CYTOGENETIC ANALYSIS OF PERIPHERAL BLOOD LYMPHOCYTES OF WORKERS OCCUPATIONALLY EXPOSED TO BENZENE IN A FUEL STATION IN ERBIL CITY- IRAQ

Hazha Jama Hidayat, Mohammed Sdeeq Ismael and Hewa Husen Hasan
College of Education, Biology Department, University of Salahaddin, Kurdistan Region – Iraq.
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Abstract:

The objective of the present study was to evaluate genotoxic effects in petrol station workers, who were previously exposed occupationally to benzene in comparison with a selected control group, through inhalation and skin contamination, by using the cytokinesis- blocked micronucleus (CBMN) and chromosomal aberration tests carried on peripheral blood lymphocytes. The study included (35) male petrol station worker and 25 control subjects. Metaphase chromosome preparations were analyzed, chromosome aberrations in peripheral blood lymphocytes, were examined. The results showed that the frequency of cell with aberrations in exposed workers was significantly higher (P≤0.01) than that of control subjects. Most chromosome aberration found in exposed group was acentric fragment, chromosome break, dicentric. Furtheremore, also the frequency of micronucle(MN) was highly significant in benzyne exposed groups as compared with control person (P≤0.01). In conclusion significant excess of chromosomal aberrations in workers who exposed occupationally to benzene, where compare to the matched controls.

Key words: *Genotoxicity, lymphocytes, petrol station workers ,chromosomal aberrations.*

Introduction

In recent years, risk of human exposure to various mutagens and carcinogens are alarmingly high and the incidances are increasing day by day. The air, water, food and the occupational areas are contaminated with a variety of suspected mutagen and carcinogen (channarayappa, 2010).

Benzene is an organic compound found most often in air as a result of emissions from burning coal and oil , gasoline vapors at gasoline service station , motor vehicle exhaust, cigarette , wood burning fires and another source (U.S. Environmental Protection Agency .2002). Benzene is classified as a known carcinogen based on occupational studies in adult that demonstrated increase incidence of several types of leukemia in exposed adult . Benzene has also been showed to be genotoxic(causeDNA damage) to experiment animal studies (Yokozawa et al. 2007). Acute exposure to relatively high concentration of benzene (benzol) may result in central nerve system disturbance consistent with solvent exposure, drowsiness, dizziness , headache , tremor , delirium ataxia , loss of consciousness , respiratory arrest and death (Vasiliouet al.2006) A characteristic effect of benzene exposure is a plastic anemia , resulting from suppression of bone marrow tissue(Syder et al. 2005).

Evaluation the mutational pattern induced by benzene on P53 gene in human type II like alveolar epithelial A54q cell *in vitro*. A total of 17 mutations were linked to benzene expouser : deletion and single substitutions. Benzen induced micronuclei, chromosomal aberrations, and DNA damage in Chinese Hamster ovary (Billet *et al*. 2010, ATSDR, 2015, Pandy et al. 2009).

Materials and Methods

The current study was carried out on peripheral blood lymphocytes obtained from (35) workers occupationally exposed to benzene and its derivatives for 32 to 60 years from different petrol station in Erbil/city. The unexposed control group consists of (25) male volunteers.

Characterization of sample

The exposed group consisted of 35 workers (all male) from fuel stations of Erbil city. The unexposed group consisted of 25 healthy individual. Fortunately, all individual of both groups not alcohol drinkers . Some individual of exposed group were smokers and unexposed group were non- smokers. The median age of both groups was (30) years. All individual answered questionnaire about their occupational and non-occupational exposure and confounding life style factors. Profile of exposed and control groups are shown in table (1)

Table (1): General characteristic of the exposed and control subjects

Parameters Exposed staff Controls	Exposed Subjects	Control
Sample size		
Age	35	25
Median	30	32
Range	25-53	22-52
Years of employment		
Median	9	8.5
Range	30-Jan	25-Jan
Smoking status		
Never smokers	25(85%)	17(68%)
Current smokers	10(14.7%)	8(32%)

Cytogenetic assay

The cytokinesis-blocked micronucleus assay was carried out according to Fenech (1993). Lumphocytes separated from whole blood cultures were initiated by the addition of 5 mL RPMI-1640 medium containing 10% fetal bovine serum (Sigma-Aldrich, United Kingdom), 25mM HEPES, penicillin (100 U/mL) and streptomycin (100 U/mL), 25mM L-glutamine, and phytohemagglutinin (2%). Duplicate cultures for each case were incubated for seventy-two hours at 37 C°. Colchicine (final concentration, 10 mg/mL) was added and incubated for forty-five minutes before the end of the culture.

The cells were harvested and slides were prepared under standard conditions (incubated with 0.075M KCL for twelve minutes and then cells were fixed with methanol:acetic acid 1:3), and mixed. The suspension was dropped onto clean slides and stained with Giemsa. Thousand binucleated cells per individual were scored for the presence of micronuclei. MN were scored according to the criteria described by Fenech(1999). The nuclear division index (NDI) was calculated according to the formula suggested by Eastmondand Tucker (1989).

Statistical analysis

All groups were compared using Student t test (SPSS for Windows, 13.0) to evaluate the influence of exposure, on MN and Chromosomal aberration frequencies of both groups. A value of $p \leq 0.05$ was considered to be statistically significant.

Results

Our results showed significant increases in the number of chromosomal aberrations in peripheral lymphocytes with a mean of 52±0.32 in the exposed group as compared with unexposed group with a mean of 5±0.20 (Fig-1-). The most frequent chromosome aberration found in the exposed group chromatid break, acentric fragment, and dicentric (Fig-2-)(Fig-3-).

Table (2) shows the results of cytogenetic analysis in unexposed controls and benzene exposed workers. Statistical analysis showed the presence significant difference ($p<0.05$) in frequencies of chromosomal aberrations between exposed group. In the control group the mean frequency of cells with total chromosomal aberrations was 5±0.118 where as in the exposed workers, the frequency was 52±0.081. (Fig-2-). In exposed individuals the, mean value of chromosomal aberration were chromatid break(32±0.29), chromatid gap (12±0.123), and dicentric (10±0.112) (table-2-).The statical analysis of data showed that there was a significant increase in the frequency of various types of chromosomal aberrations in exposed individual.

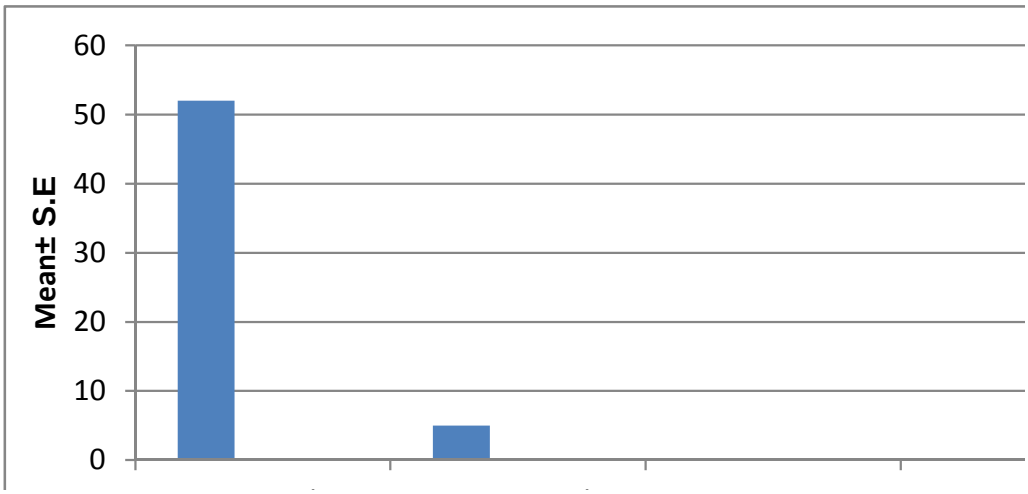

Fig. (1): Shows the difference between the exposed and the respective control groups in relation to total chromosomal aberration (CA) in peripheral Lymphocytes.

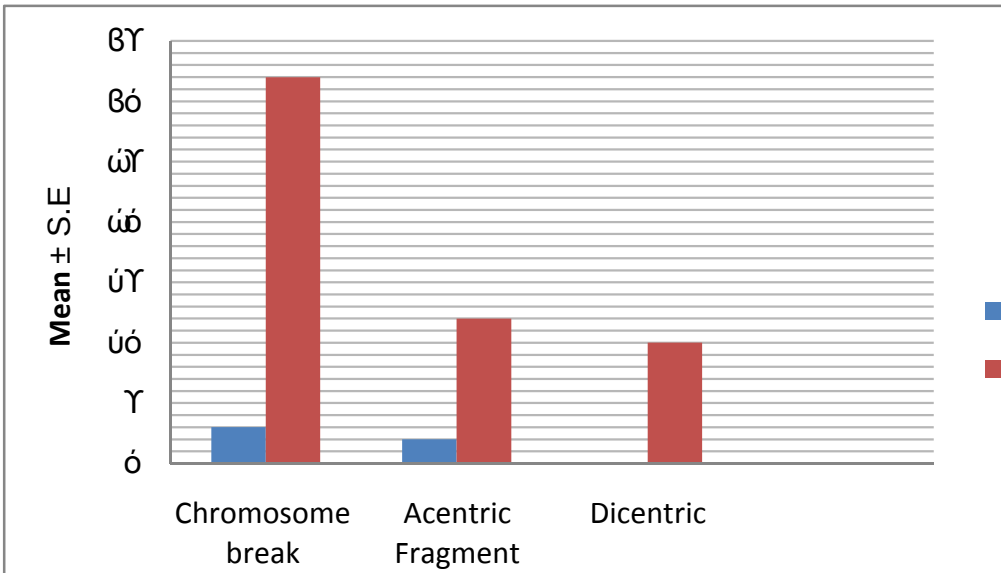

Fig. (2): Frequency and types of chromosomal aberrations in peripheral blood of workers exposed and unexposed to benzene

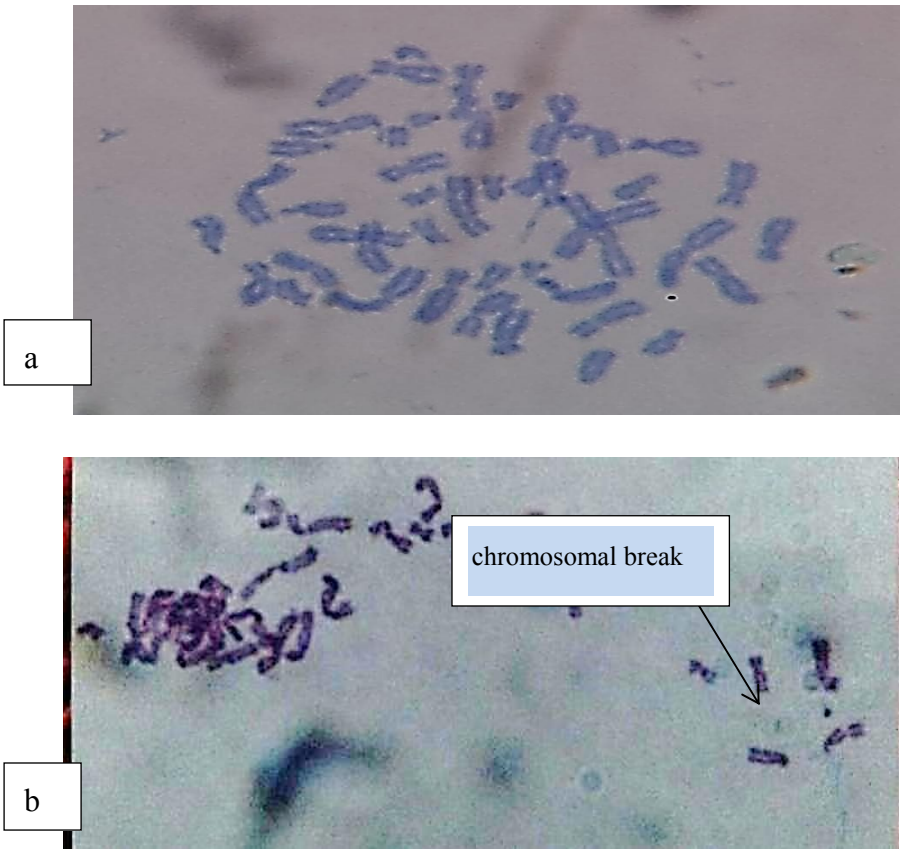

Fig. (3): show the structural chromosomal among workers exposed to benzene in fuel station (a) normal chromosome (control) (b) chromosomal break .

Table (2): Mean ±S.E Frequency and Types of Chromosomal aberrations in Unexposed and Exposed group

Types of chromosomal aberration	Unexposed	Exposed	P value
Chromosome break	**3±0.120**	**32±0.203**	**<0.05**
Dicentric	**0±0.00**	**12±0.130**	**<0.05**
Acentric fragment	**2±o.110**	**10±0.112**	**<0.05**
Total aberration	**5±0.118**	**52±0.080**	

The results of the MN assay, reported a total number of MN per 1000 BN (binucleated) cells, are shown in (Table 3). The mean MN frequency was increased significantly ($p < 0.05$) in benzen-exposed group compared with the control group (8.88 +3.44 versus 2.50+2.00 MN/1000 BN cells; Figure 1). The mean frequency of MN was higher in exposed smokers than in exposed non-smokers 6.14 +1.20versus 5.58 + 2.8175 /1000 BN cells, $p > 0.05$), but a statistical difference was not detected. Among current smokers, a higher but not significant MN frequency was found in the exposed persons than in controls (6.14 +1.20versus 5.58 + 2.81MN/1000 BN cells, $p > 0.05$). The number of smokers in exposed groups was higher than the controls. According to these results, smoking status seems to affect MN frequency but further studies are needed in larger populations. Regarding to NDI, no significant overall difference was found between exposed subjects and controls (1.94 + 0.09 versus 1.95 + 0.10, $P > 0.05$).

Table(3): The frequencies of MN and SCE in peripheral lymphocytes of exposed and control subjects

Group	sample size	MN/1000 BN cells (mean + S.D.)	NDI (mean + S.D.)	PRI (mean + S.D.)
Exposed staff				
All subjects	35	8.88 +3.44	1.94 + 0.09	1.90 + 0.17
Current smokers	25	6.14 +1.20	1.93 + 0.09	1.92 + 0.16
Never smokers	10	5.58 + 2.81	1.95 + 0.09	1.89 + 0.18
Controls				
All subjects	25	2.50+2.00	1.95 + 0.10	1.91 + 0.13
Current smokers		5.40 + 1.08	1.94 + 0.13	1.93 + 0.12
Never smokers	20	3.50 + 1.75	1.96 + 0.09	1.90 + 0.13

BN, binucleated; MN, micronucleus; NDI, nuclear division index; PRI, proliferation index; deviation.

A Each group in exposed subjects was compared with the corresponding group in controls. Additionally, current smokers and never smokers were compared to each other in their subgroups.

B Statistically significant when compared with all control subjects (Student t test, $p< 0.05$).

Discussion

In the present study, we tried to assess the genotoxicity of benzene on petrol station workers by detecting several bioparameters, in which all parameters used in this study showed increased values in the expoused workers as compared to control . Workers occupationally exposed to long term benzene and its derivatives showed a significantly increased frequency of metaphase cells with structural chromosomal aberration including (chromatid break, acentic fragment and dicentric) as compared with the control population.

The relation between benzene exposure and chromosomal aberration has been reported previously, found a higher incidence of aneuploidy and a long term deletion of chromosomes 5- and 7 in the lymphocytes of Chinese workers, who were exposed occupationally to benzene (Aida et al 2012). Several group of investigators have reported significant associations between occupational exposure to benzene and increased rates of chromosomal aberrations (e.g., breaks, deletion, translocation) for a number of chromosome in peripheral blood lymphocytes or sperm (Ji et. al. 2012). Zhang et. al.(2014) evaluated the frequency of micronuclei in peripheral blood lymphocytes from 385 benzene expoused shoe factory workers and 197 unexposed controls. Lau et. al. (2009) reported significantly increase percentage of micronuclei in bone marrow cells of adult mice following single intraperitoneal injection of benzene at 400mg/kg.

Benzene hematotoxic and carcinogenic effects are dependent upon benzene metabolisim reactive metabolites produced in liver and bone marrow can lead to production of reactive oxygen species and damage to tubuline, histone proteins, topoisomerase II , other DNA associated proteins, and DNA itself as well as clastogenic effects such as strand brakeage , mitotic recombination, chromosome translocations, and aneuploidy, also induced alteration in selected gene expression , DNA damage snd altered DNA repair capacity and increases in chromosomal aberration (ATSDR, 2015).

Conclusion

Since benzene has a relatively common environmental and occupational genotoxic effect on human health. chromosomal abnormalities are increased in the workers during a long term exposed to benzene long-term in our study. This study found a significant excess of chromosomal aberrations and miconucli in workers who exposed occupationally to benzene, where compare to the mulched controls.

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پوختە:

ئامانجی ئەم توێژینەوەیە بۆ زانین و هەژمارکردنی کاریگەری ژەهراوی بوونی بۆماوەیی لە سەر کرێکارانی وێستگەی سووتەمەنی ، ئەوانەی رۆژانە بەرکەوتوویی بەنزین دەبن لە ویستگەکان و بەراورد کردنیان لە گەل گروپی کۆنترۆل (کەسی ئاسایی) لە ڕووی هەڵژین و پیس بوونی پێست، ئەمەش بە بەکارهێنانی تێستی cytokinesis-blocked micronucleus (CBMN) و تێکچوونی کرۆمۆسۆمەکان لە خانە لیمفاوییەکانی خۆێن.

توێژینەوەکە ئەنجام درا لە سەر (35) پیاوی کرێکاری وێستگەی سووتەمەنی و (25) کەسی ئاسایی،دوا بە دوای شیکردنەوەی کرۆمۆسۆمەکان لە قۆناغی Metaphase ،تێکچوونی کرۆمۆسۆمەکان دیاریکرا لە خانەکانی خوێن.لە توێژینەوەکە بە دەرکەوت ڕێژەی خانەکانی تێکچوونی کرۆمۆسۆمیان تێدایە لە کرێکارەکانی وێستگەی سووتەمەنی زۆر زیاترە لە گروپی کۆنترۆل لە ئاستی ($P\leq0.01$). جۆرەکانی تێکچوون یان گۆرانکاری کرۆمۆسۆمی کە لە ناو کرێکارانی وێستگەی سووتەمەنی بەدی کران بریتی بوون لە :پارچەی بی ناوەندە بەش ،شکانی کرۆمۆسۆمی بە پارچە، کرۆمۆسۆمی جووت ناوندە بەش. هەروەها ڕێژەی micronucle (MN) زۆر زیاتر بوو لە نێو کرێکارانی وێستگەی سووتەمەنی بەروارد لە گەڵ گروپی کۆنترۆل لە ئاستی ($P\leq0.01$).

دەرئەنجامی ئەم توێژینەوەیە وا دەردەخات کەوا جیاوازییەکی زۆر هەیە لە ڕێژەی تێکچوونی کرۆمۆسۆمی لە نێو کرێکارانی وێستگەی سووتەمەنی بە بەراورد لە گەل گروپی کۆنترۆل.

الخلاصة:

الهدف من هذه الدراسة لتقييم الآثارالسامة للجينات في عمال محطة وقود،الذين تعرضوا لمادة البنزين فى الماضي مقارنة مع مجموعة السيطرة (الغير معرضين لمادة البنزين) من خلال الاستنشاق وتلوث الجلد، أجريت الاختبارات باستخدام cytokinesis-blocked micronucleus (CBMN) والانحرافات او التشوهات الكروموسومية من الخلايا الدم الليمفاوية.

واجريت الدراسةعلى (35) من العمال الذكور الذين يعملون فى محطة بنزين و 25 اشخاص عاديين (غير معرضين للبنزين). وقدتم تحليل كروموسومات ،و ايضا تم فحص التشوهات او الانحرافات الكروموسومية في الخلايا الدم الليمفاوية. وأظهرت الدراسة أن نسبة الخلايا مصاحبة للأنحرافات بين العمال المعرضين للبنزين اعلى بكثير مقارنة بالاشخاص الغير معرضين فى مستوى دلاله ($P\leq0.01$)

معظم الانحرافات او التشوهات الكروموسوميه التى وجدت فى العمال المعرضين للبنزين المتضمنة:جزءلامركزي،وكسركروموسوم،الكروموسوم ثنائي المركزي و ايضا كان نسبة micronucle (MN) بشكل كبيرفي العمال المعرضين للبنزين بالمقارنة مع الاشخاص الغير معرضين فى مستوى دلاله ($P\leq0.01$).