Community Research

Lymphocyte chromosome breakage in low benzene exposure among Indonesian workers

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Abstrak

Latar belakang: *Benzena sudah digunakan sejak lama dalam industri dan penggunaannya cenderung meningkat sehingga memerlukan pengendalian. Walaupun benzena industri dikendalikan < 1 ppm, namun efeknya masih terlihat, antara lain berupa patahan kromosom limfosit. Penelitian ini bertujuan mengevaluasi patahan kromosom limfosit sebagai efek pajanan benzena kurang 1 ppm di lingkungan kerja, dan faktor risiko yang berhubungan.*

Metode: *Studi potong lintang dilakukan di industri migas T pada September 2007 - April 2010. Populasi terdiri 115 pekerja tetap bagian produksi dan kantor pusat. Data tentang jenis pekerjaan, lama pajanan benzena, dan jumlah asupan antioksidan diperoleh dengan wawancara. Perilaku kerja dinilai dengan pengamatan langsung. Patahan kromosom limfosit diperiksa dengan metode ”banding”. Analisis bivariat dengan* chi-square *dan rasio odd digunakan untuk analisis hubungan faktor risiko dengan patahan kromosom limfosit, sedangkan* multivariat stepwise forward *digunakan untuk menentukan faktor risiko determinan.*

Hasil: *Patahan kromosom limfosit keseluruhan terjadi pada 72 dari 115 subyek (62,61%). Prevalensi pada pekerja produksi adalah 68,9%, sedangkan pada pekerja administrasi 40% (p > 0,05). Asupan sumber antioksidan rendah meningkatkan risiko patahan kromosom limfosit (p = 0,035; $OR_{adjusted}$ = 2,90; CI = 95% 1,08-7,78). Faktor yang meningkatkan patahan kromosorm limfosit adalah jenis pekerjaan (p = 0,010; OR_{crude} = 3,32; CI = 95% 1,33-8,3) dan pajanan benzena kronik di tempat kerja (p = 0,014; OR_{crude} = 2,61; CI = 95% 1,2-5,67), sedangkan perilaku kerja menurunkan terjadinya patahan kromosom limfosit (p = 0,007; $OR_{adjusted}$ = 0,30; CI = 95% 0,13-0,72).*

Kesimpulan: *Prevalensi patahan kromosom limfosit di lingkungan kerja dengan pajanan benzena di bawah 1 ppm masih cukup tinggi. Pajanan benzena kronik di tempat kerja, jenis pekerjaan, dan asupan antioksidan berhubungan dengan patahan kromosom limfosit. Perlu pengendalian kadar benzena lingkungan agar tetap di bawah 1 ppm dan kebiasaan konsumsi antioksidan perlu digalakkan.*

Abstract

Background: Benzene has been used in industry since long time and its level in environment should be controled. Although environmental benzene level has been controlled to less than 1 ppm, negative effect of benzene exposure is still observed, such as chromosome breakage. This study aimed to know the prevalence of lymphocyte chromosome breakage and the influencing factors among workers in low level benzene exposure.

Methods: This was a cross sectional study in oil & gas industry T, conducted between September 2007 and April 2010. The study subjects consisted of 115 workers from production section and head office. Data on type of work, duration of benzene exposure, and antioxidant consumption were collected by interview as well as observation of working process. Lymphocyte chromosome breakage was examined by banding method. Analysis of relationship between chromosome breakage and risk factors was performed by chi-square and odd ratio, whereas the role of determinant risk factors was analyzed by multivariate forward stepwise.

Results: Overall lymphocyte chromosome breakage was experieced by 72 out of 115 subjects (62.61%). The prevalence among workers at production section was 68.9%, while among administration workers was 40% (p > 0.05). Low antioxidant intake increases the risk of chromosome breakage (p = 0.035; $OR_{adjusted}$ = 2.90; 95%CI 1.08-7.78). Other influencing factors are: type of work (p = 0,10; OR_{crude} = 3.32; 95% CI 1.33-8.3) and chronic benzene exposure at workplace (p = 0.014; OR_{crude} = 2.61; 95% CI 1.2-5.67), while the work practice-behavior decreases the lymphocyte chromosome breakage (p = 0.007; $OR_{adjusted}$ = 0.30; 95% CI 0.15-0.76).

Conclusion: The prevalence of lymphocyte chromosome breakage in the environment with low benzene exposure is quite high especially in production workers. Chronic benzene exposure in the workplace, type of work, and low antioxidant consumption is related to lymphocyte chromosome breakage. Thus, benzene in the workplace should be controlled to less than 1 ppm, and the habit of high antioxidant consumption is recommended.

Keywords: antioxidant, chromosome breakage, low benzene exposure

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Benzene has been used in industries since long time ago and is produced as byproduct in the oil and gas industries. Thus, benzene is continously exists in nature. Benzene is known as carcinogenic, so its level should be controlled to as low as possible. It is recommended that the environmental level of benzene is less than 1 ppm. In Indonesia, the use of benzene is estimated to be about 20 - 40% of all other chemicals (Indonesian Bureau of Statistics).[1] With the increasing demand for energy, exploration of oil and gas has been increasing as well, so that more and more benzene is produced and more and more workers are being exposed to benzene. One of the objectives of occupational health and safety program is to control benzene level below treshold limit value (TLV), i.e less than 1 ppm.[2-4]

Many studies have been conducted on the health effect of benzene exposure to workers. In the early nineties, benzene has been known to have genotoxic effect and could lead to development of leukemia on workers.[2,3] Studies on the effects of low benzene exposure showed that more than 60% of traffic police force in India, who are alcohol drinkers and 38.46% of non-vegetarian communities had abnormal lymphocyte chromosomes. They were exposed to benzene at less than 5 ppm from air pollution.[5] Exposure from the environment or workplace will enter the human body through inhalation, and then benzene will be metabolized by the liver and produces intermediate metabolites such as phenol, hydroquinone, and cathecol which have free radical properties and have the capacity to bind to proteins in the body. In case of protein in bone marrow, free radical attack may induce lipid peroxidation and lipolysis. Chromosomes of lymphocyte can also be affected which can lead to chromosome breakage which potentially transform to malignancy and cell disruption.[6-11] This lymphocyte chromosome breakage can lead to other serious health problems, like leukemia and immune dysfunction.

In an attempt of preventive measures, the regulation has required that the TLV for benzene has been decreased significantly in the last 40 years. American Conference of Governmental Industrial Hygienist (ACGIH) had ever taken the TLV value of 50 ppm before, and then periodically decreased untill finally reached 0.5 ppm since 2006,[2-4] while in Indonesia, the TLV for benzene was set at 10 ppm before 2011.[12] In many countries, the use of Benzene has been totally banned for industrial use. Regarding that many Indonesian workers do not use protection appropriately, even in the environment with high benzene level, thus they are prone to toxic effect of benzene.

In this study, we are interested to conduct a more comprehensive study on the pravalence of lymphocyte chromosome breakage as well as the factors that influence its occurrence among Indonesian workers in low level benzene exposure.

METHODS

This was a cross sectional study conducted between September 2007 - April 2010 in a gas and oil industrie in East Kalimantan, Indonesia, with benzene exposure lower than 1 ppm. The population for this study was all employees from the production area and head office with the total sample of 210. The employees in the production area were usually exposed to benzene and those in the head office were not exposed. From 210 employees, we randomly took 115 who met selection criteria and to obtain sufficient sample size for multivariate analysis.[13]

The inclusion criteria included male, full time workers who have been working in the production area or head office for more than 1 year, and agreed to participate in this study by signing informed consent. Whereas those who was absent during data collection and had abnormal liver function (SGOT $\geq$ 35 mg/dL, SGPT $\geq$ 40 mg/dL) or abnormal renal function (ureum $\geq$ 50 mg/dL, creatinin $\geq$ 1.5), were excluded. These exclusions were made to avoid confounding effect of liver and kidney dysfunction on chromosome breakage.

Data collection was done by interview, observation of work practices and working environment. Body mass index (BMI) based on Asia Pacific criteria was measured by weight and height, and was normalized to benzene exposure in the workplace for measuring the degree of chronic benzene exposure using the semi-quantitative method.[14] Blood sampling was done for liver and renal function, as well as for lymphocyte chromosome examination. Blood examination for liver and renal function was performed at Prodia Laboratory, while benzene biomonitoring and environment was done at Australian Laboratory Services (ALS), Malaysia. Lymphocyte chromosome breakage was examined at the laboratory of Eijkman Institute, Jakarta, using the chromosome banding methode for identification of chromosome number and chromosome abnormality.

Dependent variable in this study was lymphocyte chromosome breakage, while independent variables include age, type of work, working history, risky behavior, practice/behavior & management, antioxidant consumption, BMI, chronic benzene exposure at workplace and exposure elsewhere other than workplace. Antioxidant consumption (vitamin A,C and E) was estimated by daily food intake recall for 7 days by using nutrisurvey analysis, and then compared to daily intake, followed by determination of cut-off point for good and not good criteria. Work practice/behavior was judged by observation of appropriate use of personal protection equipment for benzene pevention, such as masker, gloves, goggles and wearpack.

The protocol of this study has been approved by the Ethics Committee, Faculty of Medicine, Universitas Indonesia (No. 376/PT02.FK/ETIK/2008). Data analysis for relationship of risk factors and chromosome breakage was performed by bivariate analysis using chi-square and odd ratio, and the role of determinant risk factors was analyzed by multivariate forward stepwise analysis.[15,16] The statistical package for social science (SPSS) 11.5 for windows was used in this study.

RESULTS

This study recruited 115 subjects selected from 210 employees aged from 18 - 55 years with the median of 30 year, most of them (61.7%) were < 30 years. Almost 80% of them worked at production area and have been working there less 10 years. Benzene exposure was calculated using semiquantitative method based on scoring system which was derived from measurement of the exposure in the workplace and normalized to body mass index (BMI).

Forty percent of the workers were categorized as having low exposure with exposure rate (ER) ≤ 9.3 ppm years, and 60% as high exposure rate (ER > 9.3 ppm years). It is found in this study that 72 out of 115 workers (62.61%) had lymphocyte chromosome breakage. The prevalence of lymphocyte chromosome breakage among the workers at production section was 68.9%, while among administration workers was 40%. This difference was not statistically significant (Table 1). Most of the breakage occurred at the centromere parts of chromosome 3 and 6 (Table 2).

Bivariate analysis showed that lymphocyte chromosome breakage have significant relationship with type of work (p = 0.010; OR_{crude} = 3.32; 95% CI = 1.33-8.30), antioxidant consumption (p = 0.033; OR_{crude} = 2.74; 95% CI = 1.06-7.03), work practices behavior and management (p = 0.008; OR_{crude} = 0.33; 95% CI = 0.16-0.76) and benzene exposure at the workplace (p = 0.018; OR_{crude} = 2.53; 95% CI = 1.16-5.49). While age, work history, lifestyle, benzene exposure elsewhere and other exposures, showed no significant relationship (Table 3).

Table 1. Frequency of lymphocyte chromosome breakage among workers in administrative and production section (n = 115)

Location of work	Chromosome breakage	
	No n (%)	Yes n (%)
Administration office	15 (60.0)	10 (40.0)
Production section	28 (31.1)	62 (68.9)
Total	43	72

Table 2. Location of lymphocyte chromosome breakage among the oil and gas industry workers (n = 115)

Chromosome number	Location of the chromosome breakage (n)					
	B	C	LA	SA	SLA	NB
1	5	2	3	0	0	110
2	2	0	2	0	0	113
3	37	24	3	10	0	78
4	3	0	2	1	0	112
5	11	9	2	0	0	104
6	23	23	0	0	0	92
7	4	2	0	0	2	111
8	0	0	0	0	0	115
9	3	1	1	1	0	112
10	5	3	2	0	0	110
11	8	5	2	1	0	107
12	5	2	2	1	0	110
13	2	0	2	0	0	113
14	2	0	2	0	0	113
15	1	0	1	0	0	114
16	1	1	0	0	0	114
17	2	0	1	1	0	113
18	2	1	1	0	0	113
19	2	1	1	0	0	113
20	1	0	0	1	0	114
21	0	0	0	0	0	115
22	1	1	0	0	0	114
XY	4	2	1	1	0	111

B: Breakage; C: Centromere; LA: Long arm; SA: Short arm; SLA: Short & long arm; NB: No breakage

Table 3. Association between characteristic and management practice, benzene exposure, others exposure and lymphocyte chromosome breakage among the oil and gas workers (n = 115)

Variables	Chromosome breakage		OR crude	CI 95%	p
	No (n = 43)	Yes (n = 72)			
	n (%)	n (%)			
Age					
≤ 30 year	24 (33.8)	47 (66.2)	1.00		
> 30 year	19 (43.2)	25 (56.8)	0.67	0.31-1.46	0.312
Type of work					
Administration	15 (60.0)	10 (40.0)	1.00		
Production	28 (31.1)	62 (68.9)	3.32	1.33-8.30	0.010
Work history					
No relation to benzene	22 (44.9)	27 (55.1)	1.00		
Relation to benzene	21 (31.1)	45 (69.9)	0.57	0.27-1.23	0.153
Risk behavior					
Low	27 (36.5)	47 (63.5)	1.00		
High	16 (39.0)	23 (61.0)	0.90	0.41-1.97	0.790
Antioxidan consumption					
High	36 (43.4)	47 (56.6)	1.00		
Low	7 (21.9)	25 (78.1)	2.90*	1.08-7.78	0.035
Work practices-management					
Good	24 (29.6)	57 (70.4)	1.00		
Not good	19 (55.9)	15 (44.1)	0.30*	0.13-0.72	0.007
Benzene exposure in the workplace					
Low (ER ≤ 9.3 ppm years)	25 (50.0)	25 (50.0)	1.00		
High (ER > 9.3 ppm years)	18 (28.4)	47 (71.6)	2.61	1.20-5.67	0.014
Other benzene exposure					
Low (ER < 1.46)	19 (35.8)	34 (64.2)	1.00		
High (ER ≥ 1.46)	24 (37.4)	38 (62.6)	0.89	0.41-1.90	0.752
Other exposures					
Low (< 8.155)	24 (44.4)	30 (55.6)	1.00		
High (≥ 8.155)	19 (31.1)	42 (68.9)	1.77	0.83-3.79	0.101

Note: ER = Exposure rate; * = OR adjusted

Multivariate analysis by forward stepwise analysis was done on 6 variables which had $p < 0.25$ during bivariate analysis. It was revealed that low antioxidant consumption was the significant determinant factor of lymphocyte chromosome breakage ($p = 0.035$; $OR_{adjusted} = 2.90$; 95% CI = 1.08-7.78, Table 4).

DISCUSSION

In the present study, the effect of low level benzene exposure on lymphocyte chromosome breakage has been conducted in Indonesian workers of oil and gas industry in East Kalimantan. We have minimized the limititation of this study by using the benzene annual exposure rate for measuring the subject's exposure.[14] The prevalence of lymphocyte chromosome breakage was found to be 68.9% in exposed subjects, while it was 40.9% among non exposed subjects. The difference was not statistically significant. The high prevalence of lymphocyte chromosome breakage, even among workers of administrative office is quite intriguing. However, it can be explained by the possibility that they are indeed still exposed to benzene less 1 ppm from the environmental pollution of the city or in the workplace environment. This prevalence is about the same as a study in India conducted among policemen,[5] but lower than the prevalence reported in the study of Piciano, et al.[17] This difference might be due to the difference in

exposure level. In the study of Piciano, the subjects were exposed to higher level of benzene (between 1 to 10 ppm), while in this study the subjects were exposed to benzene level of less 1 ppm.

Regarding the position of chromosome breakage, it was mostly found in chromosome number 3 and 6, while the study by Zhang, et al[18,19] reported that anomaly were more frequent in chromosome number 5, 7, and 9, while Sasiadek and Jagielski[20] found anomaly in chromosome number 2, 4, 6, and 9. From the gene cards of the Institute of Gene International, it is known that some diseases are related to the number of chromosome damage.[21] It is mentioned that abnormalities/diseases might occur if there is damage to the chromosome numbers 3, 6, 7, and 9. Damage to chromosomes number 3 is associated with metabolism disorders, and anomaly in chromosome 3 is more closely related to triglycerides/HDL ratio, especially for the Caucasian families in USA,[22] and increasing of the heart problems and metabolic syndrome. Anomaly in chromosome 6 indicates immunology dysfunctions, and strongly associated with psoriasis and anomaly in chromosome 7 and 9 reflects the effects of cytochrome P450, and related with autoimmune diseases or leukemia.[21]

Age was not found to be a risk factor in this study, which is different from the finding of Kim,[11] who reported that with the increase of age there was also the increase of lymphocyte chromosome breakage. Workers at the production site have 3 times higher risk of having lymphocyte chromosome breakage compared to the administrative workers, which also shows that benzene exposure is related to chromosome breakage.

Regarding the role of other influencing factors of chromosome breakage, we have studied the role of antioxidant consumption. We observed that most of the subjects (80%) had only low antioxidant consumption (data not shown) and the results of analysis showed that low antioxidant consumption significantly increases the risk of lymphocyte chromosome breakage ($p = 0.035$; $OR_{adjusted} = 2.90$; 95% CI = 1.08-7.78). The most common source for antioxidants is tea, where one cup of tea has about 30-50 mg poliphenol, which is a potential antioxidant. Green tea has long been known as a high antioxidant source. Teaflavin in black tea has stronger antioxidant than in vitamin C and vitamin E.[23,24]

Among the subjects that had long-term exposure to benzene, 68.9% had lymphocyte chromosome breakage. Those who had a higher score for benzene exposure were 2.53 fold higher risks to experience lymphocyte chromosome breakage. This finding is consistent with other similar studies in East Asia, China and India, where the exposed subject has more lymphocyte chromosome breakage than non exposed.[5,6] Even though, for cytogenic damage there is no clear dose response relationship, recent studies showed that the effect of benzene is more related to total body area exposure. Thus, in this study BMI was taken into consideration as a component which determine exposure level to benzene. The work periode did not show a significant relationship to lymphocyte chromosome breakage, although it is not consistent with other studies.

Work practices-management was measured using a composite score for behavior using personal protection equipment (PPE), supervision and feedback mechanism system. This study found that good work practices and management significantly lowers the risk of lymphocyte chromosome breakage ($p = 0.008$; $OR_{crude} = 0.33$; 95% CI = 0.15 - 0.76). Not all workers use respiratory protective equipment properly. Whereas it is known that benzene entered the body mostly from inhalation rather than through the skin. Observation at the production sites showed that half masks provided by the company were not used by all workers. And before using the mask no fitting test was performed, eventhough warning signs were placed in all areas exposed to benzene and the company had standard operating procedures for those areas.

From this study, three types of implications can be generated: practical, policy and research-implications. Practical implication is related with the fact that risk factors as well as protective factors for lymphocyte chromosome breakage in workplace with low benzene exposure, were identified. So that preventive measures can be strengthened by individuals as well as by the company. For policy implication, the results of this study can be disseminated as an input for the government to lower the current treshol limit value (TLV) to 0.5 ppm for oil and gas industry. While for research implication of this study, we hope that these results will stimulates other studies on the implication of the effect of low level exposure of benzene to humans using nano-technology, as well as other studies on the effective dose of antioxidants needed to prevent lymphocyte chromosome breakage.

From the above study, it can be concluded that the prevalence of lymphocyte chromosome breakage was was still high, in workers exposed to low environmental benzene level. Low antioxidant consumption increases the risk of lymphocyte chromosome breakage. Type of work and work practices-management had significant relationships with lymphocyte chromosome breakage. It is recommended that decision makers should lower the current benzene TLV level for Indonesia for oil and gas industries. Workers should be educated on the risks of benzene exposure in the workplace and should be encouraged of good management and good behavior in work place and to increase their antioxidant consumption.

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Conflict of interest

The authors affirm no conflict of interest in this study.

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