Original Article

Genotoxic effects among petrol station workers.

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Summary:

***Background**: Benzene has been internationally recognized as a potent toxin, particularly for its effects on the blood forming system of the bone marrow and its association to a large number of haematological disorders.*
***Aims**: This study aimed to assess the cytogenetic damages related to occupational exposure to benzene by calculating the mitotic index (MI) , nuclear division cytotoxicity index (NDCI), binucleated cells ratio and chromosomal aberrations.*
***Obgectives**: Peripheral blood samples were collected from 30 benzene exposed workers and 10 from unexposed- controls- persons. 20 out of the exposed workers were occupationally exposed to benzene from 2 to more than 4 years. The rest of workers were exposed to benzene from one month to one year.*
***Results**: The mean MI in the benzene exposed workers (5.72 ± 0.62) was found significantly higher than in controls (4.03 ±0.37). The highest MI mean was calculated in the exposure group 4 to above years.The NDCI of the exposure workers (3.38 ±0.54)was also significantly higher than in control (2.04 ± 0.76).Lower NDCI mean was calculated in the 4 to above years exposure.*
***Conclusions**: Chromosomal aberrations were observed in the exposed group. Polyploidy , anuploidy (5 monosomy and 9 trisomy) and structural aberration (del 8q) were detected in the exposed groups 0-3 years.*
MN frequencies were significantly increased in relation with length of employment. According to the MN results and the chromosomal aberrations detected in the exposed groups, it could be possible that a correlation found between the elevated values of the MN and the detected chromosomal aberrations.
***Keywords:** Benzene, Chromosomal aberrations, MN*

Introduction:

There is a serious concern about the genotoxicity of the petrol derivates – especially benzene- on the petrol station workers. Because of the high volatility of benzene, inhalation is the most important route of exposure. Almost 50 percent of inhaled benzene is absorbed and 1-5 percent is absorbed from skin (1). When inhaled at high concentrations, benzene is myelotoxic and leukomyogenic for workers (2,3). It is well known that individuals occupationally exposed to benzene are at a much higher risk to developing leukaemia and several types of cancer than the normal population (4,5).
It is also known that benzene induce structural and numerical changes in experimental animals and in man (6,7).

Exposure to petrol derivates vapors is classified by the International Agency for Research on Cancer as possibly carcinogenic to humans, mainly on the basis of the established carcinogenicity of some component chemicals such as benzene (8).

The mechanism of benzene toxicity, particularly its leukemogenic effects, is far from being fully understood. Bearing in mind the leukemogenic action of benzene, blood lymphocytes appear to be a suitable cell system for biomonitoring studies. In this study, the assessment of the benzene genotoxic effect in petrol station workers peripheral blood lymphocytes was done by detecting of the mitotic index , nuclear division cytotoxicity index, micronuclei frequencies and chromosomal aberration.

According to several studies, benzene and its metabolites do not function well as mutagens but are highly clastogenic producing chromosomal aberrations (9,10), sister chromatid exchanges (11)and arising micronuclei level (12,13) and the correlation of increasing level of these factors with a heightened risk of cancer was strongly proved (14,15). Aneuploidy induction, micronuclei frequencies and mitotic index have been experimentally demonstrated both in vivo in benzene exposed workers (16,10) and in experiments in vitro (17).Most of these studies revealed that the benzene genotoxicity are early events and the chromosomal aberrations might vary depending on the target organ or cell type (18). This study was planned to find the origin of the micronuclei and to get more information about the possible relationship between benzene exposure and genetic risk . Also we investigate genetic and cytogenetic damages observed in petrol station workers exposed to benzene.

Material and Methods:
5 ml from peripheral blood using heparinised syringes was collected from 30 petrol station workers and 10 unexposed men. 0.5 ml of blood was added to 5 ml of standard supplemented RPMI 1640 medium containing 20% fetal calf serum and 2% of phytohemagglutinin (PHA)(Prepared by the Iraqi

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Center for Cancer and Medical Genetic Researches/Baghdad) . Two cultural tubes were used for each sample , one for micronuclei work and the other for cytogenetic purposes. The tubes were cultured at 37C and after 44 hrs, 4 ug of cytochalasin B / ml (Sigma Chemical Co.) was added to culture tubes of micronuclei work (19) .100 ul of colchicines (0.45 mg/ml) was added to the cytogenetic culture tubes just before 20 mins of cell harvesting.

Cells from all culture tubes were harvested after 72 hrs by centrifugation and after a mild hypotonic treatment with 3ml 0.075 M Kcl at 4C .The cells were precipitated by another centrifugation. The supernatant was discarded , the cells re-dissolved with remaining hypotonic solution and fixed with 5 ml fixative solution (3 Methanol: 1 Glacial acetic acid).Centrifugation and fixation were repeated four times at intervals of 20 min. Slides were prepared by the air-drying method. The slides were stained the following day for 10 min in 10 ml 5% buffered Giemsa solution, pH 6.8 .Three slides were prepared for each sample.

70-102 metaphases were examined to detect the chromosomal aberration for each sample .Other anomalies was determined in 500-1000 cells per individuals. The MI , NDCI values and binucleated cells ratio were calculated according to Fenech ,2000(20);Yadav and Seth,2001(21) ; Oliver et al,2006(22) and Volder et al,2006(23) . Differences between groups were assessed using a t-test.

Results:

Table-1 ,2 and 3 showed the main characteristics of the tested and control subjects. The studied individuals were classified according to their exposure periods.

Table-1:Mitotic Index (MI) in lymphocytes of petrol station workers and unexposed control.

Exposure/yrs	Number of individuals	Number of examined cells (n1)	Number of metaphases (n2)	MI±SD n2/n1x100
Control / Unexposed	15	19500	785	4.03 ± 0.37
Exposed	30	39000	2244	5.75* ± 0.62
0 --- 1	16	20800	1136	5.46 ± 0.32
2 --- 3	10	11500	700	6.08 ± 0.31
4 ---above	4	5200	408	7.85 ± 0.19
P<0.01				

Table-2:The frequency of cells with 1,2,3,4 nuclei , necrotic, apoptotic cells and nuclear division cytotoxicity index (NDCI) in petrol station workers.

Exposure/yrs	Number of individuals	Cells with tetra nuclei	Cells with tri nuclei	Cells with bi nuclei	Cells with mono nuclei
Control /Unexposed	10	30	21	126	578
Exposed	30	289	307	830	1317
0 --- 1	16	116	87	266	485
2 --- 3	10	121	139	364	516
4 ---above	4	52	81	200	316
P<0.01					

Exposure/yrs	Number of individuals	Necrotic cells	Apotptotic cells	NDCI ± SD	Binucleated cells ratio
Control /Unexposed	10	0	0	2.04 ± 0.76	16.5
Exposed	30	9	10	3.38* ± 0.54	30.26
0 --- 1	16	6	6	3.54 ±0.62	35.28
2 --- 3	10	2	3	4.3 ± 0.81	31.9
4 ---above	4	1	1	2.34 ± 0.79	30.8

Table -1 shows the data regarding the MI of both the workers and controls .It is evident that MI in exposed workers (5.75±0.62) was significantly higher (P<0.01) than in matched controls(4.03±0.37).It was maximum in workers with exposure period of more than four years . The data

of table-1 also showed gradual increasing in the MI values with the increase of the exposure periods.
The frequency of cells with different numbers of nuclei in table-2 showed a significant increase in the NDCI and in binucleated cells ratio of the exposed workers (%3.38 and %30.26 respectively) comparing to control (%2.04 and %16.5 respectively).

Significant differences were also detected between exposed group 0-1 year and 2-3 years and control .While the exposed group 4-above years did not show such difference.

When we compared the MI values from table-1 and NDCI values from table-2 we found that the exposed group in both tables always significantly increased than control. But the exposed group 4-above years showed a lower NDCI value comparing to other exposed group and nearly equal value to that in control. Theirfore , NDCI value of this group(4-above years) is not match with their MI value. However, the binucleated cells ratio of the all exposed groups indicating a double activity in the cell division(%30-%35) than in control (%16.54) which indicate an active lymphocytes proliferation.

Table-3:The distribution and number of micronuclei (MN) in lymphocytes of petrol station workers and unexposed control.

Exposure/yrs	Number of individuals	Distribution of micronuclei				Main micronuclei per 1000 binucleated cells ± SD
		1MN	2MN	3MN	Total	
Control /Unexposed	15	182	4	0	186	12.4 ± 0.16
Exposed	30	487	67	14	568	15.2* ± 0.50
0 ---- 1	16	213	41	10	264	16.5 ± 0.34
2 ---- 3	10	208	12	4	224	22.4 ± 0.43
4 ---- above	4	66	14	0	80	20 ± 0.62

P<0.01

Table-3 showed the results of the MN scoring indicating both the average total number of MN scored for 1000 binucleated cells as well as the average of binucleated cells presenting one or more MN .The second value has been considered a good parameter for measuring genotoxic effects .The over all frequency of BNMN(bionucleated cells MN) in controls was (12.4±0.16) which is in good agreement with values usually reported for control populations(24).

The results represented in table-3 showed another evidence about the existence of the correlation between the exposure period and genotoxicity.

A high significant difference was calculated between the main micronuclei of the exposed groups (15.2±0.50) and control (12.4 ±0.16).Such differences were also shown between each exposed groups (16.5-22.4) and control.

Chromosome aberrations were also detected in this study. We found three types of numerical aberrations and one structural aberration in the metaphases of the exposed groups. 3 polyploids were noticed in one sample of the exposed group 0-1 year (Figure-1) .Two aneuploidy metaphases were also noticed in the exposed groups. 5 monosomy was detected in one sample of the exposed group 0-1 year and 9 trisomy in another sample of the exposed group 2-3 years.

A deletion of a pical part of one chromatide of the long arm of the chromosome 8 was also detected in one sample of the exposed group 2-3 years (Figure-2).

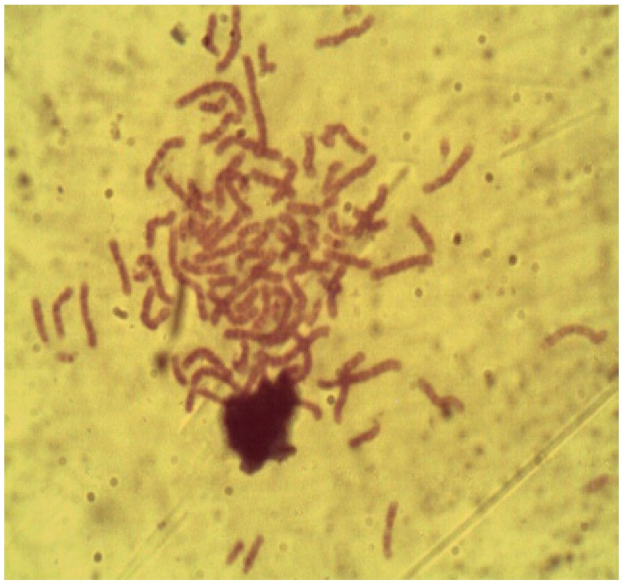

Figure-1 : Polyploid cell detected in blood lymphocytes of petrol station workers exposed to benzene.

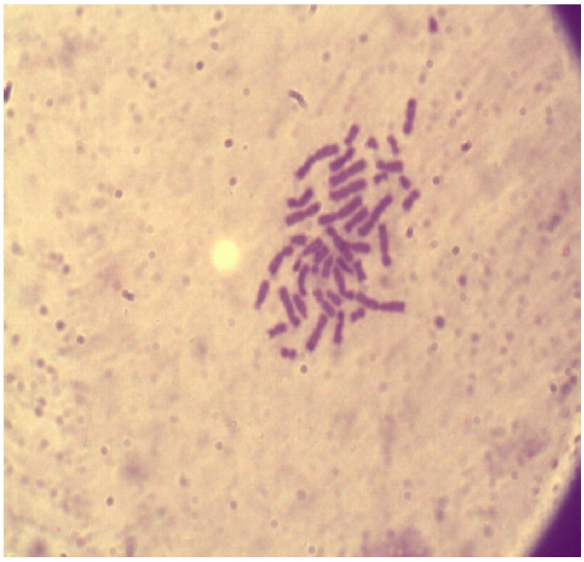

Figure-2 : deletion 8q which detected in blood lymphocytes of petrol station workers exposed to benzene.

Discussion:

During fuelling, petrol station workers may easily be exposed to extremely high levels of benzene vapour.The volume of benzene vapour inhalated per day and the ambient temperature can significantly increase the occupational risk of service station workers and attendants (25).

Exposure to benzene vapours is classified by the international agency for research on cancer as possible carcinogenic to humans, mainly on the basis of the established carcinogenicity of some component chemicals such as benzene (26,21,27,28,29). The mechanism of benzene toxicity particularly its leukemogenic effects, is far from being fully understood (1 ,30).

In the current study we try to assess the genotoxicity of benzene on the petrol station workers by detecting several bioparameters.

All parameters used in this investigation showed elevated values in the exposed workers as compared to control.

The MI and NDCI values of the exposed groups showed a significant increase .Also the binucleated cell ratio of this group showed a double values as compared to control.

These three parameters,MI,NDCI and BN ratio gave no dought provment that the benzene inhaled by the exposed groups have mutagenic activity .This mutagenic activity turn to a great toxicity in the exposed group up to 4 years exposure where is the NDCI value is decreased . In this manner, we need to assess the genotoxicity and toxic effect of benzene in large number of petrol station workers and for several years to point out the stage of toxic turning.

This findings is in conformity with results obtained by other authors (31,27).

Among all parameters used in the cytogenetic approaches, the MN assay using the cytokinesis-block method has shown a remarkable and clear results. The three exposed groups had an increased MN frequency as compared to control. On other hand a great variation was detected in the individual results of each exposed groups , unlike the control group, where the individual data were nearly homogeneous.

These results shown that the benzene inhaled by the exposed groups increased the number of BN cells containing more than 1MN/1000BN,while only 4 out of 15 controls showed more than 1MN/1000BN which reflect benzene high genotoxicity.

Also the MN frequency results showed a positive association with the exposed periods, where is the highest MN values were a attributable to those with exposed periods ranging from 2 to more years.

The cytogenetic damage in exposed humans has been reported by many authors and most of published papers are restricted to occupational exposures to benzene (32,26,10,33).

No chromosomal aberration was detected in the exposed group above to 4 years while polyploidy, 5 monosomy, 9 trisomy and del 8(q) were detected in the exposed group 0-3 years. These results indicate that both polyploidy and aneuploidy are early genotoxic events induced by benzene.

Such chromosomal aberrations were also detected in workers exposed to benzene hexane, toluene, formaldehyde and pesticides (34,24,35,36,10,37,38,39).

According to the MN results and the chromosomal aberrations detected in the exposed groups, it could be possible that a correlation found between the elevated values of the MN and the detected chromosomal aberrations, which would be interesting to confirm.

Similar results were reported by Norppa and Falck,2003 (40) who studied the effect of benzene exposure. They concluded that positive correlation between the frequencies of anuploid cells and MN cells indicate that 1 , 9 or 16 chromosomes preferentially incorporated into the MN.

However, due to the small number of exposed workers above 4 years, firm conclusions can not be made about the involvement of chromosomal aberrations and the period of exposure .

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