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R E S E A R C H A R T I C L E

Chromosomal Aberrations As an Evidence of Genetic Damage in the Offspring of Methyl Isocyante Exposed Population of Bhopal

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A B S T R A C T

Introduction: Bhopal gas tragedy was one of the worst industrial disasters that produced various short-term as well as ling-term health effects in the exposed population. Various studies have revealed the long-term genotoxicity of the exposure in the human population. However, the genotoxicity in the offspring of the exposed individuals remains unexplored. The present study is an attempt to study the genomic damage caused by MIC in the offspring of the exposed population and to know about the pattern and frequency of chromosomal aberrations in them. **Material and Methods:** Fifty healthy offspring of the MIC exposed individuals living in the vicinity of 1.5 km from the Union Carbide's pesticide plant were selected at random as the study subjects. Equal number of age, sex and locality matched non-exposed individuals were also enrolled as controls. All the subjects were non smokers and not employed at any type of chemical industry or a radiation department. Cytogenetic analysis was performed by standard protocol of lymphocyte culture as per Moorhead et al. Hundred metaphase spreads per sample were observed and the chromosome aberrations were classified according to the International Nomenclature. 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:** Chromosomal aberrations were recorded at mitotic metaphases. Mean percentage of total abnormal metaphases (TAM) in the offspring group was observed to be 54.7±0.81 which was statistically higher (4.6±0.16) than that of non-exposed controls (P<0.01). The offspring group revealed statistically elevated chromosome-type (13.9±0.17) and chromatid-type aberrations (14.26±0.2) as compared to the non-exposed group (0.4±0.54 and 0) respectively. Besides, acrocentric associations were found to be higher (13.7±4.89) in the offspring group than the controls (0.6±0.70).

Keywords: Chromosomal aberrations, Methyl isocyanate, Bhopal gas tragedy, long-term genotoxicity

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C O N T E N T S

1. Introduction

Bhopal gas tragedy of 1984 was the world's worst industrial disaster that resulted in the death of thousands of residents within days due to their exposure to the toxic methyl isocyanate (MIC) gas released from the Union Carbide's pesticide plant [1]. Various studies have been carried out so far, reporting the genotoxicity and mutagenicity of methyl isocyanate in the exposed population. Chromosomal aberrations have been found significantly higher in the exposed group as compared to the controls [2- 3]. Higher frequency of sister chromatid exchanges and chromosomal anomalies have also been reported in the MIC exposed population [4-6]. Furthermore, a gradual yearly increase of different types of cancers in the survivors of Bhopal gas tragedy has also been reported [7].

Besides genotoxicity, some studies have also been carried out initially regarding the reproductive disorders in the exposed population. It was found that 43.8% of pregnancies in exposed women resulted in still births and the unsuccessful pregnancies also involved spontaneous abortions [8]. It was also reported that the fetal loss among gas affected women was strikingly higher compared to the controls [9]. In an epidemiological study, the perinatal and the neonatal mortalities were found to be significantly elevated in the affected area [10]. Some cytogenetic studies have been carried out confirming the long term genotoxicity of Bhopal gas tragedy in the exposed population [11,12]. However, there has been no study so far on the long-term genotoxic effects of MIC exposure in the offspring of Bhopal Gas Tragedy survivors. The present study is therefore an attempt to explore the genotoxicity in the offspring of the exposed individuals born after the exposure.

2. Materials and Methods

Subject selection:

Fifty healthy offspring of the MIC exposed individuals living in the vicinity of 1.5 km from the Union Carbide's pesticide plant were selected at random as the study subjects. Equal number of age, sex and locality matched non-exposed individuals were also enrolled as controls. All the subjects were non smokers and not employed at any type of chemical industry or a radiation department. At the time of the inclusion of individuals in the study the Gas Victim Card and the other exposure related documents of their parents were checked. Detailed family history and the clinical history, if any, were recorded to minimize other confounding factors. The study was approved by the Institutional Human Ethical Committee, Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal.

Setting up of lymphocyte cultures

Heparinized venous blood samples were collected from both the study subjects as well as the non-exposed controls after an informed consent. The chromosomal anomalies were assessed on the metaphase plates of 72 hour standard lymphocyte cultures [13]. The culture medium used was RPMI 1640 (Gibco) added with the fetal bovine serum supplement (Himedia) and antibiotics: penicillin streptomycin solution (Gibco). Phytohaemagglutinin (Gibco) was used as a mitogen and the lymphocyte cultures were carried out for 72 hours in order to determine the level of structural chromosomal aberrations. 0.025% of colcemid solution (Gibco) was added to the cultures two hoursprior to harvesting. Hypotonic shock was induced by 0.075 M potassium chloride solution and the cell 27 suspension was fixed in Cornoy's fixative (3:1 v/v of methanol and glacial acetic acid). Metaphase preparations were stained with 4% Giemsa. Hundred metaphase spreads per sample were observed and the chromosome aberrations were classified according to the International Nomenclature [14].

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.

3. Results and Discussion

Chromosomal aberrations were recorded at mitotic metaphases. Mean percentage of total abnormal metaphases (TAM) in the offspring group was observed to be 54.7±0.81 which was statistically higher than that of non-exposed controls (4.6±0.16) ($P<0.01$). The offspring group revealed statistically elevated chromosome-type (13.9±0.17) and chromatid-type aberrations (14.26±0.2) as compared to the non-exposed group (0.4±0.54 and 0) respectively. Table 1 shows the chromosome-type and chromatid-type aberrations that include dicentric chromosomes and double minutes (Figure 1), acentric fragments, chromatid breaks (Figure 2) and terminal deletions (Figure 3), which were found to be statistically higher in the offspring group ($p<0.01$) than that of the non-exposed controls. Besides, acrocentric associations (Figure 4) were found to be higher (13.7±4.89) in the offspring group than the controls (0.6±0.70). The higher incidence of chromosomal aberrations in the offspring group than the non-exposed controls is in agreement with the findings of Saxena et al. [2], Ghosh et al. [3] and Malla et al. [11]. But, they reported these abnormalities in the directly exposed individuals. As of now, no such study has been carried out in the offspring

of the exposed individuals. The present study is the first of its kind to unveil the genotoxicity in the offspring of the MIC exposed individuals.

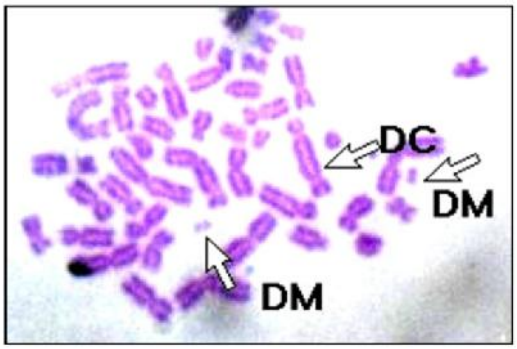

Figure 1: Metaphase of an offspring showing a dicentric chromosome and double minutes.

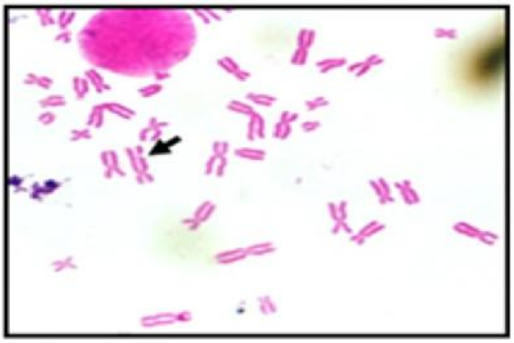

Figure 2: Metaphase of an offspring showing a chromatid break.

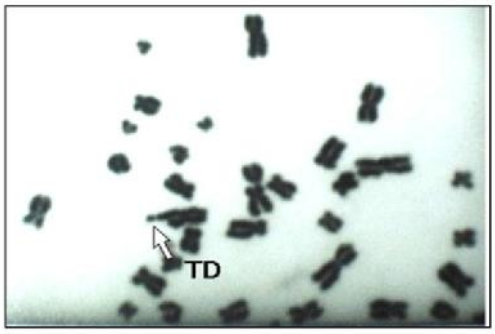

Figure 3: Metaphase of an offspring showing a terminal deletion of a chromosome.

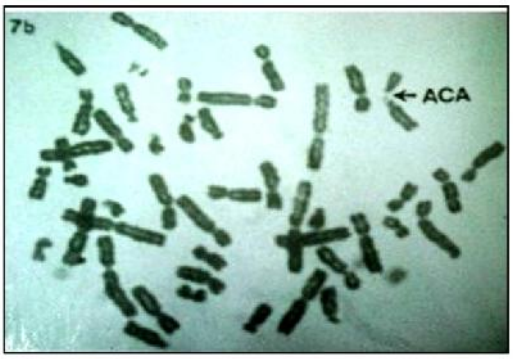

Figure 4: Metaphase of an offspring showing an acrocentric association of two acrcocentric chromosomes.

Both chromosome-type and chromatid-type aberrations were found to be statistically higher in the offspring group than the non-exposed control group. This can be attributed to the role of some chemicals in inducing DNA double-strand breaks. These breaks if not repaired, result into structural chromosomal aberrations in the individuals exposed to one or the other clastogen [15-19].

Methyl isocyanate is known to react with the exocyclic amino group of deoxycytidine, deoxyadenosine and deoxyguanosine to produce carbomoylated products [20]. Besides, Isocyanates may act as electrophilic agents and react with DNA to produce genetic damage [21]. As a carbomoylating agent, MIC might be expected to react with nucleophilic sites on cellular macromolecules, including proteins, RNA and DNA. There are three possible ways in which carbamoylation may modify the action of other factors. Firstly modification of primary DNA damage produced by other agents and transformation of DNA into a non-repairable state, for example, the simultaneous formation of methylated and carbamoylated base pairs. Secondly carbamoylation may inhibit repair enzymes resulting in decreased repair of the primary damage produced by other agents. The third way is related to the possible reaction of the isocyanates with histones leading to a loosening and dissociation of DNA-protein bonds. It is known that 1,3 bis-(2-chloroethyl)-1-nitrosourea (BCNU), which releases 2-chloroethyl-isocyanate upon decomposition, induces DNA damage through DNA aminoethylation [22].

Literature also suggests that DNA methylation is the most widely investigated mechanism of epigenetic alteration in DNA and chromatin, which occurs at the 5-carbon position of cytosine in Cp Gdinucleotides, and alteration to the chromatin packaging of DNA by post translational histone modifications [23]. Epigenetic modifications are stable in several conditions and may be inherited on to future generations or transgenerational [24]. Aberrations in DNA methylation manifests itself in two forms either through DNA hypomethylation or hypermethylation. DNA hypomethylation mediates chromosomal instability and also leads to activation of oncogene, both of these features are extensively associated with oncogenesis, and DNA hypermethylation is often related to inactivation of tumor suppressor gene during tumor initiation & progression [25]. Besides, histone modifications frequently contribute to disease development and progressions. Histone acetylation or deacetylation is one of the most common histone modifications that are involved in several diseases [26]. Aberrations in histone modifications can seriously affect gene regulation, which is a common phenomenon in disease that could potentially beheritable across generations [27]. Ample evidence suggests that MIC exposure has produced a long-term genotoxic effect in the Bhopal gas tragedy victims. Therefore, there is a possibility that this carbomoylated and methylated DNA may get inherited to the offspring and show up as chromosomal damage in the simulated lymphocyte cultures. Ample evidence shows that MIC exposure has produced long-term geneotoxic effects in

the exposed population of Bhopal. To address the long-term genotoxic effects among the survivors, Malla et al. [11] reported the frequency and pattern of chromosome instability through conventional chromosome aberration assay in the peripheral blood of exposed individuals. Their result collected during 2008- 2011 has indicated long-term genetic effects induced by MIC-exposure in 1984 in 100% of exposed cases studied. Senthilkumar et al.[28] reported micronuclei frequency in MIC exposed population as an indicator of cancer incidence.

Chromosomal aberration assay has been used to predict cancer risk in humans [29] and chromosomal aberrations have been considered as cytogenetic biomarkers as intermediate endpoints in carcinogenesis [30].

Higher percentage of chromosomal aberrations in the offspring group is therefore, a validation of the chromosomal instability in them. These chromosomal aberrations may act as the intermediate processes in the pathway of the progression of any genetic disorder like cancer. An increased risk of cancer in healthy individuals with high levels of chromosomal aberrations in peripheral blood lymphocytes has already been described in epidemiological studies carried out in Nordic countries and Italy by Bonassi et al. [31,32].

Table 1: Mean frequency of different types of chromosomal aberrations observed in the offspring group and the control group.

	Chromosome-type				**Acrocentric associations**		**Chromatid-type**		
Group	DC	MN	FR	Total	ACA	ICB	TD	ICD	Total
Offspring	3.3±4.08*	4.6±4.7*	6±.5.3*	13.9±0.17	13.7±4.89*	0.26±0.59*	7.5±7.06*	6.5±4.89*	14.26±0.2
Non-exposed	0	0	0.4±0.54	0.4±0.54	0.6±0.70	0	0	0	0

DC: Dicentric, MN: Minutes, FR: Fragments, ACA:Acrocentric Association, ICB: Intercalary Breaks, TD: Terminal Deletions, ICD: Intercalary deletion

*Statistically significant as compared to that of non-exposed group (P value<0.001).

4. Conclusion

The results of the present study suggest that Methyl isocyanate exposure at Bhopal has not only produced genotoxic effects in the directly exposed individuals but also their offspring. Extensive DNA damage studies and methylation studies of DNA repair genes in the population need to be carried out to confirm the long-term effects in the offspring. Besides, follow-up cytogenetic studies are needed in the exposed population and their offspring to identify individuals at risk. The study being the first of its kind lays the basis for further research in this area.

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