American-Eurasian Journal of Toxicological Sciences 3 (4): 245-249, 2011
ISSN 2079-2050

Chromosome Instability Among Bhopal Gas Tragedy Survivors

[1,2]Tahir Mohi-ud-Din Malla, [2,3]C. S. Senthilkumar, [1]N.C. Sharma and [2]N. Ganesh

[1]Department of Biochemistry and Genetics, Barkatullah University, Bhopal - 462026, India
[2]Department of Research, Jawaharlal Nehru Cancer Hospital and
Research Centre (JNCHRC), Bhopal - 462001, India
[3]Rajiv Gandhi Technological University (RGTU), Bhopal - 462036, India

Abstract: Bhopal gas tragedy of 1984 is the world's worst industrial disaster that led to deaths of thousands of residents as a consequence of exposure to the toxic gases released at the time of the incident. Many studies reported genotoxicity and mutagenicity in the exposed population soon after the exposure but, no study was conducted to address the long-term genotoxic effects among the survivors. The frequency and pattern of chromosome instability was therefore studied through conventional chromosome aberration assay in the peripheral blood of exposed individuals to unveil the long-term genotoxicity of the exposure. Equal number of sex and area matched exposed and non-exposed individuals were selected. Structural and numerical aberrations were recorded. The mean percentage of total aberrant metaphases in the exposed group was significantly higher ($P<0.01$) as compared to non-exposed. Besides, chromosome-type and chromatid-type aberrations were statistically elevated in the exposed group. The study concludes that chromosome instability still persists as a long-term effect in Bhopal gas tragedy survivors. The higher frequency of chromosomal aberrations may play a definitive role in the pathway of cancer progression and other genetic diseases. Hence, our study suggests that the exposed population is more vulnerable to genetic disorders and cancers.

Key words: Bhopal Gas Tragedy · Methyl Isocyanate · Chromosomal Aberrations · Genotoxicity · Long-Term Effects

INTRODUCTION

Bhopal gas tragedy, the world's worst chemical disaster had an adverse impact on humans in 1984. Methyl isocyanate (MIC), along with other gases liberated in the environment, influenced human health for ever [1]. Diverse health effects reported in the exposed population and their offsprings born after the tragedy are being the consequences of the gas exposure [2-11]. A gradual yearly increase of different types of cancers in the Bhopal gas survivors has been reported [12]. A panel of earlier studies evidenced the chromosomal instability in the exposed population revealing significantly higher chromosomal aberrations and sister chromatid exchanges in the exposed cohorts as compared to the non-exposed [13-16]. However, these studies were deficient in terms of follow up procedures to unveil the long term genotoxic effects. Keeping in view the aforementioned evidence, our present investigation aimed to evaluate the frequency and patterns of chromosomal aberrations in the Bhopal gas exposed human population.

Environmental impacts and occupational exposures are frequently assessed through cytogenetic biomarkers. Chromosomal abnormalities are the commonly used biomarkers for human biomonitoring studies. Hence, the present study was an attempt to determine the chromosomal instability in Bhopal gas tragedy survivors with special reference to long term genotoxic effects, if any. The frequency and pattern of aberrations were studied through conventional chromosome aberration assay.

MATERIALS AND METHODS

Study Subjects: The present study was conducted from 2008 - 2011 and approved by the Institutional Review Board of JNCHRC. Total hundred healthy individuals were randomly selected. Fifty each from exposed and non-exposed population (24 males, 26 females) of the age group of 25 to 50 were studied. Non-exposed were selected as controls and none of them had any conscious contact with radiation or chemical exposures. Pedigree

Corresponding Author: Tahir Mohi-ud-Din Malla, Department of Research, Jawaharlal Nehru Cancer Hospital and research Centre, Bhopal-462001, India.

charts were constructed for all the exposed and non-exposed individuals. The exposed were selected on the basis of gas victim card issued by the government, which depicts the physical presence of the individual at the time of exposure. Detailed history of exposure, acute illness, medical impairments or exacerbation of chronic diseases they had at the time of exposure was also recorded.

Lymphocyte Culture and Chromosome Aberration Assay: Lymphocyte cultures were set according to Moorhead *et al*. [17]. Peripheral venous blood was collected in the heparinized vacutainers and aseptically transferred into a sterile culture bottle with 5-8ml of RPMI-1640 medium (Sigma,USA), supplemented with L- glutamine, 10% fetal bovine serum (Himedia labs, India), Penicillin - streptomycin solution (Invitrogen, USA) and phytohaemagglutinin (Biological Industries, Israel). The cultures were incubated in a CO_2 incubator for 72 hours. 50µl colchicine was added at the completion of 70 hours to arrest the cells at metaphase. After 72 hours of incubation, the cell suspensions were centrifuged for 10 minutes at 1000 rpm. The supernatant was discarded and the pellet was treated with hypotonic solution (0.075M KCl) by gentle flushing and cyclomixed. The centrifuge tubes were incubated again at 37°C for 45 minutes. The tubes were again centrifuged carefully at 1000 rpm for 15 mins. The supernatant was removed and 5-8 ml of freshly prepared pre-chilled Cornoy's fixative was added to the pellet while mixing on cyclomixer. The tubes were allowed to stand overnight and washed with freshly prepared pre-chilled Cornoy's fixative repeatedly for 3-4 times. The slides were stained with 1% Giemsa stain [18]. All the slides were observed under 10X objective of Olympus BX60 (Phase contrast) microscope to locate the metaphases in them and for the observation of minute chromosomal aberrations 100X (Oil immersion) objective was used. Hundred well spread metaphases were analysed per sample and chromosomal aberrations were recorded in a standard format and classified according to the International Nomenclature (ISCN) [19].

Statistical Analysis: Statistical analysis was done with the help of GraphPad PRISM (Ver.4) software. Student's *t* test was performed to test the significance of difference between means of chromosomal aberrations recorded. The means were considered to be statistically significant at 1% level of probability.

RESULTS AND DISCUSSION

Structural and numerical aberrations were recorded at mitotic metaphases. Mean percentage of total abnormal metaphases (TAM) in the exposed group was observed to be 23.4±0.81 which was statistically higher (6.8±0.16) than that of non-exposed ($P<0.01$). The exposed group revealed statistically elevated chromosome-type (13.80±0.85) and chromatid-type aberrations (15.70±1.25) as compared to the non-exposed group (3.6±0.51and 3.50±0.47) respectively. Table 1 shows the chromosome-type and chromatid-type aberrations that include dicentric chromosomes, double minutes, acentric fragments, ring chromosomes, chromatid breaks and terminal deletions, which were found to be statistically higher in the exposed group ($p<0.01$) than the non - exposed. The higher incidence of chromosomal aberrations in the exposed group than the non-exposed is in agreement with the findings of Saxena *et al*. [20] and Ghosh *et al.* [16]. But, the triradial and quadriradial chromosomes reported by them were not observed in any exposed individual of our study.

On gender wise comparison of the groups, both chromosome-type and the chromatid-type aberrations were observed to be significantly increased ($P<0.01$) in the exposed males (17.0 ± 1.02 and 17.6 ± 2.10) and the exposed females (10.8 ± 1.06 and 13.9 ± 1.37) compared to the non-exposed males (2.25 ± 0.64 and 3.25 ± 0.84) and the non-exposed females (4.85 ± 0.70 and 4.54 ± 0.61) respectively. The incidence of chromosome-type aberrations in the non-exposed females was found to be statistically elevated as compared to the non-exposed males ($P<0.01$). Although, the incidence of chromatid-type aberrations was also higher in females than males, the difference between the means was non-significant. However, the trend was reversed in case of the exposed group in which the frequency of chromosome-type aberrations in the exposed males was found to be significantly higher than the exposed females ($P<0.01$). Table 2 summarizes the higher incidence of chromatid-type aberration in the exposed males than the exposed females, but the difference was not significant.

These findings were contradictory to the record of higher incidence of chromosomal aberrations in the exposed females by Ghosh *et al*. [16]. However, it is in agreement with an animal study which showed a higher incidence of micronuclei in male mice exposed to MIC *in vitro* [21, 22]. These observations suggest that males are more susceptible to chromosomal damage than the females.

Table 1: Frequency of different types of chromosomal aberrations observed in the exposed and the non - exposed group

	Structural aberrations Mean ± SE									
	Chromosome type					Chromatid type			Numerical Aberrations Mean ± SE	
Group	DC	DM	FR	RC	Total	CB	TD	Total	PP	TAM Mean ±SE
Exposed	1.28±0.20*	3.56±0.29*	5.64±0.52*	0.40±0.12*	13.80±0.85	12.24±1.09*	3.48±0.36*	15.70±1.25	2.16±0.29*	23.4±0.81
Non - Exposed	0±0	0.60±0.16	1.80±0.27	0±0	3.6±0.51	2.64±0.39	1.28±0.24	3.50±0.47	0.16±0.07	6.8±0.16

DC-Dicentric, DM - Double Minutes, FR-Fragments, RC-Ring Chromosome, CB-Chromatid Break, TD-Terminal Deletion, PP-Polyploidy, TAM - Total abnormal metaphase

*Significant at 1% level of probability as compared to the non-exposed

Table 2: Gender wise comparison of chromosomal aberrations in the exposed and the non - exposed group

Group	Chromosome type Mean±SE	Chromatid type Mean±SE	Numerical Aberrations Mean±SE
Exposed males	17.0±1.02*	17.6±2.10*	2.58±0.49*
Non - Exposed males	2.25±0.64	3.25±0.84	0.08±0.05
Exposed females	10.8±1.06*	13.9±1.37*	1.77±0.32*
Non - Exposed females	4.85±0.70	4.54±0.61	0.23±0.12

*Significant at 1% level of probability as compared to the non-exposed

An initial study conducted by Goswami [14] revealed chromosomal aberrations in 71.4% of the gas affected people of Bhopal. However, chromosomal anomalies could be observed in 100% of the MIC exposed individuals included in the present study, which indicates that the genomic instability persists at a higher intensity.

The incidence of polyploid cells as a numerical chromosomal aberration was recorded statistically higher in the exposed group than the non-exposed ($P<0.01$). This may also be due to the endoreduplication of chromosomes in which DNA synthesis is not followed by a segregation of the chromosome set. Comparing the individuals on the bases of gender, the difference between the means of the polyploid cells was found to be non-significant while comparing the non-exposed males to the non-exposed females and the exposed males to the exposed females. However, comparing the males of the non-exposed and the exposed group and the females of the non-exposed and the exposed group respectively, the means were found to be statistically higher in the exposed males as well as the exposed females than the corresponding non-exposed males and the non-exposed females ($P<0.01$).

The importance of polyploidy as a genotoxic lesion is uncertain and there have been few reports which have included data on spontaneous or induced polyploidy in routine genotoxicity screening. Mitchell *et al*. [23] reported that spontaneous polyploidy tend to be near-exact multiples of the haploid chromosome number whereas induced polyploid cells are heteroploid with a wide range of chromosome numbers. Polyploid cells observed in the present investigation were found to be heteroploid, suggesting that they may have arisen as a result of the exposure.

Increased level of chromosomal aberrations in the population exposed to various toxic chemicals and radiation is currently interpreted as an evidence of genotoxic exposure and as early biologic effects on DNA [24 - 27]. Chemically induced double-strand DNA breaks, if unrepaired, result into structural chromosomal aberrations [28 -32]. An increased risk of cancer in healthy individuals with high levels of chromosomal aberrations in peripheral blood lymphocytes has also been described in recent epidemiological studies carried out by Bonassi *et al*. [33, 34].

As the lesions induced by chemicals are mostly S phase dependent for expression in the subsequent divisional cycle, the damaged T lymphocytes may remain circulating for long periods and these aberrations can be observed only if the cells are stimulated to divide *in vitro* [35].

Measuring the frequency of chromosomal damage in humans exposed to environmental clastogens has been a priority in public health for decades. Unstable chromosomal aberrations induced in lymphocytes of atom bomb survivors of Hiroshima and Nagasaki could be detected even after about 40 years [36], unveiling the presence of long-lived lymphocytes in their peripheral blood. Likewise, there is every possibility of the presence of such lymphocytes circulating in the peripheral blood of Bhopal gas tragedy survivors indicating a long term

genotoxic effect. Significantly higher percentage of chromosomal aberrations in the exposed individuals may be due to their exposure to the toxic gases released at the time of the Bhopal gas tragedy.

CONCLUSION

Higher incidence of chromosomal aberrations in the exposed population validates chromosomal instability. The incidence of chromosomal aberrations like dicentrics and rings even after such a long period indicates persistence of clastogenic effects due to the exposure. These chromosomal aberrations may act as the intermediate processes in the pathway of the progression of any genetic disorder like cancer. Hence, our present study suggests that the 1984 Bhopal gas exposure has produced long-term genotoxic effect that persists even after two and a half decades. We conclude that the exposed population is more vulnerable to genetic diseases and must be counseled for dietary and life style changes to minimize the risk of developing any kind of genetic disorder.

ACKNOWLEDGMENTS

The authors are grateful to Shri. Madan mohan Joshi, Chairman and Mrs. Diviya Parashar, Research Co-ordinator, JNCHRC, Bhopal for providing the necessary laboratory facilities.

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