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# Micronucleus occurrence in Chinese workers occupationally exposed to benzene

## *Comparsa del micronucleo in lavoratori cinesi professionalmente esposti a benzene*

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**Summary**

***Objective.*** **To assess chromosomal damage, investigate its risk factors, and explore its association with white blood cell (WBC) decrease among benzene-exposed workers.** ***Methods.*** **A total of 312 subjects including 219 benzene-exposed workers and 93 unexposed controls were recruited, and the exposed subjects were classified according to WBC count as low, unstable and normal WBC group. Chromosomal damage in peripheral blood samples were evaluated using cytokinesis-blocked micronucleus (CBMN) assay. Statistical analysis was performed using $\chi^2$ test and Poisson regression model.** ***Results.*** **The micronucleus (MN) frequencies in the low WBC group (2.75±1.95)‰, unstable WBC group (2.49±1.85)‰ and normal WBC group (2.02±1.63)‰ were all higher values than the control group (1.22±1.12)‰ based on simple Poisson regression (P<0.01). Low and unstable WBC group has a higher average MN**

**Riassunto**

***Obiettivo.*** **Valutare il danno cromosomico, studiare i suoi fattori di rischio, e analizzare il suo legame con la diminuzione dei globuli bianchi (White Blood Cell, WBC) tra i lavoratori esposti a benzene.** ***Metodi.*** **Sono stati coinvolti in totale 312 soggetti, tra i quali 219 lavoratori esposti a benzene e 93 non esposti, e i soggetti esposti sono stati classificati, a seconda del conteggio di WBC: gruppo con basso livello WBC, con livello instabile e normale. Utilizzando il test del micronucleo con il blocco della citocinesi (Cytokinesis-Blocked Micronucleus, CBMN), è stato valutato il danno cromosomico presente nei campioni di sangue periferico. L'analisi statistica è stata effettuata usando il test del $\chi^2$ e il modello di regressione di Poisson.** ***Risultati.*** **La frequenza di micronuclei nel gruppo con basso livello WBC (2,75±1,95)‰, livello instabile (2,49±1,85)‰ e normale (2,02±1,63)‰ si dimostra maggiore rispetto al gruppo di controllo**

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**frequency than normal WBC group, too. Moreover, with the decrease of WBC count, there is a trend of increased MN abnormality rate among all exposed groups. *Conclusion.* Chromosomal damage induced by low-level benzene exposure is related with WBC decrease and might be an early predictor of hematotoxicity/ genotoxicity among the exposed population. Eur. J. Oncol., 17 (2), 71-78, 2012**

**(1,22±1,12)‰ sulla base di una semplice regressione di Poisson (P<0,01). Inoltre, i gruppi con basso livello WBC e con livello instabile hanno una più alta frequenza media di micronuclei rispetto al gruppo con livello WBC normale. Per di più, con la diminuzione del numero di globuli bianchi, si evidenzia la tendenza al manifestarsi di un tasso di anormalità con aumento di micronuclei tra tutti i gruppi esposti. *Conclusione.* Il danno cromosomico indotto da un'esposizione ad un basso livello di benzene è collegato alla diminuzione di WBC e potrebbe essere un segnale precoce di emotossicità/ genotossicità tra la popolazione esposta. Eur. J. Oncol., 17 (2), 71-78, 2012**

***Key words:*** **benzene, chromosomal damage, WBC decrease, CBMN assay**

***Parole chiave:*** **benzene, danno cromosomico, diminuzione di globuli bianchi, test del micronucleo con il blocco della citocinesi**

## Introduction

Benzene, an important component of organic solvents, is commonly used in industry. Meanwhile, benzene is a human carcinogen leading to leukemia (1-4). A possible carcinogenic mechanism might be its genotoxicity. Quite a few studies have reported that occupational benzene exposure could induce chromosomal aberrations (5-14). A review by Smith and Rothman on biomarkers in the molecular epidemiology of benzene-exposed workers proposed that specific chromosomal aberrations might provide better biomarkers for leukemia risk (15). Since white blood cell (WBC) decrease is usually an early and significant symptom of benzene-induced hematotoxicity (16), it is necessary to investigate the association between specific benzene-induced chromosomal aberrations and WBC count so as to identify early biomarkers of leukemia risk in occupationally benzene-exposed population.

Micronucleus (MN) has been an established biomarker for chromosomal damage in human populations. Previously, some studies have shown elevated MN frequencies in benzene-exposed workers (11, 13). Although MN has been correlated with overall cancer incidence (17-19), a more specific association between MN and benzene-induced leukemia still needs verification. In this study, chromosomal damage was assessed using CBMN assay among low-level benzene-exposed workers in a Chinese occupational population. By investigating its risk factors and exploring its association with WBC decrease, we hope to develop an effective biomarker and provide more evidence for screening susceptible population.

## Materials and methods

### *Subjects*

All of 312 participants, including 219 benzene-exposed workers and 93 unexposed healthy controls, came from Anhui Province, China. Eligible participants have to meet the following criteria: no history of any previous diagnosis of cancer; no X-ray test in the past two weeks; the standard questionnaire and CBMN test completion. We carried out two consecutive standard blood tests for all the participants in the past three months. Based on their WBC count, we divided the benzene-exposed workers into three subgroups: low WBC group (65 workers with WBC count less than $4.5*10^9$/L in both two tests), unstable WBC group (72 workers with WBC count less than $4.5*10^9$/L only in one of the two tests), and normal WBC group (82 workers with normal WBC count).

The cut-off point of $4.5*10^9$/L was chosen because it represents the suggested lower limit of normal WBC range in current clinical practice. Controls were frequency-matched on age (5-year intervals) and gender to workers currently exposed to benzene.

*Exposure Assessment*

Twenty-three monitoring sites in the working environment of benzene-exposed workers were set. Benzene concentration in the air was evaluated three times a day at each site.

*Epidemiological data collection*

Having obtained an informed consent from each participant, our trained staff interviewed each participant using a standard questionnaire. This questionnaire consisted of the following parts: (a) demographic background; (b) occupational history; (c) smoking and alcohol drinking history; (d) disease history and self-reported clinical syndrome. Interviews were supervised by senior officials from the Division of Occupational Health at local Centers for Disease Control and Prevention.

*Laboratory assays*

We collected a 5 ml anti-coagulated peripheral blood sample from each participant during the interview for standard blood test and Cytokinesis-blocked micronucleus (CBMN) assay. The CBMN assay was carried out according to the standard procedure described by Fenech (20), and a brief description may be found in a previous article (21).

*Statistical methods*

Statistical analysis was performed using SAS 9.1.3 software. MN abnormality rates among all groups were compared using $\chi^2$ test. Risk factors of increased MN frequency were assessed using Poisson regression model, measured by frequency ratio (FR), its 95% Confidence Interval (CI) and P-value ($FR=e^{\beta}$, e=2.718 28, β is the regression coefficient). For categorical variables, FR indicates the proportion of subjects with increased/decreased MN frequency compared with the reference group.

## Results

*General characteristics and average MN frequency*

Table 1 shows the distribution of age, gender, exposure duration (year), smoking status and alcohol consumption status among all the groups. We observed a higher proportion of male workers and younger workers (below thirty-years old) among all the groups. Compared with control group (1.22±1.12)‰, each exposed group shows a significantly higher average MN frequency (P<0.01); low WBC group (2.75±1.95)‰ and unstable WBC group (2.49±1.85)‰ both have a higher average MN frequency than normal WBC group (2.02±1.63)‰ based on simple Poisson regression (P<0.01).

*Exposure assessment*

At all of the 23 monitoring sites, benzene air concentration was less than 0.17 ppm (0.6 mg/m$^3$) for three times per day, which is below the national occupational health standard of 1.8 ppm according to the local CDC.

*Comparison of MN abnormality rates*

The frequency distribution of MN frequency in each group is found in table 2. With the 95 percentile of MN frequency in the control group (3‰) as cutoff point, we defined the subjects with MN frequency above 3‰ as abnormal, and others as normal. Based on such a definition, we calculated and compared the MN abnormality rates of all groups. A $\chi^2$ value of 19.451 (P<0.001) indicated difference of MN abnormality rates among four groups, and a P-value of 0.038 in the linear trend test suggested linear association. According to table 2, the MN abnormality rate of each exposed group was significantly different from that of the control group (P<0.05).

We further compared the MN abnormality rate among three benzene-exposed groups. A P-value of 0.08 in the linear trend test indicated that in the exposed population, there was a linear trend of increased MN abnormality rate with the decrease of WBC count.

**Table 1 -** General characteristics of benzene-exposed workers and controls

| Variable | Low WBC group (%) | Unstable WBC group (%) | Normal WBC group (%) | Control group (%) |
|---|---|---|---|---|
| Age(year) | | | | |
| <30 | 48 (73.8) | 60 (83.3) | 58 (70.7) | 57 (61.3) |
| ≥30 | 17 (26.2) | 12 (16.7) | 24 (29.3) | 36 (38.7) |
| Mean±SD | 26.15±6.23 | 24.61±5.62 | 28.06±8.26 | 30.67±9.15 |
| Gender | | | | |
| Male | 59 (90.8) | 67 (93.1) | 63 (76.8) | 68 (73.1) |
| Female | 6 (9.2) | 5 (6.9) | 19 (23.2) | 25 (26.9) |
| Exposure duration(y) | | | | |
| <5 | 39 (60.0) | 48 (66.7) | 48 (58.5) | — |
| ≥5 | 26 (40.0) | 24 (33.3) | 34 (41.5) | — |
| Mean±SD | 4.48±2.73 | 3.88±2.50 | 4.72±2.58 | — |
| Smoking status | | | | |
| Yes | 33 (50.8) | 41 (56.9) | 37 (45.1) | 28 (30.1) |
| No | 32 (49.2) | 31 (43.1) | 45 (54.9) | 65 (69.9) |
| Alcohol drinking | | | | |
| Yes | 43 (66.2) | 52 (72.2) | 38 (46.3) | 32 (34.4) |
| No | 22 (33.8) | 20 (27.8) | 44 (53.7) | 61 (65.6) |
| CBMN (‰) | 2.75±1.95 | 2.49±1.85 | 2.02±1.63 | 1.22±1.12 |

**Table 2 -** A comparison of MN abnormality rate between each exposed group and control

| WBC Group | MN frequency status | | $\chi^2$ | P-value |
|---|---|---|---|---|
| | Normal (%) | Abnormal (%) | | |
| Control | 89 (95.7) | 4 (4.3) | — | — |
| Normal | 68 (82.9) | 14 (17.1) | 7.703 | 0.006 |
| Unstable | 55 (76.4) | 17 (23.6) | 13.623 | <0.001 |
| Low | 46 (70.8) | 19 (29.2) | 19.117 | <0.001 |

*Comparison of average MN frequency and investigation of risk factors*

Firstly, we calculated the mean and standard deviation of MN frequency for each group. Compared with that of the control group, average MN frequency of each exposed group was significantly higher (P<0.0001), indicating that benzene exposure might be a risk factor for chromosomal damage.

Then we compared low WBC group and unstable WBC group with normal WBC group respectively to assess the association between benzene hematotoxicity and chromosomal damage. The result showed a possible relationship between WBC decrease and MN frequency increase, indicated by a significant difference of average MN frequency between low WBC group and normal WBC group (FR=1.36, 95% CI: 1.10-1.68, P=0.0043).

For single variant analysis, we assessed potential risk factors including age, gender, exposure duration, smoking status and drinking status in the exposed population by Poisson regression analysis. Table 3 suggested that higher age and tobacco smoking might be potential risk factors for increased MN frequency.

Based on single variant analysis, we continued to perform multi-variant analysis by Poisson regression model ($\alpha$=0.10) in the entire study population.

**Table 3 -** Single variant analysis of risk factors for MN frequency increase in exposed subjects

| Variable | WBC Group | category MN | frequency (‰) | FR (95% CI) | $\chi^2$ | P-value |
|---|---|---|---|---|---|---|
| Age (year) | Low | <30 | 2.42±1.88 | 1.00 | — | — |
| | | ≥30 | 3.71±1.90 | 1.53 (1.12-2.08) | 7.46 | **0.0063** |
| | Unstable | <30 | 2.41±1.81 | 1.00 | — | — |
| | | ≥30 | 2.83±2.13 | 1.17 (0.79-1.68) | 0.70 | 0.4038 |
| | Normal | <30 | 1.69±1.29 | 1.00 | — | — |
| | | ≥30 | 2.83±2.08 | 1.68 (1.23-2.28) | 10.73 | **0.0011** |
| Smoking status | Low | No | 2.78±1.90 | 1.00 | — | — |
| | | Yes | 2.73±2.04 | 0.98 (0.73-1.32) | 0.02 | 0.8957 |
| | Unstable | No | 1.84±1.21 | 1.00 | — | — |
| | | Yes | 2.98±2.10 | 1.62 (1.19-2.23) | 9.00 | **0.0027** |
| | Normal | No | 2.07±1.68 | 1.00 | — | — |
| | | Yes | 1.97±1.59 | 0.95 (0.70-1.30) | 0.09 | 0.7667 |

**Table 4 -** Multi-variant analysis of risk factors for MN frequency increase in all subjects

| Variable | Category | FR (95% CI) | $\chi^2$ | P-value |
|---|---|---|---|---|
| Age (year) | <30 | 1.00 | — | — |
| | ≥30 | 1.51 (1.25-1.80) | 19.31 | <0.0001 |
| WBC Group | Control | 1.00 | — | — |
| | Low | 2.51 (1.95-3.26) | 49.31 | <0.0001 |
| | Unstable | 2.34 (1.81-3.03) | 41.16 | <0.0001 |
| | Normal | 1.80 (1.39-2.33) | 0.05 | <0.0001 |

According to table 4, age and WBC group remained significant in the formula. In particular, average MN frequency of each exposed group (regardless of WBC count) was significantly higher than that of the control group adjusted for age.

Furthermore, we repeated multi-variant analysis only in the exposed population. Significant factors included age, smoking status and WBC group. In the exposed population, those with low or unstable WBC count showed higher MN frequency, thus further indicating the relationship between WBC decrease and chromosomal damage.

## Discussion

We recruited 312 subjects including 219 benzene-exposed workers and 93 unexposed controls in this study, and further divided the exposed subjects into three subgroups based on their WBC count before assessing chromosomal damage in peripheral blood lymphocytes, in order to achieve two major goals. Firstly, by comparing benzene-exposed groups with the control group, we were able to detect benzene-induced chromosomal damage and investigate its risk factors. Furthermore, by examining chromosomal damage in three exposed groups with different WBC levels, we were also able to investigate the association between benzene hematotoxicity and chromosomal damage, which would provide more insight into the mechanisms of benzene carcinogenesis in the blood system.

In our study micronucleus (MN) was selected as the biomarker for chromosomal damage. Previous studies have demonstrated that MN might be a predictor for overall cancer risk. Since MN increase

has been reported in some benzene exposed populations, we hypothesized that MN might be a potential predictor for benzene-induced leukemia. CBMN assay, as a reliable and quick technique for detecting chromosomal abnormalities, was employed in our experiments.

Moreover, we observed the MN abnormality rate of each group. An MN frequency of 3‰ was set as the cutoff point according to the 95 percentile of MN frequency in the control group, and subjects with MN frequency above the cutoff point were considered as abnormal. Difference of MN abnormality rate between each exposed group and the control group suggested higher level of chromosomal damage in exposed workers, and linear trend among the MN abnormality rates of three benzene-exposed groups suggested association between WBC decrease and chromosomal damage. Then we continued to look at the average MN frequency of each group, which was more informative than MN abnormality rate. After adjusting for potential confounding factors such as age and gender, we observed difference of MN frequency between each exposed group and the control group, as well as among all three exposed groups, thus confirming our findings mentioned above.

WBC decrease is usually the earliest sign of chronic benzene hematotoxicity. According to the analysis among the exposed population, average MN frequency has been higher in subjects with low or unstable WBC count compared with normal WBC group, and a linear trend of MN abnormality rate increase with the decrease of WBC count was present. In particular, results from the unstable WBC group suggested that even if the WBC count of a worker appeared to be "normal" in one blood standard test, his chromosomes might have already been impaired, starting the carcinogenic pathway (chromosomal damage-WBC decrease-blood system disorder-leukemia). Such findings, if confirmed by future studies, might support micronucleus as an early biomarker for benzene-induced leukemia, and further establish CBMN assay as a standard method to identify benzene exposed workers with higher leukemia risk at the earliest stage possible.

At present, benzene PC-TWA has been decreased from 12 ppm to 1.8 ppm in China, to 1 ppm in some developed countries (22), and to 0.5 ppm in USA (OSHA action level). However, still heated discussions about whether there is a safe level of benzene exposure are developing (23-25), so it is necessary to study the health effects of very low benzene exposure in the occupational population. In our study, despite the benzene air concentration of 0.17 ppm, which was far below the national standard, we still observed various levels of chromosomal damage, depending on WBC count of each subject suggesting that strict control of benzene air concentration and extensive protection for benzene-exposed workers (especially those at higher risks) are crucial in the working environment even if the level of exposure is low. Moreover, based on the demonstrated toxicity of low-level benzene exposure, benzene study may be expanded from current occupational exposure to environmental exposure to gain a more comprehensive insight in the general population.

We also observed higher age as a risk factor for increased MN frequency in the exposed population, which was in accordance with several previous findings (26-29), calling for more extensive occupational health protection for benzene-exposed workers with higher age. Moreover, MN frequency among smokers is slightly higher than that among nonsmokers in the exposed population, indicating an interaction of adverse lifestyle and occupational exposure, which calls for health education to promote healthier way of living among exposed workers.

One major drawback of this study is the incomplete exposure assessment. Without benzene air concentration in the working environment for the past few years, we were unable to calculate the cumulative exposure level of each subject. Meanwhile, biological monitoring such as benzene metabolites in the urine or blood was also lacking. In order to provide more detailed exposure status of each subject, we hope to complete data collection for exposure assessment in the near future.

In conclusion, our study demonstrated chromosomal damage induced by low-level benzene exposure in a Chinese occupational population, and risk factors for exposed workers included higher age as well as smoking, thus calling for extensive protection in the working place especially for those with higher susceptibility. Furthermore, the association between micronucleus and WBC decrease suggested

MN might be an effective biomarker for benzene-induced leukemia, which provided a reliable method for screening susceptible population.

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