

Assessment of Cytomorphological Change in Urine and Oral Mucosal Cells among Fuel Station Workers in Kassala Locality, Sudan 2018

Elharith Mohammed Mohammed Ahmed¹, Alaa Mohammed Osman², Imman Ibrahim Suliman³, Fatima Ali Mohammed⁴, Mona Ahmed Osman⁵, Bahja Mustafa Abdalgader Mustafa⁶, Abdelrahman Mohamed Sid Ahme⁷

^{1, 2, 3, 4, 5} department Of Histopathology And Cytology, Faculty Of Medicine And Health Sciences, Medical Laboratory Science Program, Kassala University, Sudan

⁶ department Of Histopathology And Cytology, Faculty Of Medicine And Health Sciences, Medical Laboratory Science Program, Kassala University, Sudan

⁷ department Of Clinical Chemistry, Faculty Of Medicine And Health Sciences, Medical Laboratory Science Program, Kassala University, Sudan

Abstract: Background: Petroleum is a mixture of several hydrocarbons of which, about 95% of the compounds in petrol vapors are aliphatic and alicyclic compounds and less than 2% are aromatics. The volatile nature of petrol makes it readily available in the atmosphere whenever it is dispensed, especially at petrol filling stations. According to the IARC (1989), exposure to gasoline vapors is stated as carcinogenic to humans. Several epidemiological studies on human populations exposed to petroleum vapors have shown that there is an increased incidence of diseases. **Aim:** To assess cytomorphological change in urine and oral mucosal cells among fuel station workers in kassala locality "2018. **Methodology:** Case control study was conducted in fuel station of kassala locality during the period from June to September 2018, subject 120, divided into 70 worker in fuel station as case and 50 non worker of fuel station as control, The age of all participants ranged from 18 to 60 years. **Results:** The result also show that found change in infection cytomorphological change in buccal with ratio 9.2% and hyperplasia 7.5%, The result of urine and buccal cytomorphological change showed more than half of case group (58.3%), The result also show that found change in infection cytomorphological in urine with ratio 14.2%, crystal with ratio 7.6% and dysplasia with ration. **Conclusions:** From the study we found high relevance between cytomorphological change in urine and buccal mucosa among fuel station workers in kassala locality in urine have been 26(21.7%), in buccal 20(16.7%) they were asymptomatic. The dominant cytomorphological change between fuel station workers were infection in urine and buccal.

I. INTRODUCTION

Petroleum is a mixture of several hydrocarbons of which, about 95% of the compounds in petrol vapors are aliphatic and alicyclic compounds and less than 2% are aromatics⁽¹⁾. The volatile nature of petrol makes it readily available in the atmosphere whenever it is dispensed, especially at petrol filling stations. According to the IARC (1989), exposure to gasoline vapors is stated as carcinogenic to humans⁽²⁾. Several epidemiological studies on human populations exposed to petroleum vapors have shown that there is an increased incidence of diseases^(3,4). Petrol vapor is not safe even when inhaled for a brief period of time during fuelling vehicles⁽⁵⁾, which puts the gas station attendants at more risk by virtue of their occupational exposure.

International Agency for Research on Cancer (IARC) Working Group determined that there was adequate evidence in humans for the carcinogenicity of diesel exhaust. In addition, the Working Group found that diesel exhaust has "a positive association (limited evidence) with an increased risk of bladder cancer." Like most other carbonaceous fuel emission, diesel and gasoline exhausts contain toxic levels of respirable particles (PM <2.5 µm) and polycyclic aromatic hydrocarbons.⁽⁶⁾ Aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (collectively labeled BTEX) are natural constituents of the petroleum stream and exposure to these agents gives rise to many cancers⁽⁷⁾ However, several epidemiological studies have been performed to investigate the association between occupation and bladder cancer incidence. These studies delivered reliable support for a small but significant increased risk of bladder cancer among petroleum workers. Although the relative risk of bladder cancer associated with these occupations is small, the public health impact may be significant, in view of the substantial number of people who were and are employed in petroleum-related occupations.⁽⁸⁾

The oral cavity has sometimes been described as a mirror that reflects the health of the individual, Changes indicative of disease are seen as alterations in the oral mucosa lining the mouth, which can reveal systemic conditions, such as diabetes or vitamin deficiency, or the local effects of chronic tobacco or alcohol use⁽⁹⁾.

Countering these changes are mechanisms to metabolise carcinogens, repair DNA damage, control growth, and defend against cancer. Cancer is a consequence of an interaction of these many factors. Diagnosis is increasingly aided by detection of cellular and now molecular changes. Treatment is increasingly looking towards chemotherapy and now gene therapy. However, there is no doubt that prevention is the most important aspect, particularly patient education and the reduction of lifestyle risk habits and environmental factors. The risk of developing oral cancer increases with advancing age, most cases occurring in people aged 50 years or over⁽¹⁰⁾.

The urothelial cells, the cells lining the surface of the urinary bladder, are comprised of a unique cell type with high plasticity and a variety of cell functions. They are the first line of bladder defense and serve as an interface between pathogens. Urothelial cells are equipped with several defense mechanisms to prevent adherence of pathogens and maintain impermeability to urinary solutes. Urothelial cells express both estrogen receptor-alpha and beta, epidermal growth factor receptor and fibroblast growth factor receptor. These receptors play a major role in urothelial cell response to injury and infection. The urothelial cells also release a number of cytokines and other immune system mediators. The ability to culture normal urothelial cells in vitro make it possible to further investigate the immunoregulatory potential of urothelial cells⁽¹¹⁾.

II. MATERIALS AND METHODS

Case control study was conducted in fuel station of kassala locality during the period from June to September 2018, subject 120, divided into 70 worker in fuel station as case and 50 non worker of fuel station as control.

A. Samples Collection

Urine sample collected in urine container and buccal sample collected by tongue depressors then smear in slide and fixed immediately, Ethical approval was obtained from kassala university faculty of medical laboratory, informed consent was taken from each participant after the full explanations about the study.

B. Ethical Consideration

An ethical clearance of this study was approved by ethical committee of kassala university. Informed consent was obtained from each participant and hospitals before taking the sample.

C. Methodology

Preparation of smear and pap stain technique.

D. Buccal Smear

Scraped of the oral mucosa and smeared rapidly.

E. Urine Smear

After urine collection next centrifugation and take perception after that spread in clean and dry slide.

F. Pap Stain Technique

Each urine and buccal smear were immediately fixed while it was wet in 95% ethyl alcohol for 15 min and eventually stained adopting Papanicolaou procedure (pap stain)(appendix). Ethyl alcohol fixed smears were hydrated in descending concentration of 95% ethyl alcohol, through 70% alcohol to distilled water, for 2 min in each stage. The urine and buccal smears were then treated with Harris' hematoxylin for 5min to stain the nuclei, rinsed in distilled water, and differentiated in 0.5% aqueous hydrochloric acid for a few seconds, to remove the excess stain. Then the smears were blued in ammoniated tap water 2 second and dehydrated in ascending alcoholic concentrations from 70%, through two changes of 95% alcohol for 2 min for each change. The smears were then treated with Eosin Azure 50 for 3 min. For cytoplasmic staining, they were treated with Papanicolaou Orange G6 for 2 min, rinsed in 95% alcohol and then dehydrated in absolute alcohol. Were then cleared in Xylene and mounted in Distrene Polystyrene Xylene(Dpx). All quality control measures were implemented all over the study procedures. Smears were first examined by light microscope at $\times 10$ followed by $\times 40$.

G. Statistical Analysis

Data from all patients were presented as percentage and (mean±SD), differences between means of patients and control groups were considered statistically significant with p-value threshold <0.05 using independent T-test. Significant correlation (r) was calculated using linear correlation test.

Table (1) relationship between cytomorphological change in buccal and study population.

Study population	Normal	Hyperplasia	Infection	p.value
Benzene working	50 (41.7%)	9 (7.5%)	11 (9.2%)	
Non Benzene working	50 (41.7%)	0 (0.0%)	0 (0.0%)	

*Result expressed as significant different conceder as p value <0.05.

Table (2) relationship between cytomorphological change in urine and study population .

Study population	Normal	Dysplasia	Crystal	Infection	p.value
Benzene working	44 (36.7%)	1 (0.8%)	8 (6.7%)	17 (14.2%)	
Non benzene working	50 (41.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

*Result expressed as significant different conceder as p value <0.05.

Table (3) relationship between cytomorphological change in buccal and productive tools.

Productive tool	Normal	Hyperplasia	Infection	p.value
Yes	10 (14.3%)	0 (0.0%)	1 (1.4%)	
No	40 (57.1%)	9 (12.9%)	10 (14.3%)	

*Result expressed as significant different conceder as p value <0.05.

Table (4) relationship between cytomorphological change in urine and productive tools.

Protective tools	Normal	Dysplasia	Crystal	Infection	p.value
Yes	7 (10%)	3 (4.3%)	1 (1.4%)	3 (4.3%)	
No	37 (37%)	14 (20%)	7 (10%)	14 (20%)	

*Result expressed as significant different conceder as p value <0.05.

Table (5) relationship between cytomorphological change in buccal and time of shower.

Times of shower	Normal	Hyperplasia	Infection	p.value
1	9 (12.9%)	1 (1.4%)	2 (2.9%)	
2_5	41(58%)	8 (11.4%)	9 (12%)	

*Result expressed as significant different conceder as p value <0.05.

Table (6) relationship between cytomorphological change in urine and time of shower.

Time of shower	Normal	Dysplasia	Crystal	Infection	p.value
1	6 (8.6%)	1 (1.4%)	3 (4.3%)	2 (2.9%)	
1_5	38 (54.3%)	0 (0.0%)	5 (7.1%)	15 (21.4%)	

*Result expressed as significant different conceder as p value <0.05.

Table (7) relationship between cytomorphological change in buccal and job year.

Job years	Normal	Hyperplasia	Infection	p.value
1_10	38 (45.3%)	5 (7.1%)	5 (7.1%)	
11_20	6 (8.6%)	2 (2.9%)	3 (4.3%)	
21_30	5 (7.1%)	1 (1.4%)	0 (0.0%)	
31_40	1 (1.4%)	1 (1.4%)	0 (0.0%)	

*Result expressed as significant different conceder as p value <0.05.

Table(8) relationship between cytomorphological change in urine and job years.

Job year	Normal	Dysplasia	Crystal	Infection	p.value 0.300
1_10	33 (47.1%)	1 (1.4%)	5 (7.1%)	9 (12.9%)	
11_20	6 (8.6%)	0 (0.0%)	0 (0.0%)	5(7.1%)	
21_30	4 (5.7%)	0 (0.0%)	2 (2.9%)	3 (4.3%)	
31_40	1 (1.4%)	0 (0.0%)	1 (1.4%)	0 (0.0%)	

*Result expressed as significant different concenter as p value <0.05 .

Table(9) relationship between cytomorphological change in buccal and nature of work.

Nature of work	Normal	Hyperplasia	Infection	p.value 0.500
Handgun	37 (52.9%)	6 (8.6%)	8 (11.4%)	
Puncture	1 (1.4%)	1 (1.4%)	0 (0.0%)	
Washer	8 (11.4%)	2 (2.9%)	3 (4.3%)	
Purser	4 (5.7%)	0 (0.0%)	4 (5.7%)	

*Result expressed as significant different concenter as p value <0.05 .

Table (10) relationship between cytomorphological change in urine and nature of work.

Nature of work	Normal	Dysplasia	Crystal	Infection	p.value 0.100
Handgun	34 (48.6%)	0 (0.0%)	3 (4.3%)	14 (20.0%)	
Puncture	2 (2.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Washer	5 (7.1%)	1 (1.4%)	4 (5.7%)	3 (4.3%)	
Purser	3 (4.3%)	0 (0.0%)	1 (1.4%)	0 (0.0%)	

*Result expressed as significant different concenter as p value <0.05 .

III. DISCUSSION

From the result, it is evident that the cytomorphological change in urine is highly relevant with working on a fuel station, about 70 workers. 26 urine smears were positive to cytomorphological change, 17 workers (14.2%) were affected by infection, 1 worker (0.8%) dysplasia and 8 workers (6.7%) crystal. Analogous study exposure of fuel station workers to fuel products increases the risk of bladder atypical changes, which may progress to precancerous and cancerous changes reported by Ahmed et al. in July 2016 in Saudi Arabia, however in the present study we aimed at evaluating the burden of such exposure through assessment of bladder atypical cytomorphological change. Although, the presence of atypia among cases was statistically significant as shown by Zeegers et al. (2001). Also from the result, it is clear that the cytomorphological change in buccal mucosa is highly connected with exposure to petroleum products, 20 buccal smears were positive to cytomorphological change. We observed 9 smears (7.5%) were affected by hyperplasia and 11 smears (9.2%) were infected. Agreeing to other studies, individuals working in fuel stations may have been exposed to volatile organic compounds from petrol, which increases cytomorphological changes in buccal mucosa, as reported by article et al. in February 2010 in Tamil Nadu, South India. Also, the use of protective tools, years of job, and nature of work can be an effect of cytomorphological change in urine and buccal mucosa among fuel station workers. At the end of this study, it is that urine and buccal mucosa cancer risk from exposure to petroleum products.

IV. CONCLUSIONS

From the study, we found high relevance between cytomorphological change in urine and buccal mucosa among fuel station workers in the Kassala locality. In urine, 26 (21.7%) were positive, and in buccal, 20 (16.7%) were positive. They were asymptomatic. The dominant cytomorphological change between fuel station workers was infection in urine and buccal.

V. RECOMMENDATIONS

We recommend the following

- 1) Increase the times of shower per day
- 2) Use effective body protectors tools
- 3) Screening program is important.
- 4) Increase the number of population (sample size)
- 5) Do advanced research in this area to detect the source of the dyskeratotic cell



REFERENCE

- [1] Gupta S, Dogra TD, Air pollution and human health hazards. *Indian J Occup Environ*, 2002: 6:89–93.
- [2] Carere A, Antocchia A, Cimini D, Crebelli R, Degradi F, Leopardi P, Marcon F, Sgura A, Tanzarella C, Zijno A, Genetic effects of petroleum fuels II: analysis of chromosome loss and hyperploidy in peripheral lymphocytes of gasoline station attendants. *Environ Mol Mutagen*, 1998;32:130–138.
- [3] Lagorio S, Forastiere F, Iavarone I, Vanacore N, Fusel-li S, Carere A, Exposure assessment in a historical cohort of filling station attendants. *Int J Epidemiol*, 1993;22:51–56.
- [4] Carletti R, Romano D, Assessing health risk from benzene pollution in an urban area. *Environ Monit Assess*, 2002: 80:135–148.
- [5] Pranjić N, Mujagić H, Nurkić M, Karamehić J, Pavlović S, Assessment of health effects in workers at gasoline station. *Bosn J Basic Med Sci*, 2002: 2:35–45
- [6] Claxton LD, The history, genotoxicity, and carcinogenicity of carbon-based fuels and their emissions. Part 3: Diesel and gasoline. *Mutation Research, Reviews in Mutation Research*, 2015: 763: 30–85.
- [7] Bråtveit M, Kirkeleit J, Hollund BE et al, Biological monitoring of benzene exposure for process operators during ordinary activity in the upstream petroleum industry. *Annals of Occupational Hygiene*, 2007;51: 487–494.
- [8] Steinsvåg K, Bråtveit M and Moen BE, Exposure to carcinogens for defined job categories in Norway's offshore petroleum industry, 1970 to 2005. *Occupational and Environmental Medicine*, 2007: 64: 250–258.
- [9] Squier, Christopher A.; Kremer, Mary J. (2001). "Biology of Oral Mucosa and Esophagus". *Journal of the National Cancer Institute. Monographs*, 2001: (29): 7–15. PMID 11694559.
- [10] Surveillance Epidemiology and End Results (SEER). SEER Cancer Statistics Review, National Cancer Institute, 1975-2004.
- [11] Moro, C; Uchiyama, J; Chess-Williams, R, "Urothelial/lamina propria spontaneous activity and the role of M3 muscarinic receptors in mediating rate responses to stretch and carbachol". *Urology*, December 2011: 78 (6): 1442.e9–15.