waste sorting. Wastes from households, markets, industries, hospitals, and other institutions are dumped together. Waste pickers spend the greater part of their days roaming these dumps without protective wears. They expose themselves to pathogen and hazardous pollutants from disposed electronics, batteries containing heavy metals, bottles with chemical residues, human fecal matter, bandages and needles from hospital wastes, and fumes from waste burning. They have high occupational health risks with serious health impact, such as respiratory and eye irritation, infectious diseases, toxic effects, and allergies. These health threats faced by waste pickers have become a critical but neglected area of research [14].

In population studies, alternatives to invasive blood collection are employed to maximize the participation rate of any studied group. Buccal mucosa cells have gained recognition as an alternative tissue sample in human monitoring for occupational and environmental toxic exposures [15] Micronuclei (MN) test with epithelia buccal cells is a non-invasive technique, which uses the formation of anomalous cells as an indicator to evaluate the cytogenetic damage in exposed population. Buccal cells can be obtained easily and non-invasively compared with lymphocytes collection [16]. The buccal epithelium cells preserves itself by constant renewal, newly epithelia cells produced in the basal layer move outwards and replace cells that are shed [17]. The formation of anomalous epithelial cells indicates cytotoxicity, which might be induced by oxidative stress, exposure to clastogens or aneugens and genetic alterations in cell cycle checkpoints [17]. Several environmental and occupational exposures studies have evaluated the genotoxic implications of various chemical compounds using MN assay of exfoliated buccal cells [18-20]. Oral exfoliative cytology has revealed to be principally valued for population mass screening purposes in human exposure monitoring. This present study therefore, aims to assess the possible genotoxic implications in waste pickers working on solid waste dumpsites in Onitsha municipal dumpsites, using the micronucleus (MN) test of exfoliated buccal cells

Methods

Study Area

Onitsha, the gateway to eastern Nigeria, is the most populous metropolitan in Anambra State, and one of the most important commercial centers in sub-Sahara Africa. It covers an area of about 49,000km2 and located on latitude 6.1°N and longitude 6.8°E in Anambra North Senatorial District. It is the most densely populated municipality in Anambra state with an estimated population of 7,425,000 [21], resulting in a significant increase in the generation of solid waste. Two major waste dumpsites in Onitsha, namely, Fegge and Obosi dumpsites were selected for this study. These sites are the main dumping sites receiving most of the solid waste from Onitsha metropolis and were chosen taken into account the number of years(more than 10 years) waste picking exercise has been practiced, and the estimated time (more than 5 hours a day) spent by waste pickers at each dumpsite.

Study Population

Obosi and Fegge dumpsites comprises of about 46 and 39 waste picker respectively at the period of this study. Sixty waste pickers (30 from each dumpsite) that met the selection criteria and gave informed consent participated in this study. They were selected in an intentional non-probabilistic manner. The control group consisted of 30 healthy, unexposed individuals selected from Iyiowa Odekpe, Anambra State, a distant population away from the dumpsites, with no known exposure to any toxicant. Study subjects were informed about the study and interviewed to obtain personal, lifestyle and clinical information such as age, alimentary habits, working duration, smoking habits, alcohol intake and health, using a predesigned questionnaire, according to the procedure published by the International Commission for Protection against Environmental Mutagen and Carcinogens [22].

Ethical Consideration

The study was conducted following Helsinki declaration and ethical clearance was approved by the State Ministry of Health, Anambra State, with reference number (MH/ANS/1473/21/110), before the collection of samples from waste pickers. Each participant willingly signed an informed consent detailing the purpose of study, benefits, confidentiality, and conflict of interest, before partaking in the study. Participation was voluntary and each subject had the opportunity to participate or opt-out at any point in the course of the study.

Exclusion criteria

Excluded from the study were subjects outside the age of 18 to 65 years, subjects that withheld consent and subjects with a history of alcohol intake, smoking, use of non-smoke tobacco, current oral infections, and dental procedures, including those exposed to X-ray radiations in the past six months.

Collection and Preparation of Buccal Cell Sample

Before the collection of buccal cell, waste pickers were instructed to rinse their mouth thoroughly with water to remove unwanted debris. Exfoliated cells from the buccal mucosa (both right and left) were obtained by scraping with a sterile wooden spatula. The mucosa was submerged in 25 ml of buffer solution (0.1 M EDTA, 0.01 M tris salt and 0.02 M NaCl) and centrifuged for 10 minutes at 1500 rpm. The supernatant was removed and replaced with fresh buffer solution. This process was repeated three times and the pellet was smeared on coded pre-cleaned slides. Smears were air-dried and fixed in 80% methanol for 10 minutes. Slides were air-dried and stained with May-Grunwald and 5% Giemsa respectively. The slides were then air-dried and observed using AmScope B120C-WM-PS100 microscope with objective 100x/1.25. Two slides were prepared for each subject, and 2000 cells evaluated per individual to determine micronucleus (MN) frequencies and other nuclear abnormalities (NA) using the HUMNxl scoring criteria [23,24]. Normal cells with nucleus appeared uniformly stained, have oval- or circle-shaped, and smaller than the cytoplasm. Micro nucleus (MN) were characterized by the presence of both a main nucleus and one or more smaller nuclear structures. Binucleated cells (BN) contained two main nuclei. Both nuclei were usually close or even in contact and are of similar size, shape and staining intensity. Karyorrhectic cells (KR) were characterized by nuclear chromatin aggregation and fragmented cell membranes. Karyolytic cells (KL) appear as a ghost-like image. The nucleus had a complete lack of DNA, which probably represented an advanced stage of cellular death. Cells with nuclear buds, called broken egg (BE), contained a nucleus with a strong constriction in one extreme. Both nucleus and nuclear buds were near and had similar characteristics in morphology.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). The data for the cytogenic properties were assessed for linear model assumptions using the global test (global validation of linear model

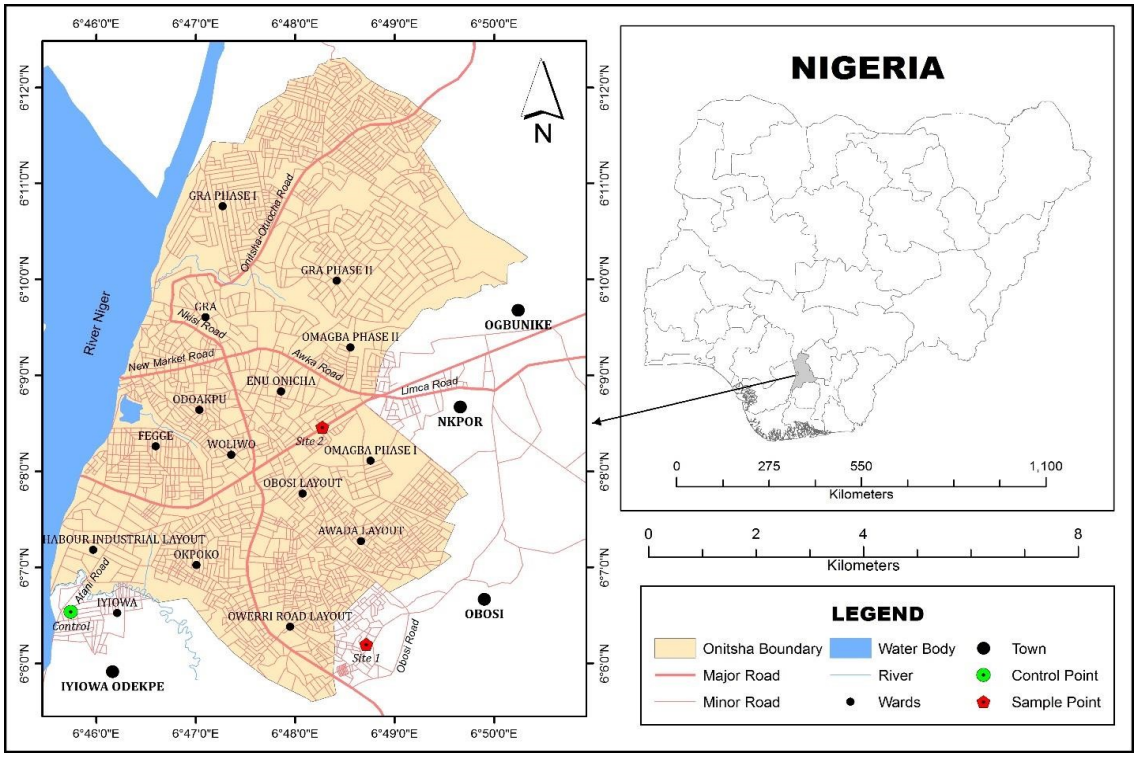

Figure 1: Map of Onitsha showing location of dumpsites.

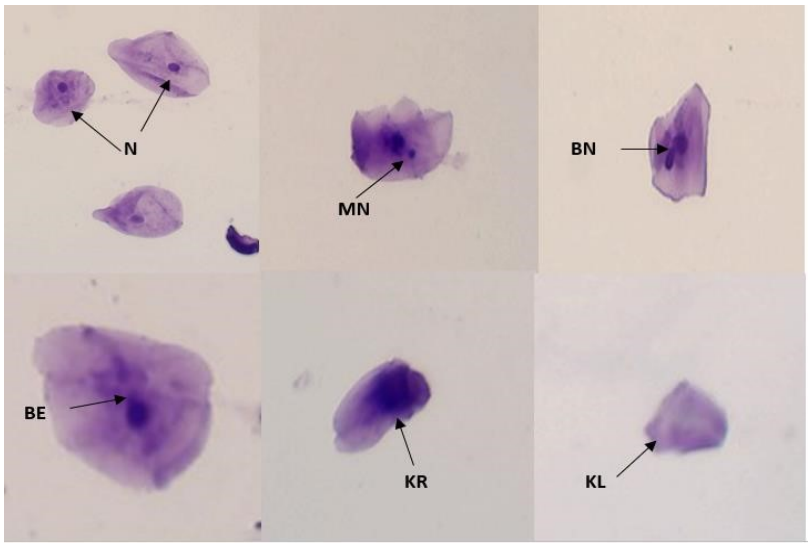

Figure 2: The epithelium cell of the oral mucosa showing, Normal cell (N), Micronucleated cell (MN), Binucleated cell (BN), Broken egg (BE), Karyolytic cell (KL) and Karyorrhectic cell (KR) (×1000).

assumptions). Variables that satisfied the assumption were analyzed using the one-way analysis of variance (ANOVA) followed by a pairwise comparison of means using the Tukey post-hoc test. Variables that departed significantly from the linear model assumptions were analyzed using non-parametric tests such as Welch's test followed by a Dunn test for pairwise multiple comparison. The Chi-square test was used to compare frequencies between groups (sex, age, hours and years of exposure) significant difference was determined at less than 5% probability ($P < 0.05$). All statistical analysis was done using R (Version 4.0.2).

Results

General Characteristics of the Study Population

The general characteristics of the waste pickers and control group are presented in Tables 1 and 2. The studied individuals were

characterized for gender, age, working hours and years. More female scavengers were observed in both dumpsite 1 and dumpsite 2 (60 % and 53 %, respectively). The control site however, had equal male and female respondents. The highest numbers of respondents in dumpsites 1 and 2 were observed for those between the ages of 26-35 (40 %, respectively), while the age group of 45years and above recorded the lowest in both dumpsites (10 % and 6% respectively). In both demographic characteristics of age and gender, there is a contrast between the number of respondent in the dumpsites and the control (Table 1).

The highest number of working hours (8-10 hours) were observed by most waste pickers in dumpsite 1 and dumpsite 2 (70% and 53% respectively) while the least number of waste pickers spend less than 4 hours in both dumpsites (10% and 17% respectively) daily (Table 2). High number of waste pickers were recorded to have spent 3-5 years and 6-10 years in dumpsite 1 while most waste pickers in dumpsite 2 have spent 3-5 years working on the dumpsite. The least number of waste pickers from both dumpsites spent more than 10 years (Table 2).

Frequencies of micronuclei (MN) and other nuclear abnormalities in the exposed and control groups

Table 3 shows the frequencies of micronuclei (MN) and other nuclear abnormalities (NA) in the exposed and control groups. The highest MN and Binucleated cell (BN) frequencies was observed in Fegge dumpsite, while the highest Karyolytic cell (KR) and Karyorrhectic cell (KL) frequencies was observed in Obosi dumpsite. The lowest frequencies of MN and other nuclear abnormalities was observed in the controls. Although the observed differences in the MN frequency between Obosi and Fegge dumpsites were not statistically significant ($P < 0.05$), a statistically significant difference in MN, BN, BE, KR and KL frequencies was observed between Obosi dumpsite and the control ($P < 0.01$) as well as between Fegge dumpsite and control ($P < 0.01$).

Frequencies of micronuclei (MN) and other nuclear abnormalities in exposed and control groups according to age and sex.

Table 4 shows that in Obosi dumpsite, the highest MN, BN, BE, KR, and KL mean frequencies (27 ± 4.58, 17.0 ± 3.0, 11.3 ± 3.06, 10.30 ± 0.58, 9.33 ± 2.52, respectively) were observed in waste pickers that are above 45 years, while the lowest (14.4 ± 5.38, 7.57 ± 2.99, 4.86 ± 1.3, 4.00 ± 1.63, 3.14 ± 1.07) were observed in waste pickers in the 18-25 years age group. Similarly, in Fegge dumpsite, the highest mean frequencies of MN, BN, BE, KR, and KL (24.5 ± 7.78, 17.5 ± 6.36, 15.5 ± 3.54, 6.00 ± 1.41, 7.50 ± 0.70, respectively) were observed in waste pickers that are above 45 years. Although the observed difference with respect to the mean MN, BN, BE, and KL frequencies in the different age groups were not statistically significant, the mean KR frequency in the > 45 years age group was significantly higher than those of other age groups. In the control group, the highest MN and BE mean frequencies (10 ± 4.56 and 4.00 ± 2.10, respectively) were observed in the > 45 age group while the lowest (7.5 ± 2.65 and 3.00 ± 1.63, respectively) was observed in the 18-25 years age group. The highest mean BN frequency (5.73 ± 1.67) was observed in the 26-30 age group, while the lowest (3.75 ± 1.26) was observed in the 18-25 years age group; the highest mean KR frequency (3.80 ± 1.30) was observed in the 36-45 age group, while the lowest (2.00 ± 0.82) was observed in the 18-25 years age group; the highest mean KL frequency (3.40 ± 1.34) was observed in the 36-45 age group, while the lowest (2.60 ± 1.12) was observed in the 26-35 years age group. The differences were, however, not statistically significant ($P > 0.05$)

Characteristics	Location						*P* value
	Obosi dumpsite (n = 30)		Fegge dumpsite (n = 30)		Control (n = 30)		
	n	%	n	%	N	%	
Gender							
Male	12	40	14	47	15	50	0.804
Female	18	60	16	53	15	50	
Age (years)							
18-25	7	23	5	17	4	13	0.472
26-35	12	40	12	40	15	50	
36-45	8	27	11	37	5	17	
> 45	3	10	2	6	6	20	

Table 1: Demographic characteristics for scavengers in dump sites and control site.

Characterstics	Location				*P* value
	Obosi dumpsite (n = 30)		Fegge dumpsite (n = 30)		
	n	%	n	%	
Hours					
< 4	3	10	5	17	0.483
04-7	6	20	9	30	
08-10	21	70	16	53	
Years					
< 3	4	14	7	23	0.289
03-5	13	43	13	43	
06-10	13	43	8	27	
> 10	0	0	2	7	

Table 2: Daily working hours and years by waste pickers at both dumpsites.

Site	Cell abnormalities				
	MN	BN	BE	KR	KL
Obosi dumpsite	19 ± 7.11	19 ± 5.24	8.43 ± 3.56	5.47 ± 2.52	5.53 ± 2.52
Fegge dumpsite	21.4 ± 5.92	21.4 ± 5.77	9.13 ± 3.81	4.43 ± 1.77	3.93 ± 1.93
Control	8.47 ± 3.25	8.47 ± 1.88	3.63 ± 2.14	2.87 ± 1.46	2.77 ± 1.19
P values	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

MN = Micronucleated cell, BN = Binucleated cell, BE = Broken egg, KL = Karyolytic cell, KR = Karyorrhecticcell.. Values expressed as mean ± standard deviation.

Table 3: Frequencies of micronuclei (MN) and other nuclear abnormalities in the exposed and control groups.

Study groups	Cell abnormalities					
Obosi dumpsite	Age (n)	MN	BN	BE	KR	KL
	18-25 (7)	14.4 ± 5.38	7.57 ± 2.99	4.86 ± 1.35	4.00 ± 1.63	3.14 ± 1.07
	26-35 (12)	15.8 ± 5.34	10.8 ± 5.91	8.25 ± 3.57	5.42 ± 2.07	5.00 ± 1.65
	36-45 (8)	24.6 ± 5.68	14.1 ± 3.4	10.8 ± 2.38	5.00 ± 2.14	7.00 ± 2.07
	> 45 (3)	27 ± 4.58	17.0 ± 3.0	11.3 ± 3.06	10.30 ± 0.58	9.33 ± 2.52
	P value	< 0.001	0.01	0.002	< 0.001	< 0.001
	Sex (n)					
	Male (12)	22.5 ± 5.82	13.0 ± 5.08	9.08 ± 3.60	6.42 ± 2.84	6.00 ± 2.89
	Female (18)	16.6 ± 7.05	10.6 ± 5.26	8.00 ± 3.56	4.83 ± 2.12	5.22 ± 2.26
	P value	0.02	0.22	0.42	0.09	0.42
Fegge dumpsite	Age (n)					
	18-25 (5)	18.4 ± 4.28	14.0 ± 4.36	9.20 ± 5.22	3.80 ± 0.84	4.60 ± 2.41
	26-35 (12)	20.2 ± 5.53	13.4 ± 6.97	8.00 ± 3.28	4.25 ± 2.09	3.33 ± 1.44
	36-45 (11)	23.5 ± 6.49	12.5 ± 5.18	9.18 ± 2.93	4.64 ± 1.75	3.64 ± 1.69
	> 45 (12)	24.5 ± 7.78	17.5 ± 6.36	15.5 ± 3.54	6.00 ± 1.41	7.50 ± 0.70
	P value	0.32	0.73	0.08	0.5	0.02
	Sex (n)					
	Male (14)	20.1 ± 4.03	12.4 ± 6.07	7.86 ± 3.03	4.21 ± 1.63	3.79 ± 2.01
	Female (16)	22.6 ± 7.12	14.4 ± 5.51	10.2 ± 4.16	4.62 ± 1.93	4.06 ± 1.91
	P value	0.26	0.35	0.09	0.54	0.54
Control	Age (n)					
	18-25 (4)	7.5 ± 2.65	3.75 ± 1.26	3.00 ± 1.63	2.00 ± 0.82	2.75 ± 0.96
	26-35 (15)	8.13 ± 3.14	5.73 ± 1.67	3.67 ± 2.44	2.93 ± 1.67	2.60 ± 1.12
	36-45 (5)	8.4 ± 2.41	5.60 ± 2.70	3.60 ± 2.07	3.80 ± 1.30	3.40 ± 1.34
	> 45 (6)	10 ± 4.56	4.0 ± 1.26	4.00 ± 2.10	2.50 ± 1.05	2.67 ± 1.51
	P value	0.63	0.09	0.88	0.28	0.65
	Sex					
	Male (15)	9.13 ± 3.14	5.13 ± 1.60	3.00 ± 1.25	2.87 ± 1.25	2.53 ± 1.06
	Female (15)	7.8 ± 3.32	5.07 ± 2.19	4.27 ± 2.66	2.87 ± 1.68	3.00 ± 1.31
	P value	0.16	0.92	0.11	1	0.29

Table 4: Frequencies of micronuclei (MN) and other nuclear abnormalities in exposed and control groups according to age and sex.

Table 4 also shows that while the highest mean frequencies of MN, BN, BE, KR and KL (22.5 ± 5.82, 13.0 ± 5.08, 9.08 ± 3.60, 6.42 ± 2.84, 6.00 ± 2.89, respectively) in Obosi dumpsite were observed in the male waste pickers, those of Fegge dumpsite were observed in the female waste pickers (22.6 ± 7.12, 14.4 ± 5.51, 10.2 ± 4.16, 4.62 ± 1.93, 4.06 ± 1.91, respectively). In the control group, the mean MN and BN frequencies in the males were higher (9.13 ± 3.14 and 5.13 ± 1.60, respectively) than those of the females. Conversely, the mean BE and KL frequencies in the females (4.27 ± 2.66 and 3.00 ± 1.31) were higher than those of the males. These differences were, however, not statistically significant (P > 0.05).

Frequencies of micronuclei (MN) and other nuclear abnormalities in exposed groups according to years and hours of exposure to dumpsite wastes.

With respect to years of exposure to dumpsite wastes, Table 5 shows that the waste pickers that were exposed to dumpsite waste for 6-10 years had the highest mean MN, BN, BE, KR and KL frequencies (25.1 ± 4.65, 14.7 ± 4.21, 9.92 ± 3.45, 6.54 ± 2.76, 6.85 ± 2.88, respectively). Conversely, waste pickers that were exposed to dumpsite waste for less than 3 years had the lowest mean MN, BN, and BE frequencies (9.5 ± 1.29, 5.0 ± 1.41, and 5.25 ± 2.06, respectively) while those exposed for 3-5 years had the lowest mean KR and KL frequencies. The statistically significant (P = 0.04) difference that was observed between waste pickers exposed for less than 3 years and those exposed for 3-5 years was in the mean MN frequency but not statistically different in other nuclear abnormalities. The observed difference in the mean MN, BN and KL frequencies between waste pickers exposed for less than 3 years and those exposed for 6-10 years, those of 3-5 years and 6-10 years were statistically significant (P < 0.05). No statistically significant

Exposed groups	Cell abnormalities					
Obosi dumpsite	Years (n)	MN	BN	BE	KR	KL
	< 3 (4)	9.5 ± 1.29	5.0 ± 1.41	5.25 ± 2.06	4.75 ± 0.50	4.75 ± 2.06
	3-5 (13)	15.8 ± 4.34	10.4 ± 4.66	7.92 ± 3.4	4.62 ± 2.33	4.46 ± 1.61
	6-10 (13)	25.1 ± 4.65	14.7 ± 4.21	9.92 ± 3.45	6.54 ± 2.76	6.85 ± 2.88
	P value	< 0.001	0.001	0.05	0.12	0.04
	Hours (n)					
	8-10 (21)	17 ± 6.45	10.8 ± 5.34	7.86 ± 3.44	4.81 ± 2.02	4.81 ± 1.54
	4-7 (6)	23.8 ± 7.03	12.3 ± 5.72	9.67 ± 4.08	6.67 ± 2.07	7.33 ± 4.27
	< 4 (3)	22.7 ± 8.02	15.3 ± 1.53	10.00 ± 3.46	7.67 ± 4.93	7.00 ± 2.00
	P value	0.07	0.35	0.41	0.07	0.05
Fegge dumpsite	Years (n)					
	< 3 (7)	19 ± 3.92	18.0 ± 6.43	7.43 ± 2.44	4.86 ± 1.57	4.29 ± 2.06
	3-5 (13)	21 ± 5.55	10.8 ± 3.31	8.54 ± 3.99	3.77 ± 1.83	3.54 ± 1.56
	6-10 (8)	22.6 ± 7.69	13.2 ± 5.97	10.40 ± 3.34	5.00 ± 1.93	3.62 ± 2.13
	> 10 (2)	27.5 ± 3.54	15.0 ± 9.90	14.00 ± 5.66	5.00 ± 0.00	6.50 ± 2.12
	P value	0.31	0.05	0.12	0.37	0.21
	Hours (n)					
	8-10 (16)	22.4 ± 6.22	14.6 ± 5.55	8.94 ± 4.12	4.75 ± 2.02	4.38 ± 1.86
	4-7 (9)	20.3 ± 5.55	11.8 ± 5.70	8.56 ± 2.35	3.67 ± 1.00	2.78 ± 1.09
	< 4 (5)	20 ± 6.2	12.8 ± 7.01	10.80 ± 5.12	4.80 ± 1.92	4.60 ± 2.70
	P value	0.6	0.51	0.56	0.31	0.09

MN = Micronucleated cell, BN = Binucleated cell, BE = Broken egg, KL = Karyolytic cell, KR = Karyorrhectic cell. Values expressed as mean ± standard deviation.

Table 5: Frequencies of micronuclei (MN) and other nuclear abnormalities in exposed groups according to years and hours of exposure to dumpsite wastes.

difference was observed in the mean frequencies of BE and KR across the waste pickers exposed for different years. In Fegge dumpsite, the highest mean MN, BE, KR, and KL frequencies (27.5 ± 3.54, 14.00 ± 5.66, 5.00 ± 0.00, and 6.50 ± 2.12, respectively) were observed in waste pickers exposed for more than 10 years while the highest mean BN frequency was observed in waste pickers exposed for less than 3 years (18.0 ± 6.43). the lowest mean MN and BE frequencies were observed in waste pickers exposed for less than 3 years while those exposed for 3-5 years recorded the lowest mean BN, KR, and KL frequencies. These differences were, however, not statistically significant (Table 5).

Table 5 also shows that waste pickers that spend 4-7 hours at the Obosi dumpsite recorded the highest mean MN and KL frequencies (23.8 ± 7.03 and 7.33 ± 4.27), while those that spend less than 4 hours recorded the highest mean BN, BE, and KR frequencies (15.3 ± 1.53, 10.00 ± 3.46, and 7.67 ± 4.93). In Fegge dumpsite, the highest mean MN and BN were observed in waste pickers that spend 8-10 hours, while the highest mean BE, KR, and KL were recorded in waste pickers that spend less than 4 hours. These differences observed in the two exposed groups were not statistically significant.

Discussion

Informal waste picking and recycling as a livelihood strategy poses occupational health risks to the waste pickers that roam the dumpsites since they operate in an adverse working condition and disregard the rudimentary ethics of occupational safety. In this study, the genotoxic effects of occupational exposure to waste pickers in two major waste dumpsites in the Onitsha metropolis were evaluated using the micronucleus test of epithelia buccal cells. The majority of the sampled respondents from both dumpsites were between the ages of 26-35 years, while the least ranged above 45 years. This age statistics confirmed that healthy young individuals participate more in this enterprise than the aged, who are often termed as fragile and weak. In agreement with this, [25] reported that this trade as a source of livelihood is most suited for healthy young men that can endure to carry loads of material daily. Also, with the increased unemployment rate and recent decline in the economy in Nigeria, an increasing number of young individuals engage in such informal work as waste picking, as a means of livelihood and survival. Although male dominance in the waste picking occupation has been reported due to the stress associated with the trade, which makes females (who are considered as the weaker sex) unfit, the majority of the respondents from both dumpsites were females. This observation may be attributed to the exclusion of respondents that have a habit of smoking, alcohol intake and the use of tobacco from the study. Lifestyle habits such as smoking and alcohol intake have been reported by previous authors to induce a high degree of nuclear abnormalities. They have also been implicated in oral carcinogenesis [26-28].

This study established that the frequencies of Micronucleus (MN) and other nuclear abnormalities (NA) increased significantly ($P < 0.01$) in the exfoliated buccal epithelial cells of waste pickers from the two dumpsites compared to those of the control group. MN and NA are sensitive indicator for cytogenetic and chromosomal damage in mitotic cells and reflect genotoxic exposure to clastogenic and aneugenic compounds [29]. The detection of these nuclear anomalies serves as a biomarker to predict increased risk to cell degenerative diseases including cancer [30]. The epithelium cells of the oral mucosa are maintained by continuous renewal and migration from the basal layer to replace shed cells [31]. These cells are the first target site for hazardous agents entering the body through inhalation or ingestion routes [32,33], resulting to MN formation and chromosomal damage [31]. [34] Reported a relationship between buccal cell alterations and the formation of malignant tumors. The micronucleus test in oral epithelia cells is, therefore, one of the easiest and most sensitive means of evaluating the degree of genomic damage in the human population study, and can be used as a suitable biomarker of occupational exposure.

Micronuclei (MN) are formed in actively dividing epidermal squamous cells as a result of mitotic spindle dysfunction or structural

aberrations resulting to fragments of chromosomes, which failed to incorporate in the daughter nuclei during cell division [35]. The presence of a high frequency of MN in the epidermal cells of these waste pickers is a clear indication of genetic damage in these cells. This result agrees with the findings of previous authors who reported an association between increase formation of MN and occupational exposure in foundry workers, building construction workers, tannery workers, petrol station attendants, and automobile spray painters [36,37].

Increased frequencies in other nuclear abnormalities were observed in the waste pickers. Their presence complements the scoring of MN and further accentuate the possible genotoxic effects of waste dumpsites pollutants. The occurrence of binucleated cells (BN) is an indication of toxic effects on a cell's protein structures, which result in failed cytokinesis during the last nuclear division in the epidermal basal cell layer. Cells with two nuclei appear as an indication of cytokinesis disturbance. A significantly higher frequency of chromosomal damage has been reported to occur in BN with failed cytokinesis than cells with complete cytokinesis (Jois et al., 2010). The presence of Broken eggs (BE) or cells with nuclear buds might be associated with the nuclear budding process which involves sharp constriction of the nuclei at one end (Sanchez-Siles et al., 2011). The BE is indicative of chromosomal instability and DNA damage. The occurrence of Karyorrhectic cells (KR) originated after cytotoxic damage [38]. This involves nuclear disintegration and chromatin disorder (Jois et al., 2010), resulting in a dense network of condensed chromatin cells and subsequent fragmentation of the nucleus [39]. Karyolytic cells (KL) indicate the morphological manifestation of necrosis, which is associated with the late stage of the cell death process. The nucleus appears ghost-like with depleted DNA [39].

The Observed differences in frequencies of MN and other NA in waste pickers at Fegge dumpsite (FDS) and Obosi dumpsite (ODS) were not statistically significant ($P < 0.05$), a statistically significant difference was observed between the dumpsites and the control ($P < 0.01$). The constant burning of waste materials by waste pickers in both dumpsites could attribute to the observed significant frequencies of MN and other NA. The waste pickers seek metals from waste by burning the pile of wastes. They also engage in plastics and electronic waste burning to obtain valuable components and smelt/remold copper materials for easy transportation. These dumpsites pose a serious threat not only to the lives of waste pickers but also to the nearby residents. Highly hazardous compounds are released into the surrounding environment from uncontrolled waste dumpsites and have shown to be toxic to human health [40]. For instance, pollutants such as methane, lead, benzene, asbestos and cadmium released from dumpsites are classified as highly carcinogenic for humans. The WHO estimated that environmental exposure contributes to 19 % of cancer occurrence globally [41], and epidemiological studies have statistically associated cancer and congenital malformations to waste exposure in dumpsites. Poor adherence to occupational and health safety measures was observed in the waste pickers from both dumpsites. Most times, without any protective wears such as gloves, boots, and masks, waste pickers roam through high piles of waste while standing close to lorries unloading wastes or machines spreading and compacting the wastes. Constant injuries from cuts by sharp objects like needles and broken glass, as well as bites from insects and rodents were reported. Due to their poor hygiene, the waste pickers are possible carriers of pathogens from waste and could serve as means for the transmission and spread of these disease-causing pathogens to the general populace.

It is essential to understand that in most bio monitoring studies involving the human population, observed variabilities in genetic alterations are probably because of genotoxic factors in daily life, individual genetic and immune system differences, gender, and age. This might explain the observed genotoxic variability in waste pickers from both dumpsites and the control site, considering that the hygienic habits, daily food, and drink intake of all respondents from the dumpsites and control site were not fully controlled. This makes evident the sensitivity of the MN test as demonstrated by an increase in genotoxicity indicators. In the present study, no gender-related significant differences in the induction of MN and other NA was observed in the waste pickers from both dumpsites. Previous authors have designated gender as a significant variable in biomonitoring assessment using the micronucleus test [42,43]. The difference in the frequency of MN in some biomonitoring assessment have been reported to be significantly higher in women. [44] Reported 1.2 to 1.6 more micronuclei in women than in men. [45] Also reported a high ratio of 1.5 MN in women hairdressers compared to men. In addition, some studies have reported that males are generally more sensitive to MN induction than females [46,47]. However, other studies have indicated no gender-related differences in MN induction, which supports our present findings [48-50].

The association between increased frequencies of MN and age has been acknowledge [41]. In this study, an association between aging and induction of genetic alterations was established. The highest increase in MN and other NA frequencies were observed in waste pickers of above 45 years range while the lowest was recorded in 18-25 years age group. Increased significant difference was also observed between the ages of waste pickers from both dumpsites and the control site. This outcome might be attributed to the longer duration of exposure (in years) for most of the older waste pickers whom might have spent more years in these dumpsites than the younger waste pickers. Similar to this finding, [52] reported a positive correlation of MN frequencies with ages. In addition, previous studies have reported an association between aging and induction of chromosomal instability [38,53]. Contrariwise, [54] reported no association between the formation of MN and age in a study involving gas station attendants. Additionally, increase of MN and other NA induction was observed in waste pickers with the highest years of exposure and working hours from both dumpsites. Understandably, this finding can be attributed to the extended period of exposure to pollutants in the waste dump as these waste pickers spend prolong time (hours and years) in these dumpsites. In conformity with this finding, [55-58] reported a correlation between working time and formation of MN in individuals working in car and battery repair garages. This result suggests that as the duration of exposure of waste pickers to dumpsites increases, the formation of MN and NA in oral mucosal cells also increases. Micronucleus test is, therefore, a suitable tool in mutagenic investigation to ensure occupational health and for early detection of genetic damage and risk for degenerative diseases [59-61].

Conclusion

Waste picking is a growing enterprise in major cities in Nigeria, and waste pickers play a vital role in waste recycling and management though exposed to numerous health problems. The induction of increasing frequency of oral epithelial cells with MN and NA observed in this study proved that waste pickers could be under risk of cytogenetic damage from a complex mixture of pollutants in dumpsites to which the waste pickers are exposed. Given the significance of waste picking as a waste management strategy, the health of waste pickers

must be considered. A more plausible approach to alleviate the health risk at these sites is by developing a good waste governance, supportive policy and implementation of basic work safety procedures. Since the induction of Micronucleus and other nuclear anomalies are considered a principal mechanism of both cytotoxicity and genotoxicity, extensive studies to directly quantify the genetic damage in waste pickers are recommended to strengthen the findings of this present study.

Conflict of Interest

The authors declare no conflict of interest.

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