

"Genetics and Epigenetics of Lung Function among the victims of Bhopal Gas Disaster"

Aim:

To assess the effects of genetic and epigenetic variations on lung functions of the victims of Bhopal gas Tragedy.

Background:

The Bhopal Gas Tragedy is the world's worst industrial catastrophe which occurred in pesticide plant in Bhopal releasing 40 tons of toxic gas into the surrounding atmosphere leaving around half a million people to the toxic gases. This uniquely exposed population was ideal for genetic and epigenetic epidemiological studies because both the exposed and unexposed populations surviving at the same place would have provided an outstanding opportunity to understand the gene-environment interactions and their impact on lung function. Therefore, it was proposed to investigate the association of recently identified novel genome-wide association study genes related to lung function and COPD together with normal physiological range of lung function measures (like FEV1) among the exposed victims of Bhopal Gas Tragedy.

Objectives:

1. To identify and recruit victims of Bhopal Gas Tragedy and their unexposed sibs.
2. To collect the data related to lung function and risk factors for COPD together with peripheral blood samples for DNA extraction from the victims of tragedy and their unexposed sibs.
3. To find out the allele frequency of the selected candidate gene polymorphisms among the population of victims of Bhopal Gas Tragedy.
4. To detect the extent of genetic association between the selected polymorphisms and lung function among victims of Bhopal Gas Tragedy.
5. To estimate the effect size of associated polymorphisms on lung function among the victims.
6. To undertake a genome-wide appraisal of DNA methylation in a sub-set of highly exposed individuals (with and without poor lung function) and their matched unexposed sibs to identify novel differentially methylated sites.

Criteria for Sample Selection:

1. Subject recruitment from the exposed area.
2. Subject exposed during the tragedy.
3. Age between ≥ 30 to ≤ 70 years.
4. Both males and females.
5. Husband and wife can also be recruited (Non-blood relatives).
6. Sample size: 4500 unrelated exposed individuals (1500 each from severe, moderate and mild area)

Work Done:**Recruitment:**

We had prepared the job description and eligibility criteria for the advertisements of required project positions i.e. 3 Research Assistants and 1 Lab Technician. Over 100 applications were received which were screened on the basis of essential eligibility criteria and finally selected through a short entrance exam followed by an interview on 23rd November 2013 in NIREH, Bhopal. The recruitment process was completed by mid of December, 2013, after the joining of three research assistants and one technician. The responsibilities of the research assistant's were to visit the field site and identify the subjects, as per the list provided by NIREH, obtain the informed written consent for the collection of required information based on the questionnaire and measurements. The responsibilities of the technician was to draw the peripheral blood and further process it and finally store it at bio repository in NIREH.

Purchase of equipments: The purchase of the required equipments was completed by NIREH by April 2013 (as per the purchasing guidelines of General Financial Rules). The purchase of equipments by NIREH took five months that too with the help of four research assistants recruited in the project for preparing advertisements, waiting for the formulation of NIREH's purchase committee and final procurement (*see the list of the equipments given below*).

S. No.	Item	Quantity
1	Spirometers	2
2	Laptops (for analyzing spiograms)	2
3	Blood Pressure machines	2
4	Stadiometers	2
5	Measuring Tape	2
6	Weighing machines	2

Training of Project staff: In order to bring uniformity and to ensure the high standards in data quality, we organized 4 days extensive training program in NIREH, Bhopal from 5th to 8th May, 2012. The staff was trained to identify and recruit eligible participants, rapport establishment in the field, troubleshooting, etc. One day visit in the field area was also made to ensure that staff is working well in the field itself. The training of project staff focused on five areas

- a. Questionnaire
- b. Anthropometry
- c. Spirometry
- d. Blood pressure
- e. Blood sampling, bar-coding, systematic storage and aliquoting

Between 5th and 8th May the training session was conducted under the supervision of Dr Vipin Gupta and Dr Aastha Aggarwal:

- Day 1: lectures were conducted and questionnaire was discussed thoroughly in detail.
- Day 2: spirometers were installed and the PFT practice session was conducted on staff members of NIREH. Both pre & post spirometry tests were performed. Their anthropometry (height, weight and circumferences) and physiological (blood pressure) measurements were taken.
- Day 3: the project staff along with Dr Vipin Gupta and Dr Astha visited the field area to enrol the subjects simultaneously.
- Day 4: five subjects were recruited in the study and Questionnaires were filled on gas exposure, anthropological profile, socio-economic status, occupation history, physical activity, tobacco & alcohol use, environmental smoke exposure, indoor air pollution, disease history and dietary recall and health expenditure of the enrolled subjects. Spirometry was performed for all the subjects. Blood samples from the subjects were collected and brought to the lab for processing. After separation of plasma, the blood was stored at -20°C.

Post field work the Questionnaire was again discussed and relative queries were solved.

There was a gap in staff recruitment and their training because purchase of required equipments and consumables required for training and field work took almost **five months** (by NIREH). Meanwhile, project staff entered the data in NIREH's old data for preparing the list of potential participants. Our staff practiced spirometry in the PFT lab of NIREH, practiced questionnaire and spent time in finalizing the random list of eligible study participants.

Finalization of questionnaire, field protocol and bar-codes: The questionnaire for data collection was finalized and printed before conducting the training programme. Similarly, we prepared comprehensive field protocol for fieldworkers. Further, after feedbacks received during training, we have made the required changes in the field protocol and questionnaire based on the responses from the field-workers. Before the training programme we had generated site-specific bar-codes.

Pilot work: After completing pre-pilot work like recruitment of the project staff, purchase of the required equipments and consumables, we started the pilot work. A ten days pilot work was conducted (i.e. 7th to 17th May, 2014) to start the project and to find out the possible problems while conducting the fieldwork by randomly visiting households in severely affected areas, explaining about the study and inviting the participants to NIREH for data collection.

Sample collection: The ten days of pilot work was assessed by Prof. V. K. Vijayan and suggested to generate a statistically random list of participants from the NIREH's existing data-base. Finally, Dr. Sushil Kumar generated a statistically random list of 3000 participants (750 from each severe, moderate, mild and control areas) according to our study criteria. We resumed the fieldwork again from 27th May, 2014, using statistically generated random list of participants, started visiting the field area again, identifying the participants, explaining them about the study, inviting them to NIREH for data collection and finally recruiting them next day in NIREH after taking their consent. Since the participants come to NIREH for providing data and physical measures and spend almost 4 hours in the institute, we reimburse the participants for the loss of their daily wage and time spent as per the suggestions of ethical committee.

Between May 8th 2014 and June 6th 2014, in all 110 samples were collected from J.P Nagar, Locality 1 (Severe Area). After obtaining informed consent from the enrolled subjects, 14 ml of peripheral blood was drawn from the subject. The blood was processed in the laboratory and approx. 2 ml each of serum and plasma were separated. This aliquot of serum and plasma was then stored at -80°C freezer at NIREH, Bhopal. Blood sample collection was stopped w.e.f June 6th 2014 as per the instructions of ICMR, New Delhi.

Database and Data Dictionary: The excel based comprehensive database was prepared for simultaneous entry of the filled questionnaires. We have also prepared data dictionary for all the variables included in the questionnaire for future reference.

Purchase of consumables for DNA extraction: We purchased the required plastic-ware and chemicals required for DNA extraction which was planned to be performed in DNA Extraction Laboratory, to be established in NIREH, Bhopal (see the attached appendix).

Surprisingly, ICMR started reviewing the project again through epidemiological committee and finally terminated the project in November, 2014.

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