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Evaluation of Micronucleus in Exfoliated Epithelial Cells and Seminal Fluid Quality in Petrol Station Workers in Samarra City-Iraq

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Abstract---The use of cytogenetic parameters, including micronuclei and seminal fluid quality, is one of the most important indicators relied upon to determine the harm that a person may suffer during his work. Benzene is an important organic product. When exposed to it continuously, it may lead to many disorders. As well as exposure to gasoline, vapors can cause damage to the DNA, which is one of the possible causes of cancer in humans. The study was conducted on 80 individuals, 50 workers in filling stations who are permanently exposed to petroleum derivatives by inhaling them while refueling vehicles, and {the control group} also consisted of 30 individuals collected from rural areas. Cells of Exfoliated buccal and seminal fluid were obtained from each subject to examine. The frequency of micronucleus among workers in filling stations results showed a significant increase compared to the control group. The hesitance of micronuclei among workers in filling stations was (106 ± 3.23) compared to the control group which was (27.6 ± 3.26) , as well as the results obtained showed that there were statistically significant differences between semen indicators for workers in filling stations compared to the control group, there is exception.

Keywords---epithelial cells, micronucleus, quality, Samarra city-Iraq, seminal fluid.

Introduction

This page should begin with the introduction of your article and follow the rest of Benzene is one of the hazardous factors that poison the workers exposed to it in petroleum filling stations, its severity depends on the duration of exposure, its concentration in the air and age and health of the person exposed. Benzene is a carcinogen and damages the composition of the DNA. Roth, et al., 2002 showed in their study that workers who used to work in the petrochemical industry could still suffer genotoxic damage, even after exposure has been removed for several years. Various hazardous substances can enter the body through the Oral cavity. It may cause a systemic condition as impact of [Koh et al., 2014]. Individual health might be reflected by oral mucosa with pathological changes [Torres-Bugarín et al., 2014]. Oral mucosa is the communicated path between the lining moist and outer portion. Thus, the basic use of the oral mucosa is protecting the oral cavity deep tissues. The body considers oral mucosa like a barrier used against the potential carcinogen's substance. Then the damage susceptible by manifests and other pathological changes before a systemic condition reflecting [Torres-Bugarín et al., 2014].

Moreover, the study by Koh, et al., at 2014 in Korea, demonstrated an increase in the incidence of oral cancer and increased mortality among workers at a refinery and petrochemical complex [Koh et al., 2014]. [Mrdjanovic, et al., 2014] also found that there were high frequencies in the formation of micronuclei (MN) and in their sister chromatid exchange in the oil refinery workers. While Jamebozorgi, et al., 2016 did not find an increasing of MN frequency when vocational exposure to a low level of benzene [Jamebozorgi et al., 2016]. The micronucleus test (MN) is one of the most reliable and sensitive tests for detecting mutations and screening for genotoxic. It is a vital sing cause of genetic damage and can be performed quickly and relatively inexpensively [Lovreglio and Maffei, 2014].

Micronucleus is a mass of chromatin outside the nucleus seen in the cytoplasm, its size is smaller or equal to the size of the nucleus and it is of light color, and it arises either form a defect in the migration of chromosome during the anaphase of cell division, or when the cell is exposed to clastogenic substances that cause damage to the DNA which results chromosomal fragments lacking a centromere do not participate in division and are not linked to the spindle filaments. Semen analysis is a clear indication for assessing the genotoxicity of substances that males may be exposed to in the workplace. Since sperm is a single cell that can be obtained easily, it is produced in large numbers and carries the genetic material to next generation. Moreover, the quantity and quality of semen is often used as evidence of male reproductive function. Therefore, the study of human semen is valuable indicators for identifying toxins resulting from occupational exposure. This study is aimed to evaluation cytogenotoxic damage due to the exposure of workers in a petrol station to petroleum derivatives, depending on the replication of micronucleus in exfoliated epithelial cells lining the mouth and seminal fluid quality.

Subjects and Methods

This study was implemented on the workers at petrol stations at Samarra, Iraq. The period was from March 15, 2019 to July 31, 2019. The study sample consisted of 80 individual's distributors to two groups. Group one: The petrol station workers group included 50 people from 22-58 years, who were randomly selected. Each individual fills a form of questionnaire included: regarding information, marital status, medical history, duration of smoking and exposure to alcohol habit. While, Group two: (The control group) also consisted of 30 individuals collected from rural areas and considered to be matched to the workers not exposure to benzene or any toxic chemicals or even smoke of Tobacco. Moral considerations. The approvals of petrol stations were obtained, as well as obtained from studied samples to participate voluntarily in the study with full withdrawal right, with free medical examinations and ensure the confidentiality not to be identified by data.

Evaluation of micronucleus

The workers in the petrol filling station and control group have been directed to rinse their mouths very well to remove unwanted remains before collecting exfoliated buccal epithelial cells, and then used a sterile wooden spatula to collect cell samples from the mucosa. The collected samples from the mouth were smeared on clean sterile glass slides. They were left at lab in the air to dry. Then, fixed with Methanol: Acetic Acid (3:1). After that, slides stained with Giemsa, each slide was evaluated to determine Micronucleus. Three slides were examined for each individual. To determine the frequency, 1000 total cells per individual were scored.

Seminal fluid samples

Samples of semen fluid were collected by masturbation after a 3-day abstinence period, the samples were analyzed by the conventional method to assess the volume and count of sperms, motility and morphology of sperms according to WHO (World Health Organization) standards (2010).

Statistical analysis

All data was conducted using a t-test to determine the significant differences between the means $\pm$ standard deviation (S.D.) of the two study groups at the level of significance $p < 0.05$. The SPSS software program used for statistical analysis was (Version 20.0) used on computer.

Results

Our current study was designed to compare the mean values of micronuclei using Giemsa stain between the petrol station workers group and control group. The results showed, the frequency of micronucleus (MN) was significantly increased to the petrol station workers group (Table 1), in comparison with the control group (Fig.1 and 2) at level ($p < 0.05$). Furthermore, the results indicated a significant difference in the indicators of semen quality between the group of workers at

petrol station and the control group. Except for volume (ml) and PH, which were not significant between the two groups (Table 2).

Table 1
The frequency of Micronucleus (MN) in petrol station workers group and control group (mean $\pm$ S. D)

Group	Micronucleus (MN) (mean $\pm$ S. D)	p- value
petrol station workers (n=50)	106 $\pm$ 3.23	< 0.05
Control (n=30)	27.6 $\pm$ 3.26	

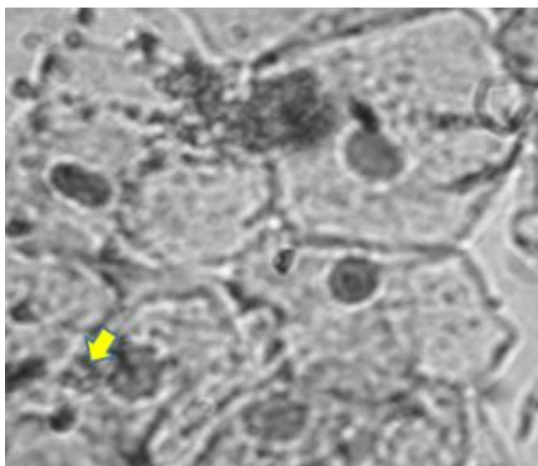


Figure 1. Shows exfoliated buccal epithelial cells in mouth control group stained with Giemsa Stain x400

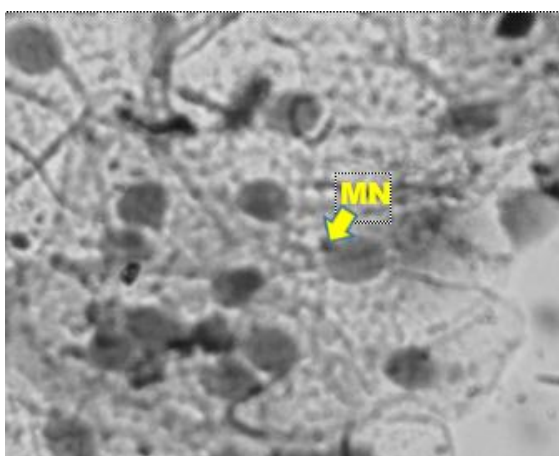


Figure 2. Shows exfoliated buccal epithelial cells in mouth with micronucleus (MN) petrol station workers group stained with Giemsa Stain x400

Table 2
Shows the means value $\pm$ standard deviation (S.D.) for each parameter,
Petrol station workers and control groups

parameters	petrol station workers (n=50) mean $\pm$ S.D	Control (n=30) mean $\pm$ S.D	p- value
Volume(ml)	2.96 $\pm$ 0.48	3.20 $\pm$ 0.39	N.S
pH value	7.22 $\pm$ 0.21	7.38 $\pm$ 0.24	N.S
Count(million/ml)	27.75 $\pm$ 3.29	49.56 $\pm$ 3.64	< 0.05
Motility%	19.7 $\pm$ 2,78	50.8 $\pm$ 2.91	< 0.05
Morphology% (Normal sperm)	19.4 $\pm$ 2.31	88.9 $\pm$ 3.98	< 0.05

Discussion

Occupational exposure to dangerous chemicals for a long period leads to a range of health problems, ranging from allergic reactions to these chemicals to cancer [Goethel et al., 2015]. The petrol station workers who are part of filling vehicles with fuel are considered the most vulnerable to health effects because of their exposure to organic compounds such as toluene, benzene and ethyl benzene either through fuel vapors or during fuel dispensing, they work in filling fuel for more than eight hours a day without wearing equipment personal protection [Shaikh et al., 2018]. In addition to the possibility that they may be exposed to car exhaust emissions directly through inhalation or through skin contact, as most gas stations in Iraq are located on the main streets, which makes them more vulnerable [Dian and Marwan, 2020].

For evaluating genetic damage to workers in filling stations in the city of Samarra, the indicators of micronucleus formation and semen quality were adopted to identify the genetic damage that may occur to them because of that exposure. The results of the study showed a significant increase in the frequency of micronucleus among workers in filling stations compared to the control group. The frequency of micronuclei among workers in filling stations was (106 $\pm$ 3.23) compared to the control group which was (27.6 $\pm$ 3.26). This was confirmed by Dian et al., in 2020 published paper which indicated a significant increase in the frequency of micronuclei among workers at filling stations in Dohuk-Iraq, comparison to the control group [Dian and Marwan, 2020]. Paper by [Hidayat et al., 2016] also indicated an increase in the frequency of micronuclei and chromosomal abnormalities in circulating blood lymphocytes for workers in filling stations in Erbil-Iraq compared to the control group.

The reason for the increased frequency of micronuclei for workers in filling stations, it may be due to the complex mixture of aliphatic and aromatic hydrocarbons in the composition of petroleum with a high volatility [Metgud et al., 2015]. Some of these hydrocarbons show mutagenic or carcinogenic properties, and most of them cause changes in the structure of the DNA or lead to genetic damage and genetic instability, which causes chromosomal abnormalities and the formation of micronucleus. Benzene and the rest of the hydrocarbons is one of the main causes of genetic mutations, chromosome damage and genetic toxicity, as

workers in petrol station are permanently exposed to large quantities of it by inhaling it through the nose or by inhaling what is volatilized from it through the mouth when refueling cars, which leads to an increase in the repetition of micronuclei in the scaly buccal cells of the mouth [Uppala et al., 2015].

Micronucleus (MN) is characteristically seen in the exfoliated epithelial cells such as buccal mucosa during cancerous and precancerous conditions. And since micronuclei are produced through two pathways, one of them is the damage that occurs when the cell divides and the spindle filaments are not formed correctly, and the second is the presence of damaged or missing chromosomes for the centromere, which leads to its delay in the dissociative phase of division and consequently its survival outside the nucleus of the daughter cell [Sridhar et al., 2017]. The regenerate of oral epithelium is fast (7-16 days). Therefore, micronuclei reflect genotoxic damage that occurred 1-3 weeks earlier in basal dividing layer. Therefore, micronucleus is one of the early diagnostic tests that are very useful to verify the health of the individual and the extent of damage caused to him because of occupational exposure to these substances.

Moreover, the results of this study showed that there statistically significant differences between semen indicators for workers in filling stations compared to the control group, except the semen volume and pH, it shows no significance in the two groups according to Table (2). This agreement with Priyanka et al., paper published in 2013, and those who found significant differences between effects of benzene metabolites were evaluated on semen samples freshly collected from men who had demonstrated fertility, and the criteria for toxicity studied were sperm motility, sperm vitality, and DNA integrity. The low percentage of both count, motility and morphology (normal sperm) working in filling stations is happened because of many reasons, such physiology of low testosterone, or because of function defect of mitochondria which on the main source of the production of molecules ATP which affect motility, or due to the presence of teratozoospermia as a result of exposure to free radicals [Sias et al., 2015], or hereditary causes such as chromosomal abnormalities and point mutations.

The current investigations revealed the role of benzene metabolites, which are phenol-hydroquinone and catechol, for motility, vitality, and the nuclear safety of human sperm [Morgan et al., 2016]. Steiber et al., at 2004 was mentioned exposure to a microenvironment of phenol hydroquinone, resulting from benzene metabolism it might inhibit the metabolic active enzyme that hinders ATP production, thus reducing sperm motility, and free radicals from benzene metabolism affect mitochondrial membranes, thus inhibiting the production of ATP molecules. In addition, our study showed a decrease in the number and shape of natural sperms for workers in filling stations, as gasoline can affect the number and shape of spermatozoa through its effect on the sperm DNA. The paper of Evenson et al. 2002 indicated that exposure to a number of chemicals, including benzene; it leads to abnormal chromatin formation incompatible with fertility, which eventually led to abnormal chromatin structure incompatible with fertility.

Chen and Eastmond at 1995 in their published paper, also stated that exposure to phenol-hydroquinone in the laboratory resulted from the metabolism of

benzene led to the production of free radicals, which was reflected in a decrease in motility, vitality and loss of the integrity of the DNA of sperm. In agreement with our findings, the ASTDR (Agency for Toxic Substances and Disease Registry) report at 1997 indicates that the genotoxicity of benzene to cells is caused by its effect on the inhibition of topoisomerase enzymes and the function of microtubules, which stimulates the occurrence of cytotoxicity, affects the DNA, and thus reduces cell proliferation and division.

Conclusion

The petrol station workers who are part of filling vehicles with fuel are considered the most vulnerable to health effects due to their exposure to organic compounds such as benzene, which is one of the dangerous factors that poison workers exposed to it in petrol filling stations. This has been confirmed by using the micronucleus test, which is one of the very useful early diagnostic tests to verify the health of the individual and the extent of damage to him due to occupational exposure to these substances. There was a significant increase in the repetition of micronuclei among workers in filling stations, which indicates damage to the structure of DNA. In addition, the toxic effect of exposure to benzene in the petrol station workers is clear on the quality of semen in terms of motility, number and shape of normal sperm depending on the obtained data. However, further in-depth studies are warranted to elucidate the toxicity of benzene and its metabolites on reproductive cells. In particular and the rest of the body tissues in general.

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