Original Article

Evaluation of Cytogenetic Alterations in Patients of Chronic Kidney Disease

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Abstract

Introduction: In recent years, there has been a rise in chronic kidney disease (CKD), and it has been estimated that by 2040, CKD will be the fifth most common cause of death globally. In addition to diabetes, hypertension, obesity, hyperlipidemia, and nonalcoholic fatty liver disease commonly associated with CKD, exposure to various toxins as a result of pollution or industrial disasters is also discussed as a cause for multi-organ pathology including kidneys. Although few cytogenetic studies were undertaken to assess the genetic damage in survivors of the disaster, no studies are available on the cytogenetic damage of toxic-gas exposed population having CKD. Therefore, the present multi-group cross-sectional study was undertaken to assess the independent role of CKD as well as toxic gas exposure on cytogenetics. **Methods:** 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. **Results:** The results of the study showed no statistically significant difference in cytogenetic damage between gas-exposed and non-exposed patients of CKD, whereas significantly higher cytogenetic damage was observed among gas-exposed participants having CKD compared to gas-exposed participants free from CKD, suggesting that cytogenetic changes could be due to CKD itself. **Conclusions:** Thus, to conclude, the cytogenetic alterations observed in the study can be partly attributed to the disease itself.

Keywords: *Chromosomal aberration, chronic kidney disease, genetic damage, micronuclei*

Introduction

In recent years, there has been a rise seen in chronic kidney disease (CKD), and it has been estimated that by 2040, it will be the fifth common cause of death globally. In 2020, there were 697.5 million cases of CKD worldwide with a prevalence rate of 9.1%.[1] The global all age prevalence increased to 29.3% during 1990–2017 with stable age-standardized prevalence, and 1.2 million deaths occurred due to CKD in 2017. Therefore, it is essential to enhance awareness about the importance of preventive measures among population, professionals, and policymakers.[2] The CKD is commonly connected with diabetes, hypertension, obesity, hyperlipidemia, and nonalcoholic fatty liver disease.[3,4] These factors not only act as initiators, but also promoters for kidney disease.[5] Exposure to various toxins as a result of either pollution or industrial disasters is also discussed as a cause for multi-organ pathology including kidneys. Several studies after the infamous 1984 Bhopal gas tragedy also highlighted the need for continuous observations for the assessment of long-term effects in all important organ systems of toxic gas-exposed individuals.[6-9]

In addition to the elementary diagnostic parameters to detect CKD, an exploration of the role of epigenetic mechanisms for their translational efficacy as novel therapy for CKD is also advocated.[10] Post disaster, though the Indian Council of Medical Research (ICMR) made attempts to assess severe effects of toxic gas on the exposed population,[11] no studies were undertaken on the cytogenetic damage of toxic gas-exposed population having CKD. Therefore, this is the first attempt to evaluate cytogenetic damage through chromosomal aberration (CA) analysis and micronuclei (MN) assay among CKD patients and survivors exposed to toxic gas.

Materials and Methods

This cross-sectional study was designed as a multi-group study to assess the independent role of CKD as well as toxic gas exposure on cytogenetic alterations. The study was approved by

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the Institutional Ethics Committee (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 sister chromatid exchange (SCE) and MN frequencies in control and CKD patients, at 0.05 level of significance and 80% power, the sample size was calculated as 175 subjects in each of the four groups that 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 pregnant/lactating females and those with congenital anomalies and malignancies, all other diagnosed cases of CKD including those with hypertension and diabetes, aged 30 years or more, and reporting at the government and or private tertiary care centers 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 nonexposed. Thus, a total of 608 participants were recruited, which comprised 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 groups III and IV.

The standard methods were adopted for preparation of CA analysis and MN assay.[12-14] For cytogenetic study, 3 ml peripheral blood sample was collected in sterile sodium heparin vacutainer by venipuncture. Peripheral blood (0.5 ml) was added to 4 ml Rosewell Park Memorial Institute (RPMI) 1640 medium supplemented with 20% fetal bovine serum and phytohemagglutinin (PHA) and maintained at 37°C for 72 h. For each individual, the cultures were set up in duplicates and in two separate sets. One set was used for chromosomal analysis, while another set was used for MN assay. The values for cytogenetic parameters, such as the frequency of MN, nuclear division index (NDI), mitotic index (MI), frequency of dicentrics, rings, chromatid breaks, and fragments, were recorded.

The severity of CKD was classified on the basis of estimated glomerular filtration rate (eGFR). The statistical analysis was done using the statistical software Statistical Package for the Social Sciences (SPSS) 25.0. The frequencies of cytogenetic parameters were expressed as mean and standard error. For the purpose of analysis, the study variables were arbitrarily dichotomized, that is, age (<45 and ≥45), gender, history of exposure present or absent, and CKD present or absent, while the outcome variables were cytogenetic parameters. The mean of cytogenetic parameters according to study variables was compared using Student's *t*-test. Group-wise comparison of cytogenetic parameters was done by one-way analysis of variance (ANOVA) followed by post hoc least significant difference (LSD). The significance level was set at $P < 0.05$.

Results

The present study included 365 male (168 with CKD and 197 without CKD) and 217 female (98 with CKD and 119 without CKD) participants. Table 1 shows 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 MN, chromatid breaks, and fragments and significantly lower mean MI than those aged <45 years. According to gender, females had higher mean MI, chromatid breaks, and fragments compared to males. Further, those exposed to toxic gas and those having CKD had higher mean values of MN, NDI, dicentrics, rings, chromatid breaks, and fragments compared to those not exposed to toxic gas and those free from CKD, respectively.

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 MN and NDI values in Group I, Group II, and Group III compared to Group IV. However, the increase was less in females of Group I and Group II. No significant difference was observed in males and females of Group III compared to those in Group IV, but significant increase was noted in total subjects (male and female combined) in Group III. When non-exposed CKD group (Group II) and exposed CKD group (Group I) were compared, there was no significant difference in the values of MN frequency and NDI. However, the values of MN frequencies and NDI were significantly higher in exposed CKD groups (Group I) compared to exposed non-CKD (Group III) group. But no significant difference in the values of NDI was observed in females of exposed CKD group (Group I) compared to exposed non-CKD group (Group III).

Further, significant changes ($P < 0.0001$) in the values of MI in Group I, Group II, and Group III were observed compared to Group IV. Similarly, significant ($P < 0.005$) difference was also noticed for the values of MI in females of Group I, Group II, and Group III compared to females of Group IV.

The frequency of dicentrics showed a highly significant difference ($P < 0.0001$) in Group I, Group II, and Group III compared to Group IV. Further, highly significant ($P < 0.005$) difference was also noticed for the values of dicentric frequencies in males of Group I and Group II compared to Group IV males. Though a significant ($P < 0.05$) difference was noticed for values of dicentric frequencies in females of Group I and Group II compared to Group IV females, no such significant difference was observed in Group III compared to Group IV.

Further, a significant difference ($P < 0.05$) was observed in the frequencies of rings in males and

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		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		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		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		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*

CKD=chronic kidney disease, MI=mitotic index, MN=micronuclei, NDI=nuclear division index

Table 2: Group-wise comparison of cytogenetic parameters

Groups	*n*	MN	NDI	MI	Dicentrics	Rings	Chromatid breaks	Fragments
Group I	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 II	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 III	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 IV	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		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

CKD=chronic kidney disease, MI=mitotic index, MN=micronuclei, NDI=nuclear division index. *Significant when compared with Group 3. @ Significant when compared with Group 1. #Significant when compared with Group 4. %Significant when compared with Group 2

females of Group I compared to males and females, respectively, of Group IV, and the difference was more pronounced ($P < 0.005$) when total subjects of Group I were compared with the total subjects of Group IV. However, there was no significant difference observed for values of frequencies of rings in Group II and Group III compared to Group IV.

Similarly, though the frequencies of chromatid breaks and fragments showed a significant increase ($P < 0.0001$) in Group I and Group II compared to Group IV, no such significant difference was observed in Group III compared to Group IV. No significant difference was observed for the values of MI, dicentrics, and rings of Group I compared to Group II. However, a highly significant ($P < 0.005$) increase was observed for the frequencies of chromatid breaks and fragments in Group I compared to Group II. On comparing Group I and Group III, no significant difference was noted for MI and rings, but a significant increase was observed for the frequencies of dicentrics ($P < 0.005$) and chromatid breaks and fragments ($P < 0.0001$).

Table 3 shows the cytogenetic parameters according to the severity of the CKD. It can be seen that a significant increasing trend was found for MN, chromatid breaks, and fragments. The post hoc analysis suggested that those having eGFR <15 had significantly higher mean MN compared to those having eGFR 15–29 ($P = 0.02$) and those having eGFR 60–89 ($P = 0.22$). Similarly, post hoc analysis of chromatids break showed that those having eGFR <15 had significantly higher chromatid breaks compared to those having eGFR 30–44 ($P = 0.014$) and those having eGFR 45–59 ($P = 0.047$). The post hoc analysis of fragments also showed that those having eGFR <15 had significantly higher fragments compared to those having eGFR 30–44 ($P = 0.018$) and those having eGFR 45–59 ($P = 0.041$). Though an increasing trend was observed for other parameters, it was statistically non-significant.

Discussion

In the present study, no significant difference was observed in the 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, suggesting that the cytogenetic alterations could be due to the disease itself. Several earlier studies have also shown that CKD patients had higher levels of genetic damage.[15-19]

Table 3: Distribution of cytogenetic parameters according to the severity of CKD

Groups	*n*	MN	NDI	MI	Dicentrics	Rings	Chromatid breaks	Fragments
Kidney failure (eGFR <15)	102	6.95±3.68	2.42±0.99	4.85±1.34	0.17±0.4	0.06±0.24	2.80±1.78	3.79±1.69
Severe decrease in GFR (eGFR: 15-29)	30	5.30±2.63	2.87±1.11	5.03±1.16	0.27±0.52	0.10±0.31	2.40±1.73	3.40±1.77
Moderate to severe decrease in GFR (eGFR: 30-44)	14	6.07±3.43	2.57±1.02	4.21±1.22	0.07±0.27	-	1.57±1.83	2.64±1.50
Mild to moderate decrease in GFR (eGFR: 45-59)	5	4.80±2.68	2.80±1.30	4.60±2.19	-	-	1.20±1.30	2.20±1.64
Mildly decreased GFR (eGFR: 60-89)	5	3.80±2.39	1.60±0.55	4.20±0.45	0.02±0.45	-	2.20±1.09	3.00±1.41
F, P		2.46, 0.04	2.21, 0.07	1.29, 0.28	0.81, 0.51	0.60, 0.66	2.49, 0.04	2.54, 0.04

CKD=chronic kidney disease, eGFR=estimated glomerular filtration rate, GFR=glomerular filtration rate, MI=mitotic index, MN=micronuclei, NDI=nuclear division index

Overproduction of reactive oxygen species in CKD patients may lead to DNA damage. The imbalance between antioxidant defense mechanisms and excess production of oxidants is obviously augmented in CKD.[20] The conditions of hypertension and dyslipidemia are also augmented by CKD that, in turn, encourages progression of kidney failure.[21] It has been noted that epigenetic alterations are linked with inflammation and cardiovascular ailment in CKD patients.[22] The increased angiotensin II levels found in CKD patients that enhance premature aging might directly impact the pathophysiology and therapeutics in CKD.[19] 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.[23] The conventional and molecular cytogenetic findings are too important in management of CKD, possibly for reducing genomic instability.[24]

It was observed that advanced CKD patients showed more DNA damage, and such damage was increased after hemodialysis in type 2 diabetes mellitus.[25,26] An exposure of metals in CKD patients can lead to reduction in kidney functions.[27] Thus, the confounders can be lifestyle, living environment, nutritional factors, drinking water, and occupational exposure to other toxicants.[28] Ipek *et al*.[29] had opined that alterations in NDI value are directly 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.[30] Coimbra *et al*.[31] have made a recent observation that CKD patients have 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. Higher chromosomal damage was reported in toxic gas-exposed women.[32] The types of abnormalities recorded were chromosome breaks, gaps, dicentrics, rings, and triradial and quadriradial configurations. Malla *et al*.[6] observed that the mean percentage of acrocentric associations in the toxic gas-exposed population was significantly higher compared to controls. The persistence of genomic instability in terms of higher CAs and atypical lymphocytes was also noticed in toxic gas-exposed population of Bhopal.[33,34] A pilot follow-up study conducted after 30 years of the tragedy reported stable or clonal rearrangements even after 30 years in increased SCE and decreased replicative index seen immediately after toxic gas exposure.[35] It also demonstrated a correlation between age, exposure status, and cytogenetic alterations in toxic gas-exposed individuals. However, the cytogenetic alterations observed may not be solely attributed to toxic gas exposure because the effects of confounding variables too contribute to the genetic damage.

Thus, to conclude, the cytogenetic changes reported here are similar to earlier studies, which can be partly attributed to the CKD itself and partly to the toxic exposure with confounding factors. Further, because of the complex interactions between environment, disease susceptibility, and genetic susceptibility, the exploration of epigenetic mechanisms to meet the challenges of CKD through novel ideas of molecular mechanisms is warranted.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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