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Original Article

Micronucleus, Nucleoplasmic Bridge, and Nuclear Budding in Peripheral Blood Cells of Workers Exposed to Low Level Benzene

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Abstract

Background: Benzene is one of the important occupational pollutants. There are some reports about the leukemogenic effects related to low-level exposure to benzene.

Objective: To study the frequency of micronucleus (MN), nucleoplasmic bridge (NB), and nuclear budding (NBUD) in the peripheral blood lymphocytes of petrochemical workers with low level exposure to benzene.

Methods: We enrolled 50 workers exposed to low-level benzene and 31 unexposed workers of a petrochemical industry. After exclusion of 3 samples, peripheral blood lymphocytes of the remaining 47 exposed and 31 unexposed workers were analyzed for the frequency of MN, NB, and NBUD by cytochalasin-blocked MN technique.

Results: MN was present in 28 (60%) exposed and 18 (58%) unexposed workers. NB was observed in 6 (13%), and 2 (7%) exposed and unexposed workers, respectively; the frequency for NBUD was 20 (43%), and 13 (42%), respectively. No significant difference was found in the observed frequencies of MN, NB, and NBUD in the peripheral blood lymphocytes between the exposed and unexposed group workers.

Conclusion: Occupational exposure to low-level benzene does not increase the frequency of MN, NB, and NBUD in the peripheral blood lymphocytes, biomarkers for DNA damage.

Keywords: Benzene; Neoplasms; Leukemia; Cell nucleus; Cytoplasm; DNA damage

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Introduction

Benzene (C_6H_6, CAS 71-43-2) is a colorless, flammable liquid hydrocarbon with a sweet odor. Benzene is one of the main health concerns for workers at high risk of exposure to the chemical. The seriousness of poisoning caused by benzene depends on its air concentration, exposure route and duration, and the age and pre-existing medical condition of the exposed person.[1] Benzene exposure has been declining over the last several decades. However, occupational exposure

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level is much higher than environmental exposure level in the general population.[2]

Benzene is a well-known carcinogen with relative hematotoxicity.[3] Occupational exposure to benzene may affect peripheral blood cell indices and induce anemia.[4] Hong, *et al*, reported a case of bilateral sub-internal limiting membrane hemorrhage after heavy exposure to benzene.[5] Neghab, *et al*, reported early liver and kidney dysfunction in occupationally exposed workers to low-level benzene, toluene, and xylene in unleaded petrol.[6] Lee, *et al*, in a study on petrochemical industry workers found that chronic low-level exposure to benzene can cause color-vision impairment.[7]

Because of the potential exposure of petrochemical workers to benzene, many studies have so far been conducted on this issue in petrochemical industries. Sahmel, *et al*, conducted a study in 2013 on a large data set of benzene air concentration at a petrochemical manufacturing facility. They analyzed measurements of 2359 air samples and found a decreasing level of benzene exposure over time.[8] Sorahan and Mohammed conducted a cohort study on 45 000 petroleum distribution and oil refinery workers in the UK. They could not find any evidence in support of the hypothesis that low-level benzene exposure could be risk for developing myelodysplastic syndrome.[9] A study conducted by Oliveira, *et al*, on workers who had previously worked in a petrochemical industry showed that they still had genotoxic damage, even when the exposure was removed for several years.[10]

Koh, *et al*, reported increased oral and pharyngeal cancers incidence and mortality among temporary maintenance workers in a refinery and petrochemical complex in Korea.[11] Mrdjanovic, *et al*, conducted a study on oil refinery workers and found significantly higher frequencies of micronuclei (MN) and sister chromatid exchanges in these workers.[12]

MN are cytoplasmic chromatin masses with the appearance of small nuclei that arise from fragments of or intact chromosomes lagging behind the anaphase stage of cell division. MN, nucleoplasmic bridge (NPB), and nuclear budding (NBUD) assays can be used to measure DNA damage in human populations.

To control occupational exposure to benzene, its levels in air, urine, and blood have been used as markers of exposure.[13] Blood and urinary levels of benzene may nonetheless be influenced by other factors. Anna Barbieri reported a strong influence of smoking on the excretion of urinary benzene.[14] This would reduce the accuracy of urinary or blood benzene levels as a predictor of leukemia in a population occupationally exposed to low-level benzene. Low-level benzene exposure would result in leukemia. Some studies suggest cytogenetic damage as a biomarker of effect among benzene exposed workers.

Analysis of MN has gained increasing popularity as an *in vitro* genotoxicity test and a biomarker assay for human genotox-

TAKE-HOME MESSAGE

- Benzene exposure is dangerous and one of the main health concerns for workers at high risk of exposure to the chemical.
- The serious damage caused by benzene depends on its air concentration, exposure route, and duration. Age and pre-existing medical condition of the exposed person are also important.
- Benzene is carcinogen and has relative hematotoxicity that cause anemia.
- Previous studies reveal a high prevalence of DNA damage with increased frequency of micronuclei and sister chromatid exchanges among benzen-exposed workers. However, we could not observe such association in those exposed to low level of benzen.

ic exposure and effect. In 1990, Yager, *et al*, conducted a study on MN induction in human lymphocytes by benzene metabolites.[15] They reported significant increases in MN formation in human lymphocytes treated with benzene metabolites hydroquinone, 1,4-benzoquinone, phenol, and catechol. Mozdarani, *et al*, suggest cytogenetic methods as reliable techniques for the detection of biological effects of occupational aromatic solvents, including benzene exposure.[16] Goethel, *et al*, in a study on gas station attendants and taxi drivers found that low levels of occupational exposure to benzene and atmospheric pollutants may be linked to genotoxicity and oxidative DNA damage.[17] Lovreglio, *et al*, could not find any increase in frequency of MN in the peripheral blood lymphocytes of workers exposed to low or very low concentrations of benzene in comparison with a control group.[18] We conducted this study to quantify MN, NPB, and NBUD as biomarkers of effect on peripheral blood lymphocytes of petrochemical workers exposed to low-level benzene.

Materials and Methods

Using a cross-sectional design, we studied 50 benzene-exposed and 31 unexposed petrochemical male workers. The exposed group consisted of 50 workers of the production unit of the company who had been exposed to benzene levels <1 ppm (lower than the permissible exposure limit), confirmed based on periodic environmental monitoring, for at least five years. The unexposed group consisted of 31 administrative workers of the petrochemical company who had not been exposed to benzene or other related derivatives. The study protocol was approved by the Research Ethic Committee of Tehran University of Medical Sciences, Tehran, Iran.

Workers with a history of chemotherapy, radiotherapy, X-ray exposure, vaccination during the previous 12 months, being afflicted by serious infections, consumption of genotoxic drugs, or supplemental vitamins during the previous six months, as well as those having vigorous sport activity for the previous seven days were excluded from the study.

After explaining the study purpose and obtaining an informed consent, 5 mL of venous blood sample was collected in heparinized tubes. All samples were stored at 4 °C and transported to the laboratory within 8 hrs. Approximately 700 µL of whole blood was added to 4.5 mL of RPMI 1640 culture medium (Sigma, USA) containing 20% fetal calf serum, 1% phytohemagglutinin (Sigma, USA) as mitogen, 100 U/mL penicillin, 250 µg/mL streptomycin, and 2 mM glutamine (Sigma, USA). All cultures were incubated for 72 hrs.

After 44 hrs of culture initiation, cytochalasin B (Sigma Aldrich, USA) at a final concentration of 6 µg/mL was added to the samples. After 72 hrs of incubation, the cells were collected by centrifugation at 1500 rpm for 10 min. The pellet cells were suspended in a hypotonic solution (KCl, 0.075 M, Merck, Germany) and incubated at 37 °C for 10 min, then centrifuged at 1500 rpm for 10 min. The supernatant was discarded and the pellet was resuspended in freshly prepared, ice-cold fixative solution containing methanol: acetic acid (3:1) (Merck, Germany). After 20 min at room temperature, the pellet was centrifuged at 1500 rpm for 10 min and resuspended in a fresh fixative solution. If the solution was unclear after additional centrifugation, the last step was repeated until a clear solution was obtained.

The fixed cells were dropped onto clean microscopic slides, air-dried, and stained in 5% Giemsa solution for 3–5 min. The slides were assessed at ×100 magnification using a Ziess (Germany) light microscope to determine the amount of MN in cytokinesis-blocked binucleate cells. For each

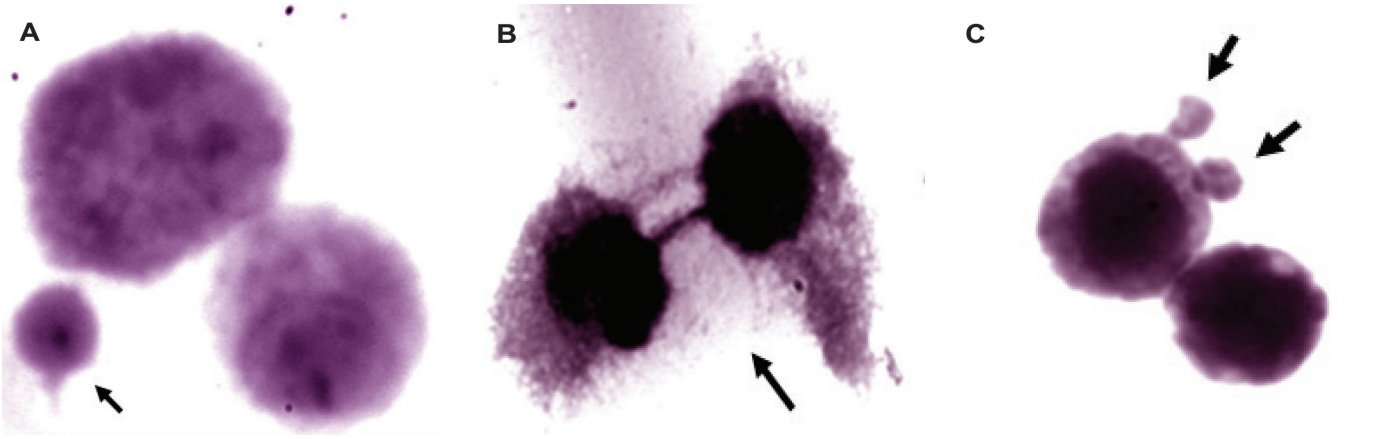

Figure 1: (A) A binucleated cell containing micronuclei, (B) nucleoplasmic bridge, and (C) nuclear budding

sample, 600 binucleated lymphocytes were evaluated to score the micronuclei frequency (Fig 1). We considered samples without any nuclear change "negative" and samples with one or more nuclear changes "positive."

The quality of three prepared slides in the exposed group was not good enough to be evaluated and thus, they were excluded from the study. Finally, we obtained 47 samples from the exposed and 31 samples from the unexposed group.

Results

Demographic data are presented in Table 1. The self-reported prevalence of smoking was 9% (n=4) in the exposed group and 7% (n=2) in the control group—not significantly different. None of the participants reported alcohol consumption.

Table 1: Demographic data in studied groups. The figures are mean (SD).

Variables	Studied Group		p value
	Exposed (n=47)	Unexposed (n=31)	
Age (yrs)	33.2 (3.2)	35.8 (5.4)	0.009
Work experience (yrs)	8.6 (2.7)	11.2 (5.5)	0.011
Body mass index (kg/m^2)	26.4 (3.4)	26.9 (3.5)	0.511

The median numbers of MN, NPB, and NBUD observed in 600 studied binucleated cells for each person in the exposed and unexposed groups are presented in Table 2. There were no significant differences between the exposed and unexposed groups in terms of the extent of DNA damage in each person. The prevalence of MN, NPB, and NBUD positive samples, surrogate markers for DNA damage, was also not significantly different between the exposed and unexposed groups (Table 3).

Linear regression analysis was used for controlling the influence of confounding variables including smoking, age, and work experience, on the effect of low-level benzene exposure on DNA damage. After controlling the confounders, no significant effect was observed between exposure to low-level benzene and the frequency of DNA damage in peripheral blood lymphocytes of studied participants.

Discussion

We found no significant difference between frequencies of MN, NPB, and NBUD in peripheral blood lymphocytes of petrochemical workers exposed to low-level benzene and a group of unexposed workers.

Basso, *et al*, conducted a study on MN

as an early biomarker for measuring the effect of some known mutagenic and carcinogenic compounds in workers employed in petroleum refineries.[19] They reported significant increase of MN in peripheral blood lymphocytes. The results of the univariate analysis of their study are inconsistent with ours. However, using a bivariate analysis, they could not find a significant difference between the MN frequency in the exposed and unexposed groups, which is consistent with our findings.

Lovreglio, *et al*, studied MN frequency in benzene-exposed fuel-tanker drivers and filling-station attendants, and did not find any increase in the frequency of MN in comparison with a control group,[18] corroborating our results. Sha, *et al*, did also not find any significant differences between cytokinesis-blocked MN in the peripheral blood lymphocytes of the exposed and unexposed groups.[20]

There are however some positive studies that are inconsistent with ours. Singaraju, *et al*, conducted a study on benzene-exposed petrol station attendants and found increased MN frequency in buccal cells of exposed subjects. They suggest the MN test in exfoliated epithelial cells is a useful biomarker for occupational exposure to genotoxic chemicals.[21] Their study was however conducted on exfoliated buccal cells, which are directly exposed to air pollutant, and their responses to direct benzene exposure may be different from those of the peripheral blood lymphocytes.

Mozdarani, *et al*, reported higher chromosomal aberration in the peripheral blood lymphocytes of benzene-exposed workers. However, their subjects were exposed to higher concentrations of benzene (15–135 ppm) than our subjects.[16]

Mrdjanovic, *et al*, conducted a study on workers exposed to some petroleum derivatives and found an increased MN frequency in this group.[12] In contrast to our study, their subjects were exposed to a wide array of petroleum derivatives, instead of benzene. This different exposure may explain the inconsistency of their results as compared with ours. Similarly, findings by Angelini, *et al*, are inconsistent with our results. They reported a significantly higher MN frequency in traffic wardens than in controls.[22] They showed that the APEX1 variant genotype is associated with a significantly lower median MN frequency in men. They concluded that genetic variation in DNA-repair genes may modulate susceptibility to benzene-induced DNA damage.

Zhang, *et al*, showed that two promoter polymorphisms in the CYP2E1 gene have significant effect on the benzene-induced MN.[23] Another researcher studied the XRCC1 polymorphism and chromosomal

Table 2: Median (IQR) number of MN, NPB, and NBUD observed in 600 studied binucleated cells for each person in the exposed and unexposed groups. MN/NPB/NBUD indicates any abnormalities (DNA damage).

Parameter	Studied Group		p value
	Exposed (n=47)	Unexposed (n=31)	
MN	1 (2)	1 (3)	0.85
NPB	0 (0)	0 (0)	0.39
NBUD	0 (2)	0 (1)	0.81
MN/NPB/NBUD	2 (3)	1 (4)	0.56

Table 3: The prevalence of MN, NPB, and NBUD-positive samples in the exposed and unexposed groups

Parameter	Studied Group		p value
	Exposed (n=47)	Unexposed (n=31)	
MN	28 (60%)	18(58%)	0.89
NPB	6 (13%)	2 (7%)	0.37
NBUD	20 (43%)	13 (42%)	0.96
MN, NPB, or NBUD	37 (79%)	22 (71%)	0.44

damage in workers occupationally exposed to benzene and concluded that diplotypes of XRCC1 could be associated with chromosomal damage induced by benzene.[24] Peng, *et al*, concluded that multiple mechanisms contribute to benzene-induced cytogenetic changes.[25]

It is well established that genomic damage may be influenced by both environmental and genetic factors. Experimental factors such as exposure to genotoxins (*eg*, benzene), medical procedures (*eg*, radiation), lifestyle factors (*eg*, alcohol consumption, smoking, drugs, and stress), and genetic factors (*eg*, inherited defects in DNA metabolism and/or repair) may influence genomic damage.

In light of studies on the correlation between some polymorphisms and benzene-induced DNA damage, the inconsistency observed among MN assays may be attributed to the effect of genetic polymorphisms. A limitation of our study was that we did not evaluate polymorphisms in DNA-repair genes, which may modulate the susceptibility to benzene-induced DNA damage.

In conclusion, the usefulness of the MN assay as a biomarker for exposure to low-level benzene is questionable. Further studies on benzene-induced MN and modulating factors are necessary.

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