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Micronucleus Assay in Exfoliated Buccal Epithelial Cells Using Liquid Based Cytology Preparations in Building Construction Workers

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ABSTRACT

Background and objective: Cytogenetic damage in exfoliated buccal epithelial cells due to environmental and occupational exposure is often monitored by micronucleus (MN) assay using liquid based cytology (LBC) preparations. This study was performed to evaluate MN in exfoliated buccal epithelial cells of building construction workers using LBC preparations.

Methods: LBC preparations of exfoliated buccal epithelial cells from 100 subjects [50 building construction workers (cases) and 50 administrative staffs (controls)] was evaluated by May-Grunwald Giemsa, Hematoxylin and Eosin and Papanicolaou stains. Student's t test was used for statistical analysis and a P value of <0.05 was considered as statistically significant.

Results: The mean frequencies of MN for cases were significantly higher than controls regardless of staining methods used. There were statistically significant differences between smokers and non-smokers of the controls as well as duration of working exposure (<5 and >5 years) and smokers and non-smokers of cases (P=0.001). However, there were meaningful differences regarding mean frequencies of MN between smokers, non-smokers, those with alcohol consumption or not in cases and controls using various stains (P=0.001).

Conclusion: There was an increased risk of cytogenetic damage in building construction workers. However, evaluation of MN of exfoliated buccal epithelial cells in building construction workers serve as a minimally invasive biomarker for cytogenetic damage. LBC preparations can be applied for MN assay as it improves the quality of smears and cell morphology, decreases the confounding factors and reduces false positive results.

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Introduction

Micronucleus (MN) is indicative of either a fragmented or whole chromosomes extruded from the main nucleus during the process of mitosis. These acentric fragments or lost chromosomes gives rise to small nuclei which appear similar to the main nuclei on staining known as MN (1,2). Therefore, MN assay is an ideal parameter to serve as a biomarker (3). High frequencies of MN have been demonstrated in exfoliated buccal cells in those occupationally exposed to toxic environmental agents such as solvents, polycyclic aromatic hydrocarbons, emissions of sugarcane straw burning, gasoline, arsenic and anti-neoplastic drugs compared to the controls (4).

Building construction workers are exposed to numbers of hazardous agents daily and for long period. Most of them are classified as carcinogenic or possibly carcinogenic namely dust, chemicals, asbestos and quartz dust of crystalline silica. Some authors have found that cement and concrete substances may be carcinogenic in nature (5,6).

Cells of buccal mucosa play role as the first barrier for inhalation or ingestion route and can metabolize carcinogenic agents to reactive chemical substances. They often considered as appropriate site for assessment of early cytogenetic damage as about 90% of human cancers are epithelial in origin (7).

Liquid based cytology (LBC) was first applied to gynecological cytology almost 25 years ago (8). Since its introduction, many laboratories have started to apply LBC to nongynecological sample and exfoliated buccal epithelial cells (9). LBC can substitute conventional smear; because it provides many advantages namely enhanced slide preparations and decreases the amount of mucus, blood and inflammatory cells. Routinely prepared slides for analysis of MN contains bacteria, staining deposits, nuclear fragments and other cytoplasmic cells and necrotic cores that can be confused with MN, which may lead to false positive results. However, slides prepared using LBC can eliminate or decrease these confounding factors; hence, LBC may serve as a reliable technique for MN analysis (7,10-12). The present study was undertaken to evaluate the frequencies of MN of exfoliated buccal epithelial cells using LBC preparations in building construction workers.

Materials and Methods

Overview of study design

This prospective study was performed in the department of pathology, Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur, Tamilnadu, India from August 2015 to October 2015. An ethical approval was obtained from the Institutional Ethical Committee. An informed written consent was taken from each patient.

Study population

A total of 100 healthy subjects, 50 building construction workers (cases) and 50 hospital administrative staffs (controls) without any type of oral lesions entered the study. All subjects were asked to complete the questionnaire to obtain demographic data including history of cigarette smoking and alcohol. Betel nut/tobacco chewers were excluded from the investigation.

Collection of specimens, staining and evaluation criteria

Prior to collection of exfoliated buccal epithelial cells, oral cavity was rinsed with water. Sterile wooden spatula was introduced into the mouth, scraped on the buccal mucosa and the sample placed in preservative solution for minimum of half an hour. Subsequently the sample was centrifuged at 1500 rpm for 5 min. The supernatant was discorded. One drop of normal saline was added to pellet and mixed well to get homogenous sample. Totally 50 uL of diluted pellet was placed on clean slides with a drop of fixative solution and stained with May-Grunwald Giemsa (MGG), Hematoxylin and Eosin (H & E) and Papanicolaou stain (Pap) using standard staining techniques. The criteria established by Tolbert et al. (13) and El-Setouhy et al. (14) were used to count the frequencies of MN in 1000 exfoliated buccal epithelial cells from each subject. The slides were examined under light microscope by pathologists. However, binucleate cells or cells with fragmented nuclei, karyolysis and nuclei appearing as broken eggs were not counted as MN.

Statistical analysis

Student's t test was used to compare the mean frequencies of MN found in all stains in both cases and controls. $P < 0.05$ was considered as statistically significant. Statistical analysis was performed using International Business Machines (IBM) Corporation Statistical Package for the Social Sciences (SPSS) Statistics for Windows (version 20.0. Armonk, New York: IBM Corporation).

Results

The mean age of cases and controls were 44.36 and 43.96 years respectively. Among 50 cases, 17 (34%) were smokers and 21 (42%) used alcohol. Nineteen (38%) were smokers and 18 (36% used alcohol among 50 controls. The mean length of working in building construction of cases was 17.34 years. The number of MN found in the present study were 854 MN with MGG, 926 with H&E and 1203 with Pap stain. The mean frequencies of MN in cases compared to controls stained with MGG (Figure 1A), H & E (Figure 1B)

and Pap stain (Figure 1C) were 18.74 vs 9.12, 19.96 vs 10.14 and 27.7 vs 14.84, respectively.

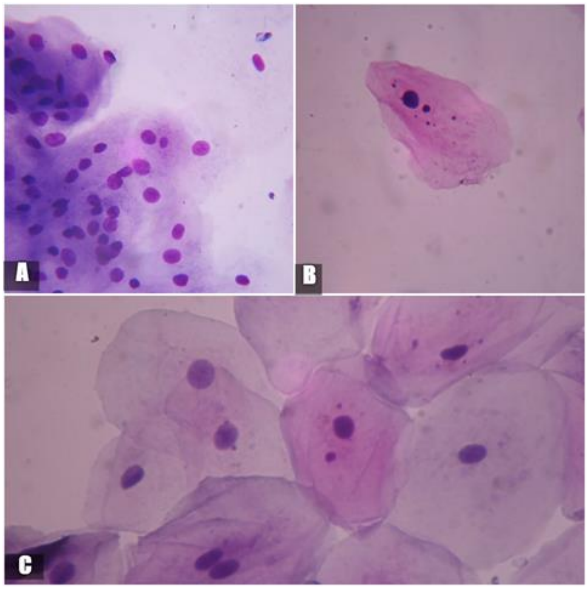

Fig 1. Liquid based cytology preparations showing exfoliated buccal epithelial cells with micronucleus. (A) May-Grunwald Giemsa, x400. (B) Hematoxylin and Eosin, x400. (C) Papanicolaou stain, x400.

These findings were statistically significant (P=0.001) regardless of staining methods used (Table 1).

Frequencies of MN by different staining methods did not reveal significant differences regarding age (<40 and >40 years) and alcohol consumption in controls and cases. Whereas, the mean frequencies of MN between smokers and non-smokers of the controls as well as duration of working exposure (<5 and >5 years) and smokers and non-smokers of cases reached a significant level (P=0.001). On intercomparison of mean frequencies of MN between smokers, non-smokers, alcoholics and non-alcoholics of cases and controls using various stains showed significant statistical differences (P=0.001) (Table 2 to Table 4).

Table 1. Comparison of Mean Frequency of MN Using Student's t Test among All Stains

Stain	Cases				Control				*P*-Value
	Number	Mean ± SI	Median	Minimur maximur values	Number	Mean ± SD	Median	Minimum-maximum value	
MGG	50	18.74 ± 7	18	8-47	50	9.12 ± 6	8	3-43	< 0.001
H&E	50	19.96 ± 6.4	19.5	9-49	50	10.14 ± 6.2	9.5	4-46	< 0.001
Pap	50	27.7 ± 6.27	26.5	17-49	50	14.84 ± 6.3	13	8-48	< 0.001

MGG: May-Grunwald Giemsa; H & E: Hematoxylin and Eosin; Pap: Papanicolaou stain;*P value <0.05 was considered as statistically significant

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Table 2. Frequency of MN Regarding Age, Smoking and Alcohol Consumption in Controls with Various Stains (N=50)

Stain	Individuals	MN (Mean ± SD)	*P*-value
MGG	**Age (year)** <40 (N=24) >40 (N=26)	8.32 ± 3.23 8.65 ± 5.78	0.8
	Smoking Yes (N=19) No (N=31)	8.74±4.43 4.55±4.05	0.001
	Alcohol consumption Yes (N=18) No (N=32)	8.69 ± 4.82 8.21 ± 4.3	0.72
H&E	**Age (year)** <40 (N=24) >40 (N=26)	9.41 ± 4.25 9.81 ± 4.63	0.75
	Smoking Yes (N=19) No (N=31)	9.51±4.98 4.69±4.06	0.001
	Alcohol consumption Yes (N=18) No (N=32)	9.56 ± 4.79 9.21 ± 4.12	0.79
Pap	**Age (year)** <40 (N=24) >40 (N=26)	12.43 ± 5.07 12.69 ± 5.55	0.86
	Smoking Yes (N=19) No (N=31)	13.79±5.82 8.14±5.24	0.001
	Alcohol consumption Yes (N=18) No (N=32)	13.98 ± 5.66 13.31 ± 5.08	0.68

MGG: May-Grunwald Giemsa; H & E: Hematoxylin and Eosin; Pap: Papanicolaou stain; *P value <0.05 was considered statistically significant

Table 3. Frequency of MN Regarding Age, Smoking, Alcohol Consumption and Years of Working Exposure in Cases with Various Stains (N=50)

Stain	Individuals	MN (Mean ± SD)	*P*-value
MGG	**Age (year)** <40 (N=20) >40 (N=30)	16.52 ± 6.12 17.43 ± 6.87	0.62
	Smoking Yes (N=17) No (N=33)	17.72±6.86 11.04±5.32	0.001
	Alcohol consumption Yes (N=21) No (N=29)	17.12 ± 6.71 16.88 ± 5.41	0.89
	Working exposure (year) <5 (N=27) >5 (N=23)	12.02 ± 4.33 17.75 ± 5.92	0.001
H&E	**Age (year)** <40 (N=20) >40 (N=30)	16.65 ± 6.11 17.87 ± 6.93	0.51
	Smoking Yes (N=17) No (N=33)	18.59 ± 6.38 11.36 ± 5.81	0.001
	Alcohol consumption Yes (N=21) No (N=29)	17.59 ± 6.44 16.06 ± 5.79	0.39
	Working exposure (year) <5 (N=27) >5 (N=23)	12.57 ± 4.27 18.85 ± 5.87	0.001
Pap	**Age (year)** <40 (N=20) >40 (N=30)	24.34 ± 5.9 25.77 ± 6.45	0.41
	Smoking Yes (N=17) No (N=33)	26.17±6.29 19.77±5.01	0.001
	Alcohol consumption Yes (N=21) No (N=29)	24.14 ± 5.42 23.32 ± 4.67	0.58
	Working exposure (year) <5 (N=27) >5 (N=23)	20.16 ± 4.67 26.43 ± 6.01	0.001

MGG: May-Grunwald Giemsa; H & E: Hematoxylin and Eosin; Pap: Papanicolaou stain; *P*-value <0.05 was considered statistically significant

Table 4. Intercomparison of Frequency of MN between Cases and Controls Using Various Stains

Intercomparison of cases and control	MGG		H&E		PAP	
	MN Mean ± SD)	*P*-value	MN Mean ± SD)	*P*-value	MN Mean ± SD)	*P*-value
Cases smokers (N=17)	17.72±6.86		18.59 ± 6.38		26.17±6.29	
Control smokers (N=19)	8.74±4.43	0.001	9.51±4.98	0.001	13.79±5.82	0.001
Cases non-smokers (N=33)	11.04±5.32		11.36 ± 5.81		19.77±5.01	
Control non-smokers (N=31)	4.55±4.05	0.001	4.69±4.06	0.001	8.14±5.24	0.001
Cases alcoholics (N=21)	17.12 ± 6.71		17.59 ± 6.44		24.14 ± 5.42	
Control alcoholics (N=18)	8.69 ± 4.82	0.001	9.56 ± 4.79	0.001	13.98 ± 5.66	0.001
Cases non-alcoholics (N=29)	16.88 ± 5.41		23.32 ± 4.67		23.32 ± 4.67	
Control non-alcoholics (N=32)	8.21 ± 4.31	0.001	9.21 ± 4.12	0.001	13.31 ± 5.08	0.001

MGG: May-Grunwald Giemsa; H & E: Hematoxylin and Eosin; Pap: Papanicolaou stain; *P value <0.05 was considered statistically significant

Discussion

Workers of building construction are exposed to various carcinogens such as quartz dust of crystalline silica, diesel fumes asbestos and heavy metals (5,15). Studies performed by Consonni et al. (5) and Tse et al. (16) showed that bricklayer/ construction workers are exposed to increased risk of lung cancer.

We evaluated the genotoxicity in the building construction workers and found higher frequencies of MN compared to the controls, regardless of staining methods used. These findings were considered statistically significant (P=0.001). Our study revealed that it is possible to analyze the frequencies of MN in exfoliated buccal epithelial cells using LBC preparations. Similar to our study, Sellappa et al. (6) found that significantly increased frequencies of MN led to more damage to DNA and repair inhibition in building construction workers.

LBC is superior than conventionally prepared exfoliated cytology in certain circumstances viz a) provides clean background by removing almost all mucus, proteins and red blood cells, b) even distribution, improved morphology of cells and nuclear characteristics) cell clusters maintenance, d) effective fixation of samples (17-20).

The first study that applied LBC preparations in exfoliated buccal epithelial cells was performed by Kujan et al. (21) who found a uniform distribution of cells in addition to improved cytomorphology and visibility of nuclear details and thereby offering a superior and unbiased sample while controlling cell density, reducing scanty preparations and eliminating air drying artefacts. A study by Hayama et al. (22) showed improved thickness of smears, distribution of cells and reduced cellular overlapping and blood on LBC preparations. Authors also concluded that LBC led to improved sample preservation, adequacy of specimens, visualization of morphology of cells and reproducibility. Hence, slides prepared by LBC technique provide good quality smears with elimination of confounding factors. Thus, application of LBC in MN assay is one way to refine and improve the assessment of this biomarker.

In the Ramos et al. (4) study, slides were stained with Feulgen/fast green, Giemsa and Pap. Of these, Feulgen/fast green was DNA specific and Giemsa and Pap were DNA non-specific and authors found that frequencies of MN in construction workers were statistically significant compared to the controls (P<0.01) regardless of staining method used. However, Giemsa and Pap stains showed greater frequencies of MN compared to Feulgen/fast green. In the current study, we stained the slides with MGG, H & E and Pap stain and all these stains were DNA non-specific. The present study demonstrated higher frequencies of MN in construction workers compared to the controls (P=0.001) regardless of staining methods used.

Cytogenetic damage can be observed in certain lifestyle factors such as smoking, consumption of alcohol, vitamin deficiencies and supplementation (7,23). Moreover, smoking may increase the frequency of MN in the exfoliated buccal epithelial cells (24,25). In exfoliated buccal epithelial cells, it is not easy to discriminate consequences of alcohol

from smoking, because either alcohol or smoking can increase frequencies of MN. However, synergistic effect of smoking and alcohol are more evident than nonsmoker and non-drinker controls (7,26). Our study found that cigarette smoking caused significant number of MN in both cases and controls. However, alcohol consumption neither in cases nor controls shows statistically significant differences. Intercomparison of mean frequencies of MN between smokers, non-smokers, alcoholics and non-alcoholics of cases and controls using various stains showed significant statistical differences (P=0.001).

Slides prepared using LBC technique in our study were in excellent quality with single layer of cells, adequate cellularity, improved cell morphology and absence of obscuring factors such as microbial colonies, inflammatory cells, mucus, staining deposits and necrotic cores. Hence, this preparation reduces the likelihood of false positive results. Residual sample can be used for advanced procedures like immunocytochemistry. Hence, LBC can be used for MN assay in those occupationally exposed to potentially carcinogenic agents.

Conclusion

There is an increased risk of cytogenetic damage in building construction workers. Our study revealed that evaluation of MN of exfoliated buccal epithelial cells in building construction workers serves as a minimally invasive biomarker for cytogenetic damage. LBC preparations can be applied in MN assay as it improves the quality of smears and cell morphology, decreases the confounding factors and reduces false positive results.

Conflict of interest

There was no conflict of interest.

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