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Status of Inflammatory Biomarkers in the Population that Survived the Bhopal Gas Tragedy: A Study after Two Decades

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Abstract: Bhopal gas tragedy is considered as one of the world's worst industrial disaster. Approximately, 3,000–6,000 people died and 200,000 injured due to the leak of 40 tons of methyl isocyanate (MIC) gas from a pesticide plant. We aimed to decipher any persistent and subtle immunotoxic effects of MIC in the survivors of the tragedy. The study was divided into 3 groups i.e. group I (n=40); Age and gender matched non-exposed healthy controls recruited from places within the geographical region of Bhopal but from unaffected zones, group II (n=40); Age and gender matched non-exposed healthy controls recruited from places well outside geographical region of Bhopal and group III (n=40); Age and gender matched MIC exposed subjects from affected zones inside geographical region of Bhopal and the status of inflammatory biomarkers (IL-8, IL-1β, IL-6, TNF, IL-10, IL-12p70 cytokines and C-reactive protein) were analysed. The results displayed a significant increase in the levels of all circulating inflammatory biomarkers in the MIC exposed group in comparison to non-exposed cohorts. A toxin induced genetic and/or epigenetic alteration seems to be the likely underlying cause. However, further studies are essential for both mechanistic understanding and clinical implications of these patterns.

Key words: Methyl isocyanate, Bhopal gas tragedy, Immunotoxicity, Inflammation, Biomarkers

Introduction

Interactions between the environment and human health are highly complex and difficult to assess. Most importantly environmental alterations resulting from human activities are often imperceptible, but perilous. Bhopal disaster occurred on the night of December 2–3, 1984; due to the release of 30–40 tons of toxic Methyl Isocyanate (MIC) gas from a pesticide plant, represents world's worst chemical accident. The MIC exposure related death toll is estimated to be 3,000–6,000, with approximately 200,000 injured[1)]. The MIC exposed subjects continue to suffer from chronic ailments such as pulmonary fibrosis, bronchial asthma, chronic obstructive pulmonary disease (COPD), emphysema, recurrent chest infections, keratopathy and corneal opacities[1–4)]. The unprecedented mortality and morbidity observed due to the accident generated global scientific interest and in this regard spectrums of multi-systemic studies were conducted on exposed individuals. However, the possible detailed immunological implications of MIC exposure

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have not received much attention. In two preliminary studies, Deo *et al*. and Saxena *et al*. have reported a significant delay of cell cycle and a decreased response to mitogen activated stimulation of proliferative lymphocytes[5, 6]. In our previous findings we observed a hyper responsive immune state in the individuals exposed to MIC *in-utero*[7]. Moreover, our group has also shown previously that *in vitro* exposure to MIC induces hyper inflammation in different culture conditions[8, 9]. The present study, aimed to decipher any persistent and subtle immunotoxic effects of MIC in the survivors of Bhopal gas tragedy through estimation of inflammatory biomarkers. In this regard, the circulating levels of cytokines IL-8, IL-1β, IL-6, TNF, IL-10, IL-12p70 and C-reactive protein (CRP) were analysed.

Subjects and Methods

Study design

The study was approved from Institutional Review Board (IRB) of the Bhopal Memorial Hospital and Research Centre. Since, 24 yr have elapsed following the exposure, a very careful and deliberate attempt was made to identify and recruit subjects to ensure that they were indeed exposed to MIC. The study comprised of three groups with no history of smoking or any significant medical history like bronchial asthma or COPD and was of equal socio economic conditions. Group-I (n=40): Age and gender matched non-exposed healthy controls recruited from places within the geographical region of Bhopal but from unaffected zones (more than 25 km from the plant); Group-II (n=40): Age and gender matched non-exposed healthy controls recruited from places well outside geographical region of Bhopal (more than 200 km from the plant); Group-III (n=40): Age and gender matched exposed individuals of 25 to 50 yr from affected zones (within 2.5 km from the plant). The exposure of the subjects to MIC was ascertained on the basis of following criteria: a) Physical presence and address of stay on the night of MIC leak from the factory; b) Distance from the factory; c) Protective measures taken, at the time of gas leak, if any; d) Remained outdoor or indoor; e) If outdoor, what was the activity profile i.e. running or exertion or walking or driving etc.; f) The overall symptom profile like respiratory, cutaneous and ocular symptoms on exposure on that night to corroborate exposure. Blood was collected by the routine venipuncture method and informed consent was obtained from all the subjects. The blood thus obtained was centrifuged at 3,000 rpm for 10 min for the separation of plasma which was used for further investigations. Statistical analysis using student's *t*-test was employed for comparison of Group-I versus Group-II and Group-I versus Group-III individually and the p value ≤ 0.05 was considered to be significant.

Multiplex Cytometric Bead Array (CBA) assay for cytokine analysis

Estimation of different cytokine levels was performed using BD™ Multiplex CBA Human inflammation kit from BD™ Biosciences, San Diego, CA, USA. Plasma was subjected for measuring the levels of cytokines IL-8, IL-1β, IL-6, TNF, IL-10 and IL-12p70. The assay was performed by ensuing, all the necessary instructions from the manufacturer. Data acquisition and analysis were carried out on a flow cytometric platform using BD™ CBA software (BD™ Biosciences, San Jose, CA, USA).

ELISA for CRP levels

Levels of CRP were determined by Quantikine Human CRP Immunoassay kit from R & D Systems, Minneapolis, MN, USA and by following all manufacturers' instructions. The assay employs quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CRP has been pre-coated onto a micro-plate. Standards and samples were pipetted into the wells so that any CRP present in plasma will bound to the immobilized antibody. The optical density was measured at 450 nm by ELISA reader.

Results

Analysis of cytokine levels

A significant increase in the circulating cytokine levels was recorded in the exposed group in comparison to their respective controls. The exposed group (group III) showed a marked increase in the mean levels of IL-8, IL-1β, IL-6, TNF, IL-10, IL-12p70 in comparison to their respective levels in group I (control within Bhopal) and group II (control outside Bhopal). The mean levels of inflammatory cytokines IL-8, IL-1β, IL-6, TNF and IL-12p70 in MIC exposed group were 85.78 ± 28.9, 76.42 ± 10.48, 249.5 ± 48.5, 273.61 ± 62.48 and 115.2 ± 22.97 pg/ml respectively were significantly higher than the mean cytokine levels in both the control groups (i.e. 46.87 ± 5.42, 31.16 ± 3.78, 196.26 ± 31.72, 189.91 ± 32.46, 78.8 ± 12.9 pg/ml and in group I and 45.34 ± 3.81, 30.65 ± 2.89, 195.52 ± 43.14, 187.82 ± 38.85 and 79.23 ± 18.31 pg/ml respectively in group II). However, no notable difference was found in the values of anti-inflammatory cytokine IL-10 in all 3 groups (Fig. 1).

ELISA for CRP levels

CRP levels were significantly higher in the group III (MIC exposed group) as compared to group I (control group within Bhopal) and group II (control group outside

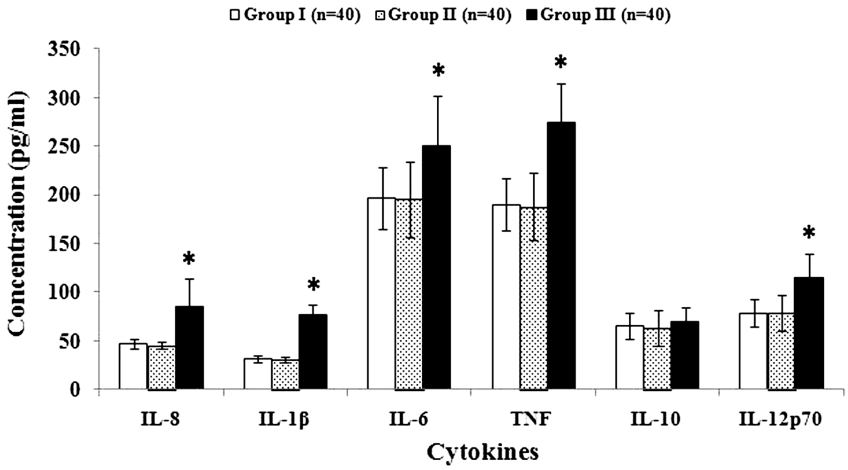

Fig. 1. Showing the circulating cytokine levels in Group I (unexposed individuals within Bhopal but more than 25 km from plant); Group II (unexposed individuals outside Bhopal more than 200 km from plant); Group III (exposed individuals within 2.5 km from plant). *$p \leq 0.05$.

Table 1. Circulating C-reactive protein levels in exposed and unexposed individuals

	Group I (n=40) (unexposed individuals within Bhopal)	Group II (n=40) (unexposed individuals outside Bhopal)	Group III (n=40) (exposed individuals within Bhopal)
Mean CRP levels (mg/ml)	0.29 ± 0.01	0.31 ± 0.03	1.07 ± 0.25*

*$p \leq 0.05$.

Bhopal). The mean level of CRP in the group III was 1.07 ± 0.25 mg/ml while in group I and group II the recorded mean levels were 0.29 ± 0.01 and 0.31 ± 0.03 mg/ml respectively (Table 1).

Discussion

Environmental chemicals are known to exert immunotoxic affects, resulting in suppression of immune responses, decreased resistance to infection and malignancies or induce hypersensitivity and auto-immunity[10]. Studies have shown that the recruitment of inflammatory cells following toxic exposure presents a risk of tissue damage through the release of toxic mediators, including proteolytic enzymes and reactive oxygen species[11]. Moreover, studies on inhalation toxicity and immune monitoring have shown that accidental or occupational exposure of certain chemicals such as organo-phosphorous pesticides, polycyclic aromatic hydrocarbons and diesel exhaust can exert pronounced effects on function and regulation of human immune system[12–14].

Application of immunological markers would be advantageous in understanding the mechanisms that underlie associations between environmental exposures and immune-mediated disorders. Detection of specific cytokines reveals the state of immune response at that given time (e.g. elevated TNF-α levels reveal a state of inflammation). The applications of these markers in adult clinical and epidemiological studies have been reviewed previously[15–17]. Moreover, a growing body of evidence has consistently shown a strong correlation of chronic subclinical inflammation with different diseases[18–23]. The present study reinforces the fact that the circulating cells of the immune system are sensitive to environmental contaminants, and effects are often manifested as changes in their activity including alteration in the inflammatory cytokines[24–27]. Recently, in our preceding study we had reported an enhanced production of inflammatory cytokines upon exposure of immune cells to isocyanates *in vitro*[28, 29]. In addition, *in-vitro* MIC exposure to lung fibroblast cells was also observed to induce inflammatory response which further leads to genomic instability[30]. In present study, we observed a significant increase in the circulating levels of inflammatory cytokines in group III (MIC exposed group) in comparison to the control group I (control group within Bhopal) and group II (control group outside Bhopal) (Fig. 1).

Another important inflammatory biomarker is CRP which is produced predominantly by liver during pro-inflammatory conditions[31, 32]. The MIC exposed group in our study reported substantially increased CRP levels in comparison with both controls. The mean CRP level

in the exposed group was 1.07 ± 0.25 mg/ml while in control group I and group II the mean levels were 0.29 ± 0.01 and 0.31 ± 0.03 mg/ml respectively (Table 1). Although, the present study reported a substantial increase in the levels of inflammatory biomarkers in the individuals exposed to MIC, whether this increases have any clinical implications will be clear by on-going follow up of the exposed individuals. A toxin induced genetic or epigenetic alteration at the level of immune stem cells can be speculated to be the likely underlying cause. However, further studies are essential for mechanistic understanding of these patterns and refinements in exposure ascertainment are needed to reinforce study design, to evaluate exposure-response relationships and study gene-environment interactions.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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