

Evaluation of Occupational Exposure To Xylene in Petroleum Workers by Assessing Urinary Methyl Hippuric Acid and Cellular Changes of Exfoliated Epithelial Cells in Buccal Mucosa Smears



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Abstract: Xylene is an important component of petrol and a widely distributed environmental contaminant. About 98% of Xylene is derived from the petrochemical and petroleum refining industries. When human get expose to xylene, which is one of the major Geno toxicants, may be associated with a range of acute/chronic diseases and cancer still literature is not available. Taking into our mind that occupational exposure to such derivatives may possess genotoxic risk or not. Hence our study aims to investigate and correlate the cellular changes in exfoliated oral epithelial cells oral smears with urinary methyl hippuric acid level estimation in petrol pump workers and to identify the significant role of xylene on oral mucosa.

Materials and Methods: Urine samples and oral buccal mucosa smears were collected from 30 healthy individual (control) and 30 petroleum pump workers (case) working in petroleum station who are above 18 years of age. The urine was collected before exposure/work shift and after completion of work shift. The urinary methyl hippuric acid (MHA) level was analyzed by using Shimadzu UV-Visible Spectrophotometer procedure. The smeared slides were stained with PAP stain and analyses the cytomorphometric changes of exfoliated epithelial cells by using Axio Vision SE64 Rel 4.9.1. Ink Software.

Results: The urinary Methyl hippuric acid level was substantially higher in cases than in controls ($p < 0.001$). The Micronuclei (MN) frequency was drastically increased in cases than in controls and was statistically highly significant ($P < 0.0001$). The frequency of MN gradually increased along with increased urinary MHA level in petroleum pump workers (case).

Conclusion: The urinary MHA level and MN frequency is a useful index to recognize the occupational exposure to the petroleum product. Thus, our study emphasizes that appropriate precaution and regular biomonitoring must be taken among

petrol pump workers which shall help to reduce their potential levels of risk associated with the occupation.

Index Terms: Exfoliated buccal cells, Urine Methyl hippuric acid level, UV Spectrophotometry, Xylene

I. INTRODUCTION

Health is very precious, and the work environment plays an important role in one's health. Air, noise, heat, and radiation are the main source of environmental pollution especially in urban areas [1].

Millions of workers in a variety of occupational settings have the potential to be exposed to hazardous substances, important among which are petroleum derivatives [2]. Many toxicological effects may be associated with the exposure to petroleum component such as benzene, toluene, ethylene and xylene, which are also known as volatile organic compounds (VOCs) [3]. Petroleum derivatives constitute a complex mix of chemicals and are well known genotoxic agents especially xylene. Exposure to petroleum vapors is classified by the International Agency for Research on Cancer as Group-2A ('probably') carcinogen to humans, mainly on the basis of well-established carcinogenicity.³ The presence of xylene in petrol and as an industrial solvent can result in widespread emissions to the occupationally exposed petrol pump workers[4]. Xylene is a colorless, flammable liquid that smells like gasoline. It is found in both natural products like coal tar and petroleum and also in manufactured products like inks, insecticides, and paints. Xylene is also used as a solvent to make other chemical compounds. People might be exposed to xylene in many ways including breathing air, particularly in areas near factories or petrol pumps [5].

The mechanism(s) of xylene as a carcinogenic agent has not yet fully understood but the hypothesis of xylene acting as an indirect genotoxic carcinogen is well-documented in literatures. Cytogenetic damage, including structural aberrations, micronuclei (MN) and sister chromatid exchanges (SCEs), are demonstrated in bone marrow cells, splenocytes,

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peripheral blood lymphocytes and oral mucosal cells of mice exposed to benzene but not proven in humans. Among all the cytogenetic biomarkers, micronuclei have been extensively used to gauge rates of chromosomal damage to genotoxic agents[6]. Exfoliated oral buccal cells have been used non-invasively to successfully show the genotoxic effects of various lifestyle factors, medical treatments and occupational exposure to potentially mutagenic and/or carcinogenic chemicals.

Methyl hippuric acid is a principal metabolite of xylene and hence its level in urine can be used to monitor external exposure in petrol pump workers.⁵ Although xylene is present at low level in urine of exposed individuals, trying to find some sensitive methodology of direct determination of trace amount of this environmental and industrial pollutant can be a suitable alternative way to overcome the problems may associate with the determination of its metabolite (methyl hippuric acid) in urine[7]. Petrol pump workers are chronically exposed to petroleum derivatives primarily through inhalation of the volatile fraction of petrol during vehicle refueling. Taking into our mind that occupational exposure to such derivatives may possess genotoxic risk. Hence our study aims to investigate and correlate the cellular changes in exfoliated oral epithelial cells using PAP stained oral smears with the UV- spectrophotometric urinary methyl hippuric acid level estimation in petrol pump workers.

II. METHODOLOGY

Methodology:

The study population selected from various petrol pumps according to inclusion and exclusion criteria and control population were healthy individuals who were not exposed to petrochemical product.

Inclusion criteria:

- Persons giving their written consent to participate in the study.
- Male employees who is above 18 years of age
- Participant working in the Mysuru city limits.
- Workers who are working in shifts not less than 8hrs.
- Worker who are working in petrol station for a period of 5ys or above.
- Workers should not have the habit of consuming any form of tobacco/alcohol.

Exclusion criteria:

- Person with history of any systemic condition or disease.
- Person with history of any chemotherapy/ surgery/ radiation therapy.
- Person having any lesions/ulcer/tumour/trauma in the oral cavity.

Urine samples were collected from 30 healthy individual and 30 petroleum pump workers working in petroleum station who are above 18 years of age. The urine was collected before exposure/work shift and after completion of a work shift. The urinary methyl hippuric acid (MHA) level was analyzed by using Shimadzu UV-Visible Spectrophotometer procedure. (Fig.1) Oral buccal mucosa smears were also

collected from the same individuals (30 healthy individual and 30 petroleum pump workers) and smeared slides were fixed in the alcohol and coded it. These smeared slides were stained with PAP stain. The coded slides were analyzed by using bright field microscope under 40X magnifications and cytomorphometric changes of exfoliated epithelial cells by using Axio Vision SE64 Rel 4.9.1. Ink Software. (Fig.2) The cellular changes in exfoliated oral epithelial cells like cytomorphometry and micronuclei were studied. 100 cells were studied in a zig-zag manner in each slide under bright field microscopy. Care must be taken to avoid repeating the same/ overlapping cells.

Micronuclei were also identified and scored only in intact epithelial cells and recorded criteria used for identification of micronuclei were followed based on Tolbert P.E. et al (1992) criteria [8]. (Fig.3)

The following criteria for scoring micronuclei:

- Rounded, smooth perimeter suggestive of the membrane.
- Less than a third the diameter of the associated nucleus, but large enough to discern shape and colour.
- Staining intensity similar to that of a nucleus.
- Texture similar to that of a nucleus.
- The same focal plane as a nucleus.
- An absence of overlap with, or bridge to, nucleus.

The obtained data were collected, and statistical analysis methods were applied like descriptive statistics, one sample t-test, paired sample t-test, Pearson's correlation.

A. Figures



Figure.1: Shimadzu UV-Visible Spectrophotometer with quartz cuvettes and digital display.



Figure 2: Cytomorphometric analysis of exfoliated epithelial cells by using software Axio Vision SE64 Rel 4.9.1. Ink

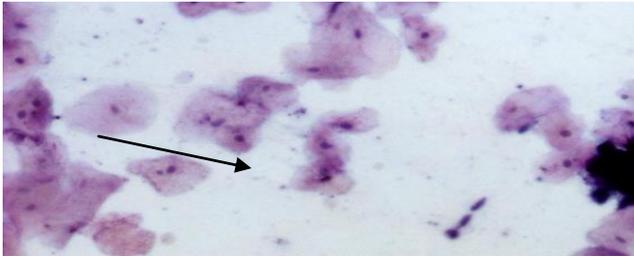


Figure 3: Micronuclei in oral exfoliated epithelial cells of PAP stained smear

III. RESULT

In the present study, the urinary MHA level was in cases 87.8 µg/ml with SD value is 29.18441 whereas 30.8 µg/ml in controls with SD value is 7.42038 was found as shown in Table I and Graph I. The urinary MHA level was high in cases than the control population.

The urinary MHA level in cases, before shifting to work the mean value was 30.8333 and after shift of work, the mean value was 87.8333 was highly significant in Table II. The urinary MHA level in cases after shift of work was more than before shifting of work in the same population similarly, the Urinary MHA level in Controls before the shift of work is 28.0667 mean value and after shift of work is 30.5000 mean values were shown as non-significant in Table III. There was no much variation in the level of urinary MHA level among control population. Comparison of urinary MHA level in both cases and controls before and after shifts of work is showing in Graph II.

The correlation of urinary MHA level, cellular diameter (CD) in cases and controls, the p-value was 0.514 and 0.607, which was non-significant shown in Table IV and Graph III. The correlation of urinary MHA level, nuclear diameter (ND) in cases and controls, the p-value was 0.234 and 0.632 which was non-significant shown in Table V and Graph IV. Similarly, the correlation of urinary MHA level, nuclear-cytoplasmic ratio in cases and controls, and the p-value was 0.514 and 0.607, which was non-significant shown in Table VI and Graph V. There was no correlation identified between cases and control population regarding urinary MHA level and cytomorphometry of oral epithelial cells.

The correlation of urinary MHA level and micronuclei (MN) among cases, the p-value was 0.000 which was significant in Table- VII and Graph VI. The urinary MHA level increase along with the increase in the number of micronuclei.

The comparison between urinary MHA level and micronuclei in cases and controls, the p-value was 0.000 which was highly significant as shown in Table VIII and Graph VII.

Table I: Mean, SD value of urinary MHA level in both case and controls

	Group	Mean	SD	Inference
Urinary MHA	Controls	30.8	7.42038	Highly

(µg/ml)	Cases	87.8	29.18441	Significant
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Table II: Urinary MHA level in cases before and after shift of work.

Variable	Group	Mean	SD	Inference
Urinary MHA (µg/ml)	Cases Before shift	30.8333	7.39680	Highly Significant
	Cases After shift	87.8333	29.18441	

Table III: Urinary MHA level in Controls before and after shift of work.

Variable	Group	Mean	SD	Inference
Urinary MHA (µg/ml)	Controls Before shift	28.0667	2.21178	Non Significant
	Controls After shift	30.5000	1.85231	

Table IV: Correlation of urinary MHA level and cellular diameter (CD) of oral epithelial cells in cases and controls.

	N	V1	V2	Pearson Correlation	P value	Inference
Cases	30	Urinary MHA (µg/ml)	CD	-0.124	0.514	Non-significant
Controls	30	Urinary MHA (µg/ml)	CD	-0.098	0.607	

Table V: Correlation of urinary MHA level and nuclear diameter (ND) of oral epithelial cells in cases and controls.

	N	V1	V2	Pearson Correlation	p value	Inference
Cases	30	Urinary MHA (µg/ml)	ND	.222	.238	Non significant
Controls	30	Urinary MHA (µg/ml)	ND	-.091	.632	

Table VI: Correlation of urinary MHA level and nuclear and cytoplasmic (NC) ratio of oral epithelial cells in cases and controls.

	N	V1	V2	Pearson Correlation	P value	Inference
Cases	30	Urinary MHA (µg/ml)	NC	-.121	.523	Non-significant
Controls	30	Urinary MHA (µg/ml)	NC	-.096	.612	

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Table-VII: Correlation between urinary MHA level and micronuclei (MN) in Cases.

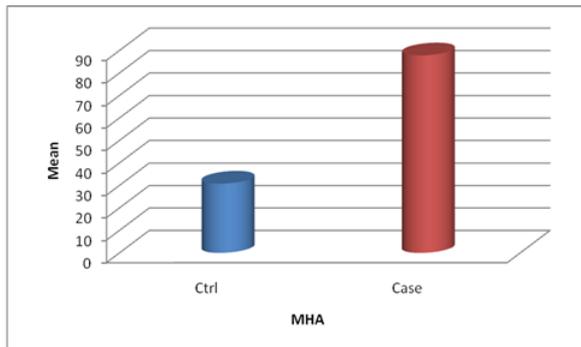
V1	V2	Pearson Correlation	N	P value	Inference
Urinary MHA (µg/ml)	MN	.808**	30	.000	Significant

** . Correlation is significant at the 0.01 level (2-tailed).

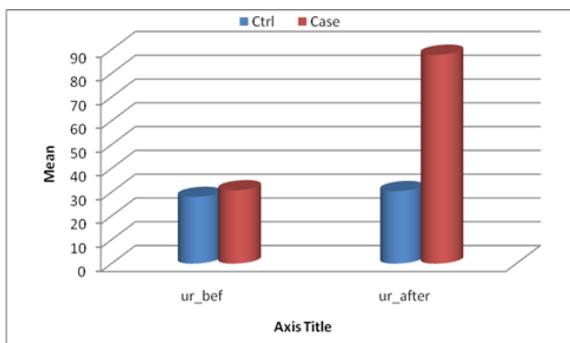
Table-VIII: Correlation between urinary MHA level and micronuclei (MN) in Cases and Controls.

	N	V1	V2	Pearson Correlation	P value	Inference
Case	30	Urinary MHA (µg/ml)	MN	.808**	.000	Highly significant
Control	30	Urinary MHA (µg/ml)	MN	.662**	.000	

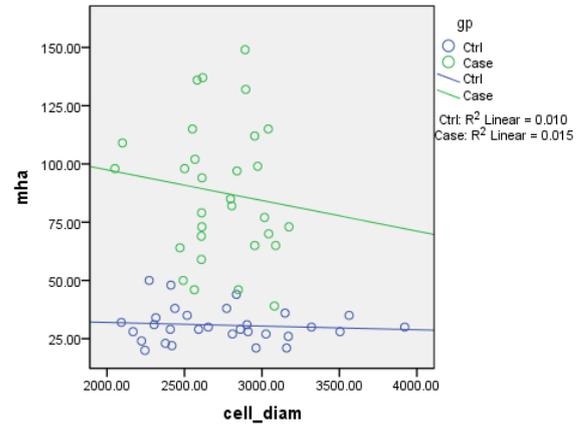
Graph I: Showing the level of urinary MHA in both Cases and Controls



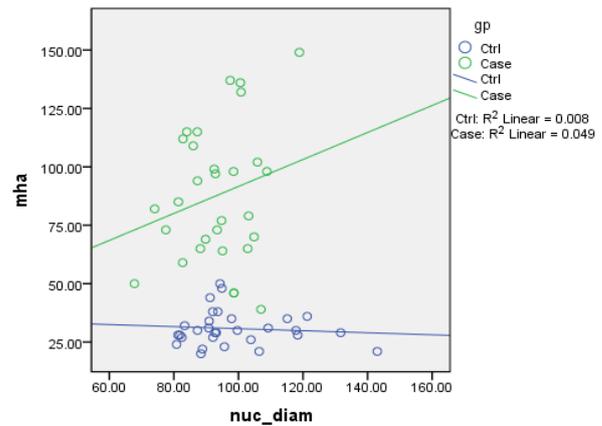
Graph II: Comparison of urinary MHA level in cases and controls before and after shifts of work



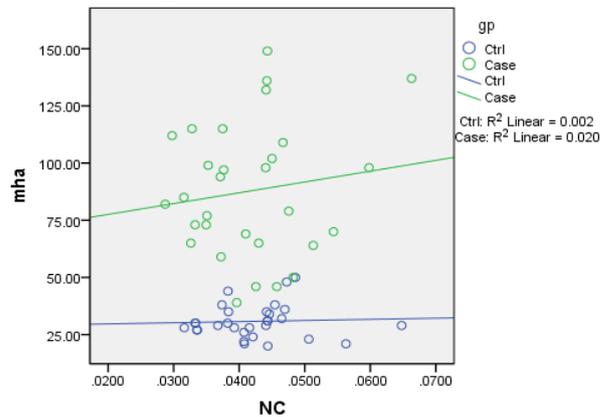
Graph III: Showing Correlation of urinary MHA level and cellular diameter of oral epithelial cells in cases and controls.



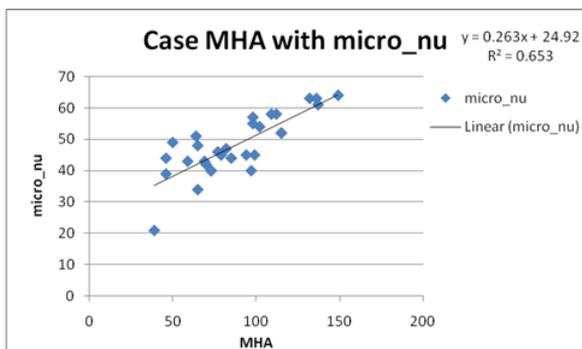
Graph IV: Showing correlation of urinary MHA level and nuclear diameter of oral epithelial cells in cases and controls



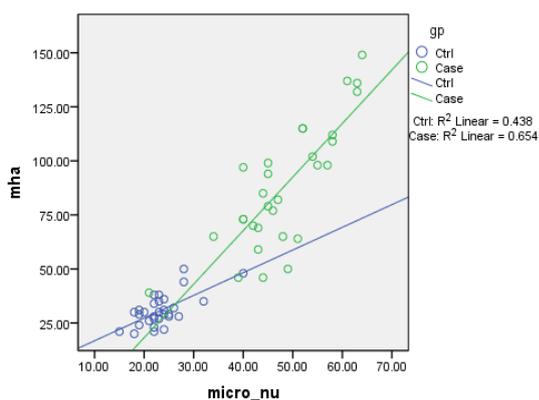
Graph V: Showing comparison of urinary MHA level and NC ratio of oral epithelial cells in cases and controls



Graph VI: Shows the urinary MHA level increase along with the increase in the number of micronuclei (MN) in Cases before and after the shift of work



Graph VII: showing the comparison between urinary MHA level and micronuclei (MN) in Cases and Controls.



IV. DISCUSSION:

Xylene is an important component of petrol and a widely distributed environmental contaminant. About 98% of Xylene is derived from the petrochemical and petroleum refining industries. Petrol station attendants are chronically exposed to petroleum derivatives primarily through inhalation of the volatile fraction of petrol during vehicle refueling. Therefore, occupational exposure to xylene in humans generally takes place in factories, refineries; petrol refueling and other industrial settings [9]. When humans get exposed to one of the major Geno toxicants like xylene have been associated with a range of acute/chronic diseases and cancer depending upon the concentration and time of exposure [10].

Methyl hippuric acid is a principal metabolite of xylene and hence its level in urine can be used to monitor external exposure in petrol pump workers[5]. Taking into our mind, the occupational exposure to such derivatives may cause genotoxic risk, hence our study attempted to investigate any correlation between cellular changes in exfoliated buccal epithelial cells that were obtained and the urinary MHA level among petroleum pump worker.

The present study consisted of 30 petroleum pump workers (case group) and 30 healthy individuals with no known exposure to petrochemical products (control group) all the above 18yr old male population. All the subjects who met the inclusion and exclusion criteria were subjected to a pair of oral buccal smear sampling and collection of the urine sample

for estimation of MHA levels. The oral buccal smears were stained with Rapid PAP stain, cytomorphometry of cells was done and the estimation of urinary MHA level was done using UV-spectrophotometer.

In our study, the urinary MHA level was found to be more in case population (87.8 µg/ml) than control population (30.8 µg/ml). Our study is in accordance with studies by Celik A et al. (2003)[3], Singaraju et al. (2012)[7], Koschsorur et al. (2000)[11], Verma et al. (2001)[12], Hein et al. (1989)[13], were also reported that the urinary MHA level in petrol station workers was significantly higher than controls. In the present study, the urinary MHA level in participant after the end of their shift was significantly higher than before starting to work. Similar to our study, Engstrom K et al. (1978)[14] who reported that a determination of methyl hippuric acid concentration in urine samples was found to be high at the end of the workday for evaluating the xylene exposure. Thus, our study found that occupational exposure to xylene is associated with increased MHA level in urine at the end of work shift compare to start of work shift.

In the present study, the correlation of urinary MHA level to cytomorphology of oral epithelial cells in case population was showed non-significant. There was not much difference was found between urinary MHA level and cytomorphology of oral epithelial cells among case and control population. Further studies may require knowing the effect of the xylene over the oral epithelial cells with a time of exposure in the larger population.

The analysis of micronuclei (MN) has gained popularity as biomarker assay for human genotoxic exposure and effect of xylene [7]. Hence, we analyzed the micronuclei along with urinary MHA level in both case and control group. In our study, the urinary MHA level and micronuclei (MN) showed highly significant in the case group than control group. Similarly studies by Celik et al. (2003)[3], Rajkokila et al. (2010)[9], Oesch et al. (1995)[15], Bukvic et al. (1998)[16], P Arul et al.(2017)[10], Benites et al. (2006)[2], Sellapa S et al. (2010)[17], and Moohammadaree A et al. (2012)[18], reported that increased frequency of MN in buccal smears obtained from the petrol pump workers than in controls and concluded that petrol pump workers were at a risk of cytogenetic damage at their work place[19].

In our study, we try to identify the relation between urinary MHA level and micronuclei frequency in epithelial cells. The frequency of MN gradually increased with increased urinary MHA level in petroleum pump workers. Hence, the micronucleus test in exfoliated epithelial cells and the urinary MHA levels in urine seem to be a useful biomarker of occupational exposure to xylene.

As demonstrated in this study, MN frequency is a useful tool to identify for the exposure of petroleum product, but we could not able asses the relationship between urinary MHA level and cytomorphometric analysis of epithelial cells among petrol pump workers. The urinary MHA level and MN frequency is a useful index to recognize the occupational exposure to a petroleum product. Molecular studies are required to identify the effect of the xylene over the oral epithelial cells in a larger population. Xylene has a significant role in humans, and act as carcinogenic agent, it may initiate the carcinogenic process often to do damaging the cellular DNA. Thus, our study emphasizes that appropriate precaution and regular biomonitoring must be taken among petrol pump workers about their health which may reduce their levels of risk associated with the occupation.

V. CONCLUSION

The hazards of xylene are well documented, but the substitutes are not so thoroughly evaluated. It may not be good to get expose to petroleum product on daily basis to larger the volume. One must be aware these petroleum products are the main pollutant that is responsible for the genotoxic effect. The result obtained in this study suggests that exposure in these workplaces may induce genotoxic effects. It is important to control and reduce the exposure to petroleum products as much as possible and it is necessary to educate the petrol pump workers about its hazardous effect to ensure the safety and healthy working atmosphere for the petrol pump workers. Hence, the petrol pump worker should use a proper personal protective equipment, so that they can prevent the hazardous effect of xylene.

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Dr. Gurupadayya currently working on drug- drug interaction and herbal drug interaction in pharmaceutical research. He worked on research projects funded by government agencies viz. UGC, AICTE and VGST, DST. Under his guidance, 7 students have completed their PhD degree and currently 1 Ph.D. student is working under his supervision. In addition he has successfully guided 36 masters' students in the field of pharmaceutical analysis. He has several research publications both in national and international journals of high profile. He is a life member of Association of Pharmaceutical Teachers of India, India Pharmaceutical Association and Pharmacist of Karnataka State Pharmacy council, India