Evaluation of cytogenetic alterations of toxic gas exposed population of Bhopal having chronic kidney disease

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Abstract

The industrial disaster of Bhopal in 1984 resulted into widespread morbidity and mortality in the vicinity of the industry and required long term surveillance for chronic health effects in those affected by the leakage of gas. Although few cytogenetic studies were undertaken to assess genetic damage in survivors of the disaster, no studies are available on cytogenetic damage of toxic gas exposed population having chronic kidney disease (CKD).Thus, the present study aimed to evaluate cytogenetic alterations in chronic kidney disease patients who wereexposed to leaked gas and to compare it with those who were not exposed to the leaked gas. The cytogenetic alterations were evaluated through chromosomal aberration analysis and micronuclei assay. The study included 608 study participants divided into four groups on the basis of history of exposure to the leaked gas and presence or absence of CKD. The results of the study showed no statistically significant difference in cytogenetic damagebetween gas exposed and non-exposed patients of CKD. However, significantly higher cytogenetic damage was observed among gas exposed participants having CKD as compared to gas exposed participants free from CKD.Thus, to conclude though the cytogenetic alterations were observed in exposed group it cannot be solely attributed to the gas exposure and the role of other confounders must also be studied.

Introduction

Industrial disasters have widespread implication in terms of population affected, magnitude of morbidity and number of bodily systems affected. The story of 1984 Bhopal Gas disaster has been no different. It has affected all important body systems directly or indirectly probably kidneys also. As a result the need for the continuous observations for assessment of long term effects in toxic gas exposed individuals has been advocated (Mishra et al., 2009; Malla et al., 2010; Shrivastava, 2011; Samarth et al., 2013).

In the recent years there has been a rise in chronic kidney diseases and it has been estimated that by 2040it will be the 5th most common cause of death globally. Therefore, it is essential to enhance the awareness for the importance of preventive measures amongpopulation, professionals and policy makers (Li et al., 2020). In 2020, there were 697.5 million cases of chronic kidney disease worldwide having prevalence rate of 9.1% (Bikbov et al., 2020). The global all age prevalence has increased to 29.3% during 1990-2017 with stable age standardized prevalence, and 1.2 million deaths occurred due to CKD in 2017.The CKD is commonly connected with diabetes, hypertension, obesity, hyperlipidemia and non-alcoholic fatty liver disease (Hruska et al., 2010; Targher et al., 2011; Targher and Byrne, 2017). These factors not only act as initiator but also promoters for the kidney diseases (Levey and Coresh, 2012). However, Indian CKD Registry published in 2012 emphasized on etiology and demographics of the country (Rajapurkar et al., 2012).Though the elementary diagnostic parameters commonly used to detect CKD include measurement of serum creatinine, GFR and urinary albumin levels (Jha et al., 2013), an exploration of the role of epigenetic mechanisms for their translational efficacy as novel therapy for CKD was advocated (Reddy and Natarajan, 2015).

The genetic damage in CKD patients could be attributed to various factors such as increased uraemic toxins and endogenous genotoxic products including oxidative stress intermediaries that results in genomic instability (Luttropp et al., 2009; Schupp et al., 2010). Genetic makeup attribute to the damaged DNA repair mechanisms. Attention is being paid to oxidative DNA damage because of its complex genetic effects contributing to early aging, neurodegenerative disorders, diabetes, atherosclerosis, mutagenesis and carcinogenesis (Hagmar et al., 2004; Rangel-Lopez et al., 2013; Quintero Ojeda et al., 2018). Generally, in CKD patients, the genomic damage has been measured applying cytogenetic techniques through evaluation of sister chromatid exchange (SCE) rates and micronuclei (MN) frequency in peripheral blood lymphocytes (Konat, 2003; Roth et al., 2008; Karaman and Keskinler, 2009; Lialiariset al., 2010; Demircigil et al., 2011; Aykanat et al., 2011; Coll et al., 2013; Guido et al., 2016). Recently, Canadas-Garre et al., (2018) has summarized the most important human studies evaluating genomic biomarkers for CKD. Anderson et al., (2019) studied the possibility of association of CKD with genetic variation of chromosome Y.

Post disaster, the Indian Council of Medical Research made attempts to assess the severe effects of toxic gas on exposed population (Sriramachari, 2004; NCRP, 2005). However, low birth weight, genetic/developmental/growth disorders and respiratory ailments were thrust areas of research. Few cytogenetic studies were also undertaken to assess genetic damage in gas exposed population (Goswami et al., 1984; Goswami 1986; Goswami et al., 1990; Ghosh et al., 1990; Malla et al., 2011, Senthilkumar et al., 2013; Ganguly and Mandal, 2017, Ganguly et al., 2018, Ganguly, 2019, Ganguly et al., 2019). However, no studies are available on cytogenetic damage of toxic gas exposed population having CKD. Therefore, this study was undertaken to evaluate cytogenetic damage through chromosomal aberration analysis (CA) and MN assayamong gas exposed survivors in the presence or absence of chronic kidney disease

Materials And Methods

This cross-sectional multi-group study was undertaken at Bhopal. The study was approved by the Institutional EthicsCommittee (IEC) of ICMR-National Institute for Research in Environmental Health Bhopal and written informed consent was taken from each participant before initiating the study. Considering a male: female ratio of 70:30 among CKD patients and SCE and MN frequencies in control and CKD patients, at 0.05 level of significance and 80% power the sample size was calculated to be 175 subjects in each of the four groups. These four groups included those exposed to gas and having CKD (Group I); those not exposed to gas but having CKD (Group II); those exposed to gas and free from CKD (Group

III) and those not exposed to gas and also free from CKD (Group IV). Excluding the pregnant/lactating females, those with congenital anomalies and malignancies, all other diagnosed cases of CKD including those in hypertensive and diabetic patients aged 30 years or more andreporting at government and/or private tertiary care centres during the study period were included.

All the participants possessing the ICMR registration card issued for the purpose of long term surveillance studies were considered as gas exposed participants while those not possessing that card were considered as non-exposed. Thus, a total of 608 participants were recruited which comprises of 167 in Group I, 116 in Group II, 162 in Group III and 163 in Group IV. However, due to failure of culture in 26 participants, the final analysis included 582 participants which included 160 in Group I, 106 in Group II and 158 each in Group III and IV.

The standard methods wereadopted for preparation of chromosomal aberration analysis and micronucleus assay (Rooney and Czepulkowski, 1992; Fenech, 2000; Samarth et al., 2012; Samarth et al., 2018). For cytogenetic study,3 ml peripheral blood sample was collected in sterile sodium heparin vacutainer by venepuncture.Peripheral blood (0.5 ml) was added to 4ml RPMI 1640 medium supplemented with 20% fetal bovine serum and phytohaemagglutinin (PHA) and maintained at 37^{o}C for 72 hrs. For each individual, the cultures were set up in duplicates and in two separate sets. One set was usedfor chromosomal analysis while anotherset was used for MN assay.The values for cytogenetic parameters such as frequency of micronuclei (MN), nuclear division index (NDI), mitotic index (MI), frequency of dicentrics, rings, chromatid breaks and fragments were recorded.

The statistical analysis was done using statistical software SPSS 25.0. The frequencies of cytogenetic parameters were expressed as mean and standard error. For the purpose of analysis the study variables were age (arbitrarily dichotomized into <45 and ≥45), gender, history of exposure and presence or absence of CKD while the outcome variables were the cytogenetic parameters. The mean of cytogenetic parameters according to study variables was compared using student's t- test. The group-wise comparison of cytogenetic parameters was done by one way ANOVA followed by post-hoc LSD. The significance levels was set at $p<0.05$.

Results

The present study included 365 males (168 with CKD and 197 without CKD) and 217 females (98 with CKD and 119 without CKD) participants. Thus, the ratio of M: F for CKD was 63:37.

Table 1 depicts the mean values for cytogenetic parameters according to study variables. It can be observed that those aged ≥45 years were having significantly higher mean of micronuclei, chromatid breaks and fragments while significantly lower mean mitotic index that those aged <45 years. According to gender females had higher mean mitotic index, chromatid breaks and fragments as compared to males. Further, in those exposed to toxic gas and those having CKD had higher mean values of micronuclei, NDI, dicentrics, rings, chromatid breaks and fragments as compared to those not exposed to toxic gas and those free from CKD respectively.

Table 1
Distribution of cytogenetic parameters according to the study variables

Characteristics	N	MN	NDI	MI	Dicentrics	Rings	Chromatid breaks	Fragments
Age<45 years	221	3.28 ± 0.23	2.27 ± 0.08	5.28 ± 0.09	0.09 ± 0.02	0.04 ± 0.01	0.95 ± 0.09	1.50 ± 0.12
≥45 years	361	4.15 ± 0.19	2.31 ± 0.06	4.99 ± 0.07	0.13 ± 0.02	0.04 ± 0.01	1.43 ± 0.09	2.12 ± 0.09
F; p-value		8.28;0.004*	0.18;0.669	5.81;0.016*	1.94;0.164	0.22;0.639	12.69;0.000*	15.51;0.000*
Gender Male	365	3.68 ± 0.18	2.29 ± 0.06	4.99 ± 0.08	0.10 ± 0.02	0.04 ± 0.01	1.12 ± 0.08	1.77 ± 0.09
Female	217	4.05 ± 0.26	2.31 ± 0.07	5.29 ± 0.09	0.13 ± 0.02	0.14 ± 0.02	1.47 ± 0.12	2.08 ± 0.14
F; p-value		1.44;0.231	0.07;0.792	6.60;0.01*	1.80;0.18	0.002;0.961	6.79;0.009*	3.96;0.047*
Exposure Absent	264	3.18 ± 0.22	2.12 ± 0.07	5.33 ± 0.09	0.10 ± 0.02	0.02 ± 0.008	0.93 ± 0.09	1.49 ± 0.10
Present	318	4.35 ± 0.19	2.44 ± 0.06	4.92 ± 0.08	0.13 ± 0.02	0.06 ± 0.01	1.52 ± 0.09	2.21 ± 0.19
F; p-value		15.87;0.000*	11.28;0.001*	12.54;0.000*	0.73;0.394	6.19;0.013*	20.22;0.000*	22.38;0.000*
CKD Absent	316	1.40 ± 0.06	1.91 ± 0.06	5.34 ± 0.08	0.05 ± 0.01	0.02 ± 0.008	0.37 ± 0.03	0.71 ± 0.04
Present	266	6.69 ± 0.21	2.75 ± 0.07	4.82 ± 0.08	0.19 ± 0.03	0.06 ± 0.02	2.30 ± 0.11	3.28 ± 0.11
F; p-value		699.7;0.000*	90.3;0.000*	21.49;0.000*	24.59;0.000*	6.53;0.011*	336.74.22;0.000*	524.26;0.000*
*Significant								

Table 2 shows the distribution of cytogenetic parameters according to different groups.The one-way ANOVA showed that there was a declining trend in the mean values for all cytogenetic parameters according to the group. The post-hoc test revealed that there was a significant increase in frequencies of micronuclei (MN) and NDI values in Group I, Group II and Group III as compared to Group IV (p=0.000). When non-exposed CKD group (Group II) and exposed CKD group (Group I) were compared, there was no significant difference in values of MN frequency (p=0.229) and NDI (p=0.576). However, the values of MN frequencies and NDI were significantly higher in exposed CKD groups (Group-I) as compared to exposed non-CKD (Group III). Further significant changes in values of mitotic index (MI) in Group I, Group II and Group III was observed as compared to Group IV(p=0.000).

Table 2
Group-wise comparison of cytogenetic parameters

Groups	N	MN	NDI	MI	Dicentrics	Rings	Chromatid breaks	Fragments
Group 1	160	6.83 ± 0.26$^{*\#}$	2.78 ± 0.08$^{*\#}$	4.87 ± 0.11$^{\#}$	0.18 ± 0.03$^{*\#}$	0.08 ± 0.02$^{*\#}$	2.59 ± 0.13$^{\%*\#}$	3.58 ± 0.14$^{\%*\#}$
Group 2	106	6.47 ± 0.34$^{*\#}$	2.71 ± 0.11$^{*\#}$	4.74 ± 0.13$^{\#}$	0.20 ± 0.04$^{*\#}$	0.04 ± 0.02	1.87 ± 0.17$^{@*\#}$	2.83 ± 0.18$^{@*\#}$
Group 3	158	1.83 ± 0.08$^{@\%\#}$	2.09 ± 0.08$^{@\%\#}$	4.97 ± 0.10$^{\#}$	0.07 ± 0.02$^{@\%}$	0.04 ± 0.02$^{@}$	0.43 ± 0.05$^{@\%}$	0.83 ± 0.06$^{@\%}$
Group 4	158	0.97 ± 0.07$^{@\%*}$	1.73 ± 0.09$^{@\%*}$	5.72 ± 0.11$^{@\%*}$	0.04 ± 0.02$^{@\%}$	0.01 ± 0.006$^{@}$	0.30 ± 0.05$^{@\%}$	0.59 ± 0.06$^{@\%}$
F; p-value		241.11;0.000	33.7;0.000	15.90;0.000	8.48;0.000	3.91;0.009	123.67;0.000	188.53;0.000
$^{@}$Significant when compared with Group 1 *Significant when compared with Group 3								
$^{\%}$ Significant when compared with Group 2 $^{\#}$ Significant when compared with Group 4								

The frequency of dicentrics also showed a statisticallysignificant difference (p=0.000) in Group I, Group II as compared to Group IV while no significant difference was observed between Group III and Group IV (p=0.394). Further analysis showed highly significant (p=0.000) difference for values of dicentric frequencies in males of Group I and Group II as compared to Group IV. Though, a significant (p<0.05) difference was noticed for values of dicentric frequencies in females of Group I and Group II as compared to Group IV, no such significantdifference was

observed in Group III as compared to Group IV. The Group I participants had significantly higher mean number of rings as compared to Group III (p=0.049) and Group IV (p=0.001) participants. However, the difference was non-significant when compared with Group II (p=0.077).

Similarly, though the frequencies of chromatid breaks and fragments showed significant increase in Group I and Group II as compared to Group IV(p=0.000), no such significant difference was observed in Group III as compared to Group IV for the frequencies of chromatid breaks (p=0.367)and fragments (p=0.116).

No significant difference was observed for values of MI, dicentrics and rings of Group I (Exposed-CKD) as compared to Group II (Non exposed-CKD). However, a highly significant increase was observed for frequencies of chromatid breaks and fragments in Group I as compared to Group II(p=0.000). In case of comparison between Group I and Group III, no significant difference was noticed for MI but a significant increase was observed for frequencies of dicentrics (p=0.002),rings (P=0.049) chromatid breaks (p=0.000) and fragments (p=0.000).

Discussion

In the present study, no significant difference was observed in cytogenetic end points between exposed CKD and non-exposed CKD patients. A significant difference was noted between exposed CKD and exposed non-CKD patients in terms of MN frequency, NDI, chromatid breaks and fragments. Several studies have shown that chronic kidney failure patients had higher levels of genetic damage (Konat, 2003; Schupp et al., 2010; Ersson et al., 2011; Rangel-López et al.,2013; Rodríguez-Ribera et al.,2014). In the present study, the cytogenetic alterations observed may not be solely assigned to toxic gas exposure because the effects of confounding variables too contribute to the genetic damage. Over production of reactive oxygen species in CKD patients may leads to DNA damage. The imbalance between antioxidant defense mechanisms and excess production of oxidants is obviously augmented in CKD (Locatelli, 2002). The conditions of hypertension and dyslipidemia are also being augmented by CKD that in turn encourages progression of kidney failure (Schiffrin et al., 2007). It has been noticed that epigenetic alterations are linked with inflammation and cardiovascular ailment in CKD patients (Dwivedi et al., 2011). The increased angiotensin II levels found in CKD patients that enhance the premature ageing might directly impact the pathophysiology and therapeutics in CKD (Rodríguez-Ribera et al.,2014).Several enhanced pro-oxidant actions also lead to CKD and these are age, hypertension, inflammation and incompatibility of dialysis membranes (Del Vecchio et al.,2011). It has been noted that a variety of factors prejudice the formation of MN in cells of CKD like age, sex, genetic makeup, physical and chemical agents as well as habitual practice of chewing and/or smoking of tobacco and drinking of alcohol (Sabharwal et al., 2015). The conventional and molecular cytogenetic findings are too important in management of CKD possibly for reducing genomic instability (Khan et al., 2016).

It was observed that the advanced CKD patients showed more DNA damage, and such damage was increased after hemodialysis in Type 2 Diabetes mellitus (Palazzo et al., 2012; Mamur et al., 2016). An exposure of metals in CKD patients can leads to reductions in kidney functions (Orr and Bridges, 2017).Thus, the confounders can be a lifestyle, living environment, nutritional factors, drinking water and occupational exposure to other toxicants (Ganguly et al., 2018). Ipek et al., (2017) opined that alterations in NDI value aredirectly related to the proliferative ability of the cell. The urinary cell free mitochondrial DNA and nuclear DNA could be employed as prognostic biomarkers for kidney outcome in CKD (Chang et al., 2019). Coimbra et al., (2020) have made recent observation that CKD patients had increased levels of circulating cell free DNA as well as different types of DNA damage.

The exposed non-CKD Group when compared to non-exposed non-CKD Group, showed significant cytogenetic damage in terms of MN frequency and NDI. In Chromosomal assay, MI showed significant decrease but no significant difference was observed for dicentrics, rings, chromatid breaks and fragments. A few reports are available on the cytogenetic alterations of the individuals exposed to toxic gas (Goswami et al., 1984; Goswami, 1986; Goswami et al., 1990). The higher chromosomal damage was reported in toxic gas exposed women (Ghosh et al., 1990). The types of abnormalities recorded were chromosome breaks, gaps, dicentrics, rings, and triradial and quadriradial configurations. Malla et al., (2010) have observed that the mean percentage of acrocentric associations in the toxic gas exposed population was significantly higher as compared to the control. The persistence of genomic instability in terms of higher chromosomal aberrations and atypical lymphocytes was also noticed in toxic gas exposed population of Bhopal (Malla et al., 2011; Senthilkumar et al., 2013). A pilot follow up study after 30 years of the tragedy by Ganguly et al.,(2019) reported stable or clonal rearrangements even after 30 yearsin the increased SCE and decreased replicative index seen immediately after toxic gas exposure.It also demonstrated a correlation between age, exposure status and cytogenetic alterations in toxic gas exposed individuals.

The study has several limitations. Firstly the toxic gas exposure was subjectively done as there were not sufficient methods for exposure assessment 37 years back when the tragedy stuck. It was crude on the basis of number of mortality occurred at the time of the accident. Secondly, as the cases were recruited through hospital, the information on many confounders of such cytogenetic changes was limited and thus limits the generalization of the findings of the study. Thirdly sequential measurement of cytogenetic parameters along with environmental measurement of various toxic pollutants would have given a better causal-effect relationship of toxic gas exposure with cytogenetic changes.

Thus, to conclude, though the cytogenetic changes are reported similar to earlier studies, it cannot be solely attributed to the exposure to toxic gas as many confounding factors may also contribute genetic damage but such studies are significant for assessing the risk (Ganguly and Mandal, 2017; Ganguly et al., 2019). Further, because of the complex interactions between environment, disease susceptibility and genetic susceptibility, the exploration of epigenetic mechanisms to meet challenges of CKD through novel ideas of molecular mechanisms is warranted.

Declarations

Authors Contribution

Ravindra M Samarth has conceptualized, analyzed the data and participated in writing the manuscript and is corresponding author. Gopesh Modi, Kishor K Soni, Shariq Ul Hasan, M.L. Banjare and Sanjay Jain contributed for sample collection, data generation and participated in writing the article. Ravindra M Samarth and RR Tiwari have revised, reviewed and approved the final draft.

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Conflict of Interest

Authors declare that no financial or non-financial interests are directly or indirectly related to the work submitted for publication.

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