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GENOTOXIC EFFECT OF ARSENIC ON CHROMOSOME STRUCTURE OF MICE

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ABSTRACT

Arsenic toxicity has become a global public health concern and has affected human health adversely in many different ways. Exposure to inorganic arsenic occurs via environmental and occupational exposures. The people residing in arsenic hit area were prone to different types of disease including skin disease, reproductive disease and cancer. Children of these areas have symptoms of severe arsenicosis and low memory status. The present study is designed to study the genotoxic effects of arsenic on chromosome structure of Swiss albino mice. The control group of 6 mice received distilled water as drinking water. The 'treatment' group received sodium arsenate 6 mg/kg body weight /day by Gavage method for 16 weeks. Mice were sacrificed and bone marrow was removed for chromosomal study. At twelve weeks administration of arsenic elongation of chromosomal arm, decrease in size of centromeres and Increase in thickness of chromosomal arm were observed. Elongated thin arm of chromosomes were observed. Less dense telomeres were also observed. At sixteen weeks administration of arsenic, serrated outer surface of chromosomes were observed. Centromeres were not distinct with clustered chromatid and fragmented arms of sub metacentric chromosomes were observed. It is concluded from the study that long term arsenic exposure causes chromosomal anomalies through heterogenous structure, elongation and finally fragmentation of chromosomes. The study on Swiss albino mice with arsenic did confirm the genotoxic effect of arsenic through chromosomal assay.

KEYWORDS: Centromere; Telomere; Metacentric; Heterogenous; Arsenicosis.

INTRODUCTION

Arsenic toxicity has become a global public health concern and has affected human health adversely in many different ways. Numerous epidemiological studies have reported that large populations in the world are being exposed chronically to arsenic with its ill effect.[1]

Exposure to inorganic arsenic occurs via environmental and occupational exposures. The toxicity of arsenic is very complicated and the toxicity varies on its oxidative state and solubility,[2] Two types of arsenic are present, trivalent and pentavalent. The trivalent compounds such as arsenic trioxide, sodium arsenite and arsenite, and arsenic trichloride are more toxic than the pentavalent compounds such as arsenic pentoxide, arsenic acid, lead and calcium arsenates.[3] The trivalent and pentavalent both forms of arsenic are found in arsenic-contaminated water.[4]

Arsenic is a pro-oxidant and thus may cause lipid peroxidation[5], protein and enzyme oxidation and glutathione (GSH) depletion, DNA oxidation and DNA adducts.[6] Arsenic generates reactive oxygen species like nitric oxide; which are known to induce poly ADP-ribosylation which is implicated in DNA repair, signal transduction and apoptosis. As a result, arsenite may induce DNA strand-breaks and Nicotinamide adenine diphosphate (NAD) depletion.[7] Hence, the genotoxic effects of arsenic compounds may be connected with an inhibition of DNA repair or the induction of oxidative stress.[8]

Metabolic methylation of inorganic arsenic to Di-Methyl Arsenate is involved in induction of DNA damage and DNA single-strand breaks resulting from the inhibition of repair polymerization[9, 10] and hence is a genotoxic-enhancing process.

It is thus likely that arsenic-mediated DNA–protein interactions may play a major role in arsenic carcinogenesis and the induced protein associated DNA-strand breaks could provide an explanation for chromosome aberration.[11] Cytotoxicity, morphological neoplastic transformation and cellular uptake were determined, to compare the effect between trivalent and pentavalent arsenic.[12,13]

The present study is designed to study the genotoxic effect of arsenic on chromosome structure of Swiss albino mice.

MATERIALS AND METHODS

Animals

The mice were reared in our animal house. The age group of mice selected for the study was 12 weeks old with 30±2 gm. body weight.

Chemicals

Sodium arsenate manufactured by Sigma was utilized for the experiment. Sodium arsenate was administered 6 mg/kg. body weight/day by Gavage method for 16 weeks.

Study groups & sampling

The control group of 6 mice received distilled water as drinking water. The 'treatment' groups (n=6) received sodium arsenate 6 mg/kg body weight/day by Gavage method for 16 weeks.

Chromosomal assay

The animals were induced with yeast extract a day before sacrifice and injected with colchicines 2 hours before the sacrifice. After the sacrifice femur bone was removed for bone marrow collection. The bone marrow was washed with 0.075 M KCl and collected in centrifuge tube. It was aspirated to make it homogeneous. This homogeneous solution was incubated in incubator at 37ºc for 15 minutes and then centrifuge at 2500 RPM for 15 minutes. Supernatant fluid was removed without disturbing the pallet. This procedure was repeated three times. In final step, cells were suspended into 1 ml carnoy's fixative. Three to four drops were taken on a clean slide and stained with 2% Giemsa stain for 10 minutes and air dried. This slide then taken for observation for chromosomal analysis.

RESULTS

In control group of mice well arranged 20 pairs of chromosome were observed. Centromere and both p and q arm of chromosome is normal in structure (Figure: I). At 12 weeks administration of arsenic, elongation of chromosomal arm, decrease in size of centromeres and Increase in thickness of chromosomal arm and many more anomalies were observed. Elongated thin arm of chromosome were observed (Figure: II). Less dense telomeres were also observed (Figure: III). At 16 weeks administration of arsenic, chromosomal fragmentations and serrated outer surface of chromosomes were evident (Figure: IV). Centromeres were not distinct. Clustered chromatid and fragmented arms of sub metacentric chromosomes were observed (Figure: V).

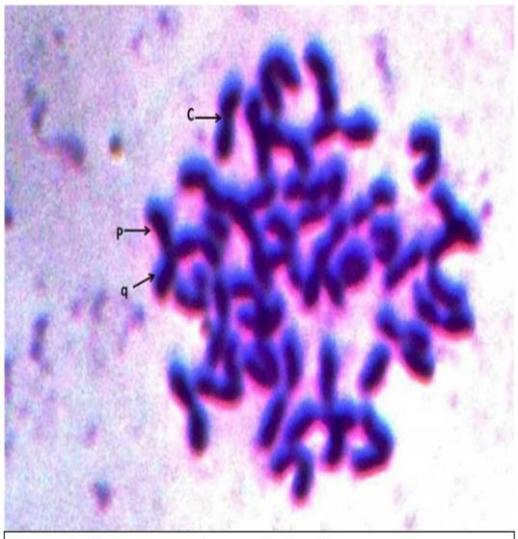

Figure-1: Chromosome of control mice. Show normal centromere (C), p and q are normal chromosome arms.

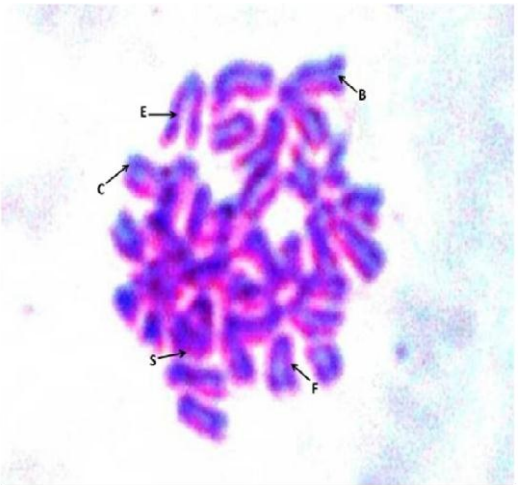

Figure-2: Chromosome of Arsenic 12 weeks administered mice show serated (S) and elongated (E) chromosome. Fragmentation (F) of chromosome was also evident.

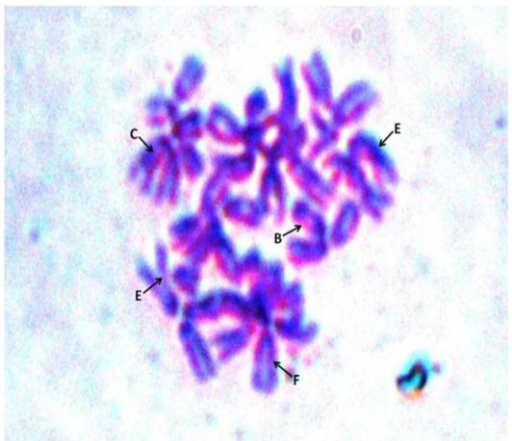

Figure-3: Chromosome of Arsenic 12 weeks administered mice show bulging (B), fragmentation (F) and elongation (E) in many chromosomes

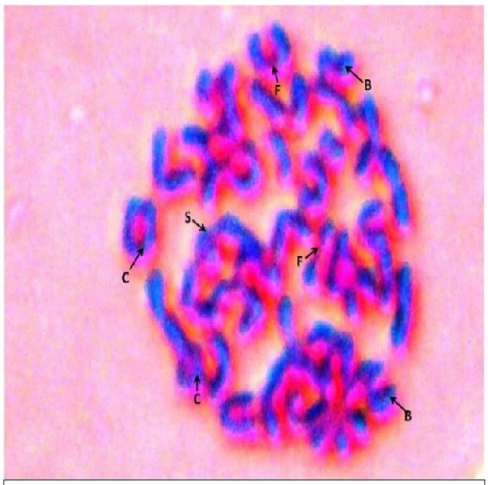

Figure-4: Chromosome of Arsenic 16 weeks administered mice show bulging (B) of chromosome membrane frequently. Complete fragmentation (F) was observed. Serrated (S) and irregular shaped chromosomal membrane was observed.

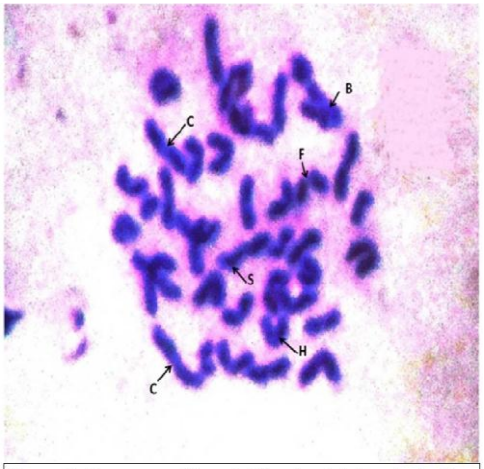

Figure- 5: Chromosome of Arsenic 16 weeks administered mice show heterogeneous (H) chromosome. Fragmentation (F) was evident. Bulging (B) of chromosomal membrane were more prominent.

Figure Legend

Figure - I: Chromosome of control mice. Show normal centromere (C), p and q are normal chromosome arms.

Figure - II: Chromosome of Arsenic 12 weeks administered mice show serated (S) and elongated (E) chromosome. Fragmentation (F) of chromosome was also evident.

Figure - III: Chromosome of Arsenic 12 weeks administered mice show bulging (B), fragmentation (F) and elongation (E) in many chromosomes.

Figure - IV: Chromosome of Arsenic 16 weeks administered mice show bulging (B) of chromosome membrane frequently. Complete fragmentation (F) was observed. Serrated (S) and irregular shaped chromosomal membrane was observed.

Figure - V: Chromosome of Arsenic 16 weeks administered mice show heterogeneous (H) chromosome. Fragmentation (F) was evident. Bulging (B) of chromosomal membrane were more prominent.

DISCUSSION

Arsenic is a well known human toxicant and carcinogenic metalloid. Exposure to arsenic and its compounds can have adverse effects on human health. Epidemiological studies based on ingestion of arsenic have been implicated in non-carcinogenic health effects in various organs and systems including cardiovascular, dermal, reproductive, neurological, respiratory, hepatic, hematological, renal, and gastrointestinal.[14]

Large numbers of in vitro and in vivo studies have been devoted to determine the genotoxicity of inorganic arsenicals.[15,16] In present study, we observed serrated chromosome arm with degeneration in centromere. Fragmented chromosomal arms with little dense telomere were also observed indicating early loss of the genes residing at telomere end and responsible for cellular stress including ageing. Serration of chromosomal surface indicates excessive heterochromatization leading to none functioning of several active genes, which adversely affects normal gene regulation in mice.

In vitro studies on human fibroblasts, leukocytes, lymphocytes and hamster embryo cells have shown that arsenic induces chromosomal aberrations and sister chromatid exchange.[17] Similar studies using human, mouse and hamster cells explored a potential enhancement of DNA damage, DNA repair enhancement or the inhibition of DNA synthesis.

Studies of humans have detected a higher than average incidence of chromosomal aberrations in peripheral lymphocytes, after both inhalation exposure[18] and oral exposure.[19] We also observed chromosomal aberrations in arsenic exposed group of mice. Genotoxic effect increases with increased duration of arsenic exposure.

CONCLUSION

It is concluded from our study that long term arsenic exposure causes chromosomal anomalies through serration of membrane, heterogenous structure, elongation and finally fragmentation of chromosome. The study on Swiss albino mice administered with arsenic did confirm the genotoxic effect of arsenic through chromosomal assay.

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