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Research Article

DNA Damage and Chromosomal Aberrations Assessment in Trace Mineral Deficient Patients

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ABSTRACT Minerals play vital roles in human health. They are important in genome stability. Deficiency of macro and micro minerals can lead to high oxidative stress which affects the genomic DNA. This study was aimed to determine the genotoxicity status in mineral deficient patients. A total of 200 blood samples were taken from the confirmed mineral deficient individuals and free of any kind of other diseases. Negative control group included healthy individuals that were not having any kind of deficiency and disease with no smoking habits and drug addiction. Individuals with any kind of disease, smoking habits, or drug addiction were excluded from the study. To evaluate genotoxic effects in mineral-deficient group, Comet assay, micronucleus and chromosomal aberration assay were performed. Among deficient minerals, sodium was found to be highest deficient mineral while phosphorus was found least deficient. Comparison of genotoxicity revealed a significant difference among all performed tests. DNA damage was observed showed highest in the mineral deficient individuals. Negative control individuals were having no significant genotoxic effects. Results of chromosomal aberrations appeared in three distinctive shapes i.e. V-shaped, Breakage or Circular. It was concluded that mineral deficient were having genotoxic effects which can lead to the cancer.

KEYWORDS DNA damage, Oxidative stress, Minerals deficiency, Chromosomal Aberration, Micronucleus

Introduction

Minerals are essential inorganic nutrients needed for enzymatic action in every living cell. Minerals assist in routine body functions such as producing energy, growing, and healing. They also function as coenzymes and participate in all enzyme reactions in the body (Tolonen, 1996). Epigenetically active enzymes require minerals as a co-factor for their proper functioning (Bell and Saffery, 2012). Minerals have some direct and indirect effects on the methylation of DNA and modification of histones. Balanced human diet is main source of both micro- and macro-nutrients to body (Ghazanfar *et al*, 2017). Imbalanced diet could result in deficiency of these minerals which can result in many disorders including anemia, cardiovascular diseases, and goiter. The deficiency of minerals causes severe diseases that lead to DNA damage.

Low concentration of minerals in humans may impair a function dependent on required mineral. Oxidative stress caused by mineral deficiency can lead to DNA damage (Ghazanfar *et al*, 2017). Increased DNA damage eventually diminishes cellular health, increases risk of degenerative diseases, and accelerates aging (Fenech *et al*, 2023). Patients with co-morbidities such as Thalassemia patients are more likely at the risk of increased oxidative stress. The patients in thalassemia face the deficiency of minerals such as calcium, magnesium (Arcasoy and Cavdar, 1975). Mineral imbalance also affects the genome at the embryonic development (Skjærven *et al*, 2016). Such chronic diseases can be overcome by countering the insufficiency of minerals by diet. The reduction of such minerals in the body also results in the production of carcinogens which leads to damage, blockage, alteration in DNA synthesis and DNA repair (Woodson, 2005). DNA methylation needs nutrients such as magnesium, zinc (Ames, 2001; Jackson and Bartek, 2009). For humans, calcium, magnesium, iron, manganese, copper, and iodine are considered as important and essential minerals. Deficiency of such minerals could lead to serious DNA damage. So current study was designed to evaluate the actual status of DNA damage in mineral deficient patients.

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Materials and Methods

Total of 200 blood samples were collected from various Government teaching hospitals after taking consent from the studied patients having confirmed mineral deficiency and from any kind of infectious disease. The questionnaire was filled by the patients about their complete history. Mineral deficiency was confirmed by their blood reports. Patients having the any kind of disease, smoking habits, drug abuse and under any kind of treatment were excluded from the study. Healthy humans (n=20) free from any kind of disease and mineral deficiency were taken as negative control. Confirmed blood samples of patients those fulfilled the criteria of study was further processed for the genotoxic assessment.

Alkaline comet assay

Alkaline comet assay was performed as described by the Kaygisiz and Cigerci (2017). Briefly lymphocytes were extracted through Histopaque (Sigma Aldrich) as per manufacturer protocol. Labeled pre-coated normal melting agarose slides were prepared. Central layer was extracted and 10X PBS was added. After centrifugation low melting agarose was added in pellet. This mixture was spread on pre coated slides. Slides were covered and left in ice for 5 minutes. Slides were kept in lysis buffer for an hour. Electrophoresis was performed for 40 min at 110 volt and 20 mV. Slides were stained with 50 µl ethidium bromide and slides were observed under the fluorescent microscope. Extent of damage was noticed according to the length of tail of cells (Ali *et al*, 2024).

Micronucleus Test

Lymphocytes (500 µl) were taken by using Histopaque and mixed with 1% KCl solution. These were kept for 5 min and then centrifuge at 1500 rpm for 5 min. Pellet was taken and added 1ml fixative 1 buffer, centrifuged it at 1500 rpm for 6 min. Then, supernatant was discarded and 1 ml 1ml fixative 2 solution was added. This was again centrifuged at1500 rpm for 6 min. Again, pellet was taken and spread it on the wet slides. Slides were allowed to dry for 24 h and stained with Giemsa (10ml Giemsa + 9ml distilled water) for 12 min. Slides were washed carefully and observed under light microscope after drying (Cigerci *et al*, 2022).

Chromosomal aberration test

Remaining lymphocytes were taken and mixed with 5ml solution of KCl (1 %). The cells were incubated at 37°C for 5 min and gently resuspended cells were centrifuged at 1500 rpm. Supernatant was discarded and resuspended pellet in 0.5ml supernatant. 5ml Carnoy's fixative was added in the pellet. This was centrifuged at 1500 rpm for 5min and supernatant was discarded. This step was performed three times. Pellet was mixed and spread over the wet slides. Slides were dried for 2-3 hours and then stained with Giemsa. Slides were observed under the light microscope after drying for 2-3 hours to observe the chromosomal aberration.

Results

Among 200 included patients, 56.25% were men and 43.75% were women. Mostly patients were deficient of sodium, calcium, chloride, potassium, and phosphorus. Highest deficiency was of sodium followed by calcium (Fig.1). While Phosphorus deficiency was found to be lowest among the patients.

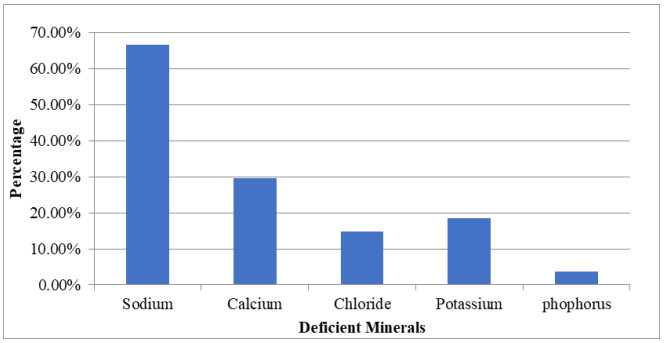

Fig1: Percentage of deficient minerals in patients. Blood samples were analyzed using Blood analyzer. Automated reports were recorded for the mineral deficiency analysis.

Mineral deficient patients showed the higher amount of DNA damage, chromosomal aberrations and micronuclei formation compared to control group. Control group had no significant number of genotoxic effects (Fig. 2). Among these genotoxicity markers, micronuclei formation was least followed by chromosomal aberration and DNA damage. Comet Assay test showed that DNA damage can be assessed in samples.

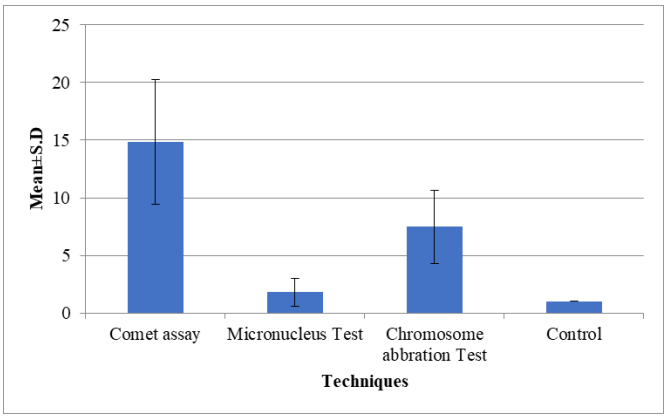

Fig. 2: DNA damage in cells through comet assay, micronucleus test and chromosomal aberrations. Values are represented in Mean and Standard Deviation (S.D.)

Chromosomal aberrations appeared in three distinctive shapes (Fig. 3). DNA breakage or fragmentation was observed in majority of the results followed by V-shaped chromosome. V shape appeared as half of the X shaped chromosome, which was indicative of half separation of chromosome from the centromere and appeared as V shape. Circular shape of chromosomes was also observed. Circular shape of chromosomes was due to the breakage of portion of a chromosome and its reattachment in circular shape.

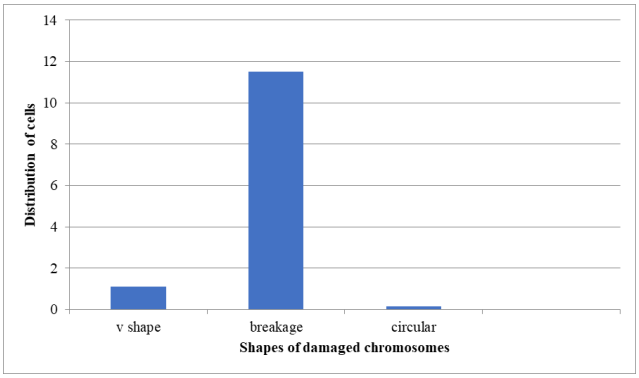

Fig 3: Number of shapes of damaged chromosomes.

DNA damage was found frequently among all the results. Therefore, Comet assay results were further explored under the florescent and compound microscopes. These results revealed tail shaped DNA damaged cells and micronucleus formation in the cells (Fig. 4 a b).

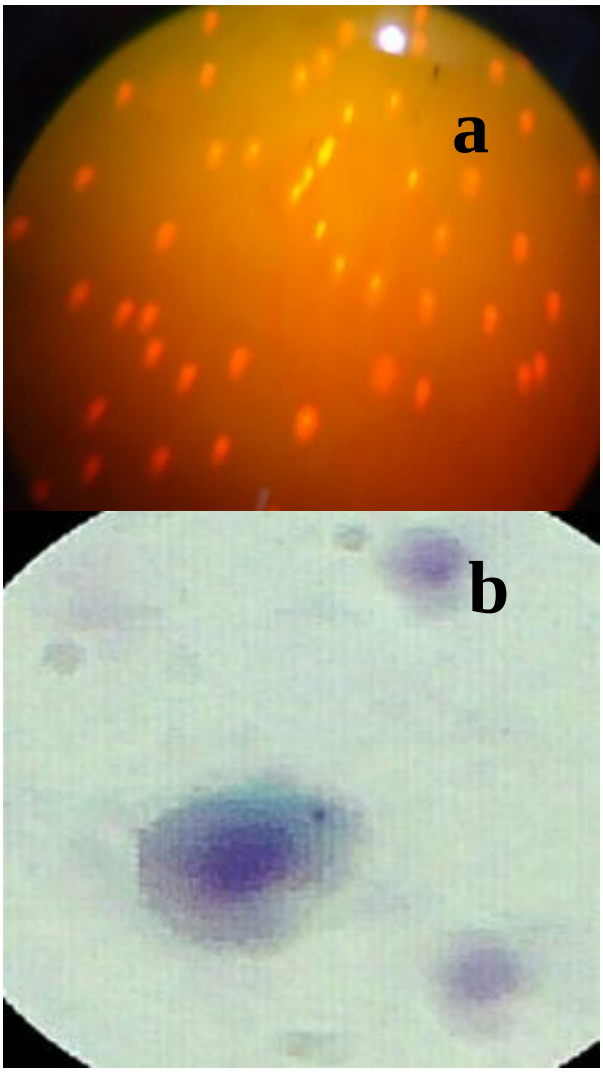

Fig4: Comet Assay results under microscopes. a. Tail shaped DNA Damaged cells under florescent microscope. **b.** Micronucleus under light microscope.

Discussion

DNA damage can be caused by poor intake of minerals. Determining the intake levels of minerals is very important to maintain the genome stability. It is essential for the prevention of many diseases such as cancer, caused by genome damage. A diet rich in essential minerals has been associated with low risk of cancer (Ballmaier and Epe, 1995; Bell and Saffery, 2012). In current study, adverse genotoxic effects have been observed in mineral deficient patients.

Minerals can block or induce those enzymes that are involved in activation or deactivation of carcinogenic and those substances which can cause DNA damage (Strickland and Groopman, 1995). Deficient intake of nutrients which are involved in DNA synthesis, repair, or methylation can affect mutation rate or changes in gene expression (Bloomfield, 1997; Collins, 2004). It also influences the cell cycle and replicatory rate (Key *et al*, 2004; Jackson and Bartek, 2009). This deficiency can also cause single and double strand breaks in DNA. The imbalance between oxidants and antioxidants can cause DNA damage and cancer (Akbari *et al*, 2008). Minerals can catalyze high number of free radicals from reactive species by using reactions and maintain the integrity of DNA (Pegg, 1990).

High deficiency of minerals could be led to high amount of damage of DNA. In previous studies, Calcium have been linked with reduced cancer risk. Because calcium binds with fat molecules in intestine that further reduces fat metabolism by bile salts and subsequent formation of carcinogen breakdown products. Calcium intake leads to lower the frequency of MN. Sodium is also required for the stability of negative charge in nucleic acid which led to genomic stability (Ballmaier and Epe, 1995; Bell and Saffery, 2012). Sodium ions neutralize the phosphate charges that cause electrostatic free energy release and strong electrostatic interactions that also stabilize DNA. Phosphorus is a basic structural component of DNA, RNA, and the genetic code which shows the importance of phosphorus in cellular growth and development (Ballmaier and Epe, 1995; Bell and Saffery, 2012). Phosphate diesters are adapted to link two nucleotides and ionize them making them more stabilized to carry genetic information. Both genetic molecules have a sugar phosphate backbone that maintains double helix (Ghazanfar *et al*, 2017). Therefore, depletion of phosphorus results in the destabilization of DNA and less bonding and ionization of nucleotides in this way they fail to carry genetic information that results in genotoxicity and causes mutations. Reduction of potassium causes DNA strand breaks in humans (Ballmaier and Epe, 1995; Ladeira *et al*, 2017). It does not cause any extensive damage to DNA alone but to some extent. DNA damage was mainly found due to base pair alteration.

In conclusion, DNA damage can increase if deficiency of minerals persists from long time. Short time deficiency shows less amount of DNA damage

Declaration of Competing Interest

The authors declare that they have no competing or conflict of interests.

Author Contributions:

Komal Ashraf: Conceptualization, Methodology, Formal analysis, Writing—original draft preparation. Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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