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Chemical Mutagenesis and Cytogenetic Chromosomal Abnormalities in a Population Living in the Aral Sea region

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Abstract

AIM: The article presents the results of a study of chromosomal mutations in residents living in the Aral Sea disaster zone, using the example of the city of Aralsk.

METHODS: The article identifies the level of chromosomal aberrations (CA) in the surveyed population and identifies the leading type of aberrations in this region.

RESULTS: Researches have shown that the main types of structural changes were chromatid breaks and single fragments of chromosomes. The results showed that in the study population, the microelement status indicates an imbalance of microelements. A correlation analysis showed a relationship between the nickel content in the blood and the increase in CAs. Furthermore, researches show a hypothesis about the pathogenesis mechanism of the formation of CAs.

CONCLUSION: Thus, the article provides information on chromosomal mutations during chemical mutagenesis.

Introduction

Environmental pollution and the consequences of its impact on public health are one of the most pressing issues for the world community. In many ecologically unfavorable regions, a difficult situation persists for a long time: air pollution, soil salinization, pollution of drinking water, which negatively affects the health of the population at various levels (molecular, cellular, organ, and systemic). Environmental pollution has a negative impact on the health status of the population [1], [2], [3], [4], [5].

The negative impact of adverse environmental factors on the health of the population has become special significance both for the whole world and for Kazakhstan.

Aral Sea shallowing and partial drying affects the interests of all countries in Central Asia and is a matter of concern on the part of the world community. This situation has been noted as an Aral Sea disaster and has led to significant economic damage and to harmful effects on human habitat.

Various researches show that pollution of the area near the Aral Sea by heavy metals affects the development of diseases of various organs and systems [6], [7], [8], [9], [10], [11], [12].

This environmental situation has a negative effect on public health, including genotoxic ones, and can manifest itself as chromosomal abnormalities such as an increase in size, a change in shape, and chromosomal aberration (CA) [13], [14].

One of the problems of hygienic significance is the problem of the genetic consequences of their effects, which are manifested at the chromosomal level and underlying the malignant transformation of cells, the increase in cases of the disease, and decrease in the body's resistance to environmental factors [15], [16].

Numerous epidemiological, laboratory, and clinical observations indicate for the presence of cause-effect relationships between environmental pollution and damage to the genetic material of the human body. These examinations were carried out both in harmful industrial conditions and in living areas, where the atmosphere, water, and soil were contaminated with mutagens [17], [18]. Assessment of the effects of mutagens on humans

in real conditions (with prolonged exposure of mutagens) is carried out mainly by cytogenetic examination of people exposed to the harmful factors.

Aralsk city of the Kyzylorda region (Population – 33,141 people) located in Southern Kazakhstan, in the zone of ecological disaster at a distance of 17 km from the Aral Sea [19]. In the south of the city is the dried-up Gulf of the Aral Sea – Bolshoy Saryshyganak. The climate is continental, arid, with large fluctuations in seasonal and daily temperatures.

The studies conducted by the "Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring" showed a high level of chemical load on the population in the conditions of the city of Aralsk [20]. The Aralsk ambient air is polluted with salts of heavy metals (nickel, manganese, lead, cuprum, zinc, iron, and silicon) that exceed the permissible level [21], [22]. In a control area – Atasu city, there is no excess of the above pollutants detected [23].

The aim of cytogenetic researches is to estimate the frequency and qualitative spectrum of chromosomal deviations in peripheral blood lymphocytes of people in reproductive age living in the territory of the Aral Sea ecological disaster zone.

Materials and Methods

Research design

Prospective medical case-control study was conducted in Aralsk city (46'48''00'' N 61'40''00'' E) and in Atasu city (48'41''00'' N 71'39''00'' E), Kazakhstan.

Conditions and sampling technique

The level of CAs and the level of microelements in the blood were considered as the main evaluated result.

We analyzed 7465 metaphase plates in 40 healthy people of reproductive age (18–45 years old) in the main group of examined persons who have been living in the ecological disaster zone (Aralsk) since they were born, which were not affected by harmful factors at the workplace.

For comparison purposes, 7020 metaphases were analyzed in 40 individuals living in the city of Atasu (control group). People living in Atasu formed a control group as they lived far from the disaster zone, but the climatic, geographical, and socioeconomic conditions were identical to compared groups.

All subject patients were divided into two groups based on matching parameters such as gender, age, duration of stay, social status, education, profession, and living conditions. The difference between the groups was in living in different ecological regions.

Genotoxic effects were studied using a modified method of peripheral blood lymphocytes cultivation by Hungerford, with the purpose of accounting frequency and types of CAs [24], [25], [26]. The main stages of preparation of chromosome preparations are venous blood sampling, lymphocyte culture, mitosis arrest at the metaphase stage, hypotonization of metaphase lymphocytes, fixation of chromosome sets on a glass slide, and chromosome coloring [26].

For each examined, at least 150 metaphase plates were analyzed. The frequency of CAs was calculated by the formula:

$$CA=(a/b)*100\%$$

Where: a – is the number of CAs;

b – is the number of metaphases.

The main purpose of the methods for processing cell cultures and chromosome preparations is the obtaining a sufficient number of metaphase plates with chromosome spread, which makes possible to assess the size, ratio of chromosome shoulder lengths, the presence of secondary constrictions, satellites, and other morphological signs of each karyotype chromosome.

The level of the trace elements in blood: (i.e., copper [Cu], zinc [Zn], cadmium [Cd], mercury [Hg], plumbum [Pb], arsenic [As], chromium [Cr], selenium [Se], manganese [Mn], iron [Fe], nickel [Ni], and iodine [I]) was carried out with MGA-915 atomic absorption spectrometer (Lumeks, Russia).

Inclusion criteria

Eighty people are without acute gastrological, bronchial obstructive, hemorrhagic, neurological and splenomegaly syndromes, infectious and severe somatic diseases, acute inflammatory processes, mental disorders, and severe physical illnesses. Persons from these groups have been living in their cities since they were born; they were not affected by harmful factors at the workplace. The survey sample included persons of reproductive age from 18 to 45 years.

Discontinuation criteria

Patients with gastrological, bronchial obstructive, hemorrhagic, neurological and splenomegaly syndromes, infectious and severe somatic diseases, acute inflammatory processes, mental disorders, persons under 18 years old and over 46 years old, working in harmful working environment were excluded from the study.

Statistical analysis

Data analysis was carried out with a Statistica 10 software package (StatSoft, USA). Processing operations have included the calculation of arithmetic

mean values (M), standard errors of arithmetic mean (m), confidence intervals, and standard deviation for variables with normal distributions. The latter was verified by the Shapiro–Wilk test and by the Kolmogorov–Smirnov test. Differences between the groups with normal distribution were found by means of parametric statistical methods and Student's t-test for two unrelated groups. Linear dependence was determined by means of the Pearson correlation coefficient (PCC) for indicators with a normal distribution.

Results

The identified CAs were divided into two main groups: Chromosome type and chromatid type. The total frequency of aberrations in the surveyed population amounted to 126 cases and was at 1.167 ± 0.149%, which is 40% higher than that in the control group of 1.011 ± 0.119%. The mean values of aberrations of the chromatid among respondents living in the area of ecological disaster zone were at 1.205 ± 0.126%. The frequency of chromatid-type aberrations (1.205 ± 0.126%) also exceeded the corresponding values in the control group (0.655 ± 0.096%) 45%. There were no significant differences between the aberrations of chromosomal type (Figure 1).

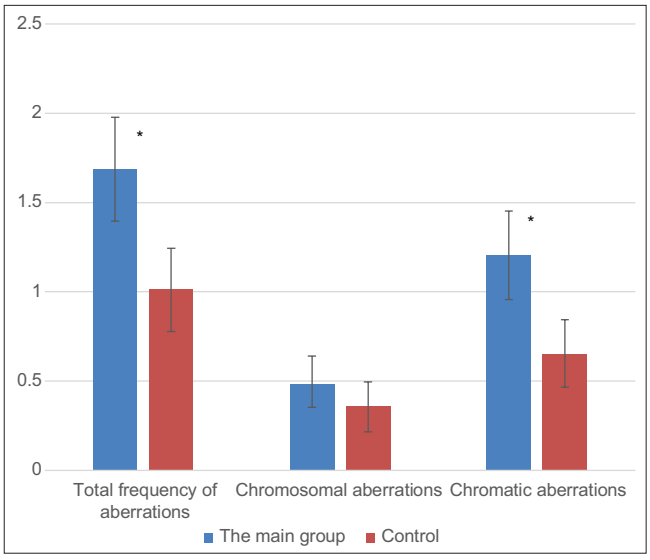

*Figure 1: Frequency and types of chromosomal aberrations in the examined individuals living in the area of the Aral Sea ecological disaster zone (M ± m%; 95% confidence intervals). The mark *denotes a significant difference in relation to control numbers by Student's t-test p < 0.05. Unit of measurement for all values is %*

Analysis of obtained data has shown that the identified chromatid type of aberrations was represented by single fragments, whose share in the total number of aberrations was 59.52% (75 cases), chromatid breaks of 7.14% (10 cases), and deletions of 4.76% (6 cases). Cytogenetic disorders in the control group, mainly represented by the same types of CAs, as in the main group: Single fragments of 49% (35 cases), chromatid breaks of 9.8% (7 cases), deletions of 5% (4 cases).

Chromosome type aberrations were represented by paired fragments, whose contribution to the total number of aberrations was 25.39% (32 cases), breaks on the centromere of 1.58% (2 cases), and translocations – is 1.58% (2 cases). In the control group of the chromosome type, there are also paired fragments of 28.16% (20 cases) of the total number of CAs, center breaks – 5.63% (4 cases), and interchromosomal translocations – 1.41% (1 case); ring chromosomes were not found (Table 1).

Table 1: Types of CAs in the examined individuals living in the area of the Aral Sea ecological disaster zone (M ± m%; 95% CI)

Indicators	The studied group		p
	Control	The main group	
Aberrations of chromosomal type			
Paired fragments	0.285±0.063 (0.160–0.409)	0.428±0.075 (0.280–0.576)	-
Breaks on the centromere	0.057±0.028 (0.001–0.112)	0.027±0.018 (0.009–0.045)	-
Translocation	0.014±0.013 (0.001–0.027)	0.013±0.012 (0.001–0.025)	-
Acentric fragment	-	0.013±0.012 (0.001–0.025)	-
Total	0.356 ± 0.071 (0.216–0.495)	0.482 ± 0.080 (0.325–0.639)	-
Aberrations chromatid type			
Chromatid breaks	0.099 ± 0.037 (0.025–0.173)	0.147 ± 0.044 (0.060–0.234)	-
Single fragments	0.498 ± 0.084 (0.333–0.663)	0.991 ± 0.114 (0.766–1.215)	0.0006
Deletions	0.057 ± 0.028 (0.001–0.112)	0.067 ± 0.029 (0.008–0.125)	-
Total	0.655 ± 0.096 (0.466–0.844)	1.205 ± 0.126 (0.957–1.453)	0.0005

M – arithmetic means. m – standard errors. CI – confidence intervals 95%. p – significant difference in relation to control group by Student's t-test. CA: Chromosomal aberration.

The research on chromosomal and chromatid types of aberrations in peripheral blood lymphocytes of patients living in the area of The Aral Sea region has shown a significant prevalence of chromatid aberrations over chromosomal, which indicates on the chemical mutagenesis. Thus, the total number of chromosomal and chromatid aberrations of people living in the ecological disaster zone was divided as follows: 71% of chromatid type of aberrations and 29% of chromosomal type. Analyzing obtained data on the types of CAs may be noted that the level of chromatid type aberrations 42% was higher than the aberrations of chromosomal type (Figure 2). As we know from the literature, the manifestations of chromatid-type aberrations are typical for chemical mutagenesis [27], [28], [29]. The total number of aberrations for the control group was 65% for chromatid type and 35% for chromosomal. Many authors support the view that chromatid-type aberrations are formed as a result of exposure to mutagens and damage to the DNA molecule in the synthetic stage [30], [31], [32].

CAs during chemical mutagenesis often occur in the S phase, even when exposed to a factor at any stage of the cell cycle. Consequently, DNA fragments detached from the whole molecule will condense into single fragments, and not into an integral chromosome (Figure 3).

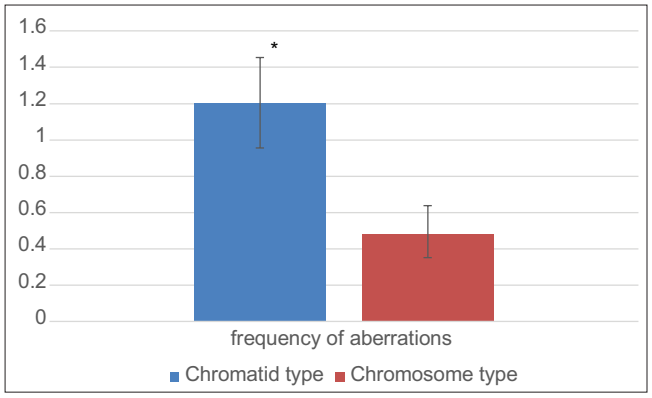

*Figure 2: Comparison of types of chromosomal aberrations in the examined individuals living in the area of the Aral Sea ecological disaster zone (M ± m%; 95% confidence intervals). The mark *denotes a significant difference in relation to control numbers by Student's t-test p < 0.05. Unit of measurement for all values is %*

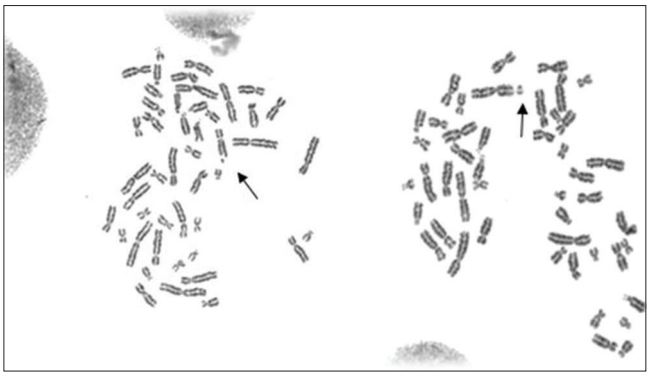

Figure 3: Chromosomal aberrations in peripheral blood lymphocytes of persons living in the area of the Aral Sea ecological disaster zone (chromatid type of aberrations on the left and chromosomal type of aberrations on the right)

The chromatid type of aberrations indicates the predominantly chemical nature of genotoxic agents, although there are exceptions for the general hypothesis, according to which the entire chromosome changes during breaks in the pre-synthetic stage and double aberrations are observed; during breaks at the post-synthetic stage, only one chromatid changes, and single aberrations are observed [32], [33], [34], [35].

Assessing the effect of genotoxic of chemical agents (include heavy metals) on the chromosomal structure was performed correlation analysis between the level of CAs and the content of microelements in the blood.

Obtained results indicate significant chromosomal mutations in examined subjects during adaptation to high chemical load. Blood test for microelements content of people living in the disaster zone has shown that concentration of heavy metals in the blood such as plumbum, nickel, and copper, which have a toxic effect, exceeded control indicators for 98%, 97%, and 41%, respectively.

Continuous chemical load also has a significant effect on the level of essential trace elements in the body of people living in the Aral Sea region. There is a decrease in such important trace elements such as selenium for 38%, zinc for 40%, and iodine – 30% in comparison with the control group (Table 2).

The conducted analysis has shown a correlation relationship between CA showings and the content of microelements in the blood. Correlation analysis showed a dependence on the total level of chromosome aberration from the level of nickel in the blood (PCC = 0.61). Based on the results of regression, a linear prognostic dependence model of total level of chromosome aberration on the nickel level in blood: y (total level of chromosome aberration) = 0.53 + 0.54 * x (regression coefficient is R = 0.61, determination coefficient – R2 = 0.36, Fisher's coefficient – F = 41.05, p < 0.01) was developed. Trace element analysis showed that the nickel level in the main group is higher than in the control group in 49.5%. The correlation between the general levels of CAs and the level of nickel in the blood consists of an increase in chromatid type aberrations (PCC = 0.56, p < 0.01), and precisely because of the increase in the number of single fragments (PCC = 0.50, p < 0.05).

Furthermore, there was revealed a correlation of aberrations of chromosomal type with cadmium (PCC = 0.29, p < 0.05); the correlation between the copper content in the blood and single fragments was revealed (PCC = 0.30, p < 0.05). A statistically significant inverse correlation between the zinc level in blood and aberrations of chromatid type was also found (PCC = 0.32, p < 0.05).

Discussion

Various studies on the genotoxic activity of chemical agents, such as heavy metals, show

Table 2: Blood trace elements content of examined subjects (main and control groups)

Indicator	Reference values	Control M ± m (95% CI)	The main group M ± m (95% CI)	p
Copper	800–1300 μg/l	966.33 ± 23.21 (919.35–1013.31)	1366.59 ± 35.65 (1294.05–1439.14)	0.01
Zinc	4000–8600 μg/l	5859.15 ± 183.45 (5250.13–5822.88)	3516.93 ± 89.93 (3333.93–3699.93)	0.01
Plumbum	Under 25 μg/dl	2.38 ± 0.34 (1.69–3.07)	4.72 ± 0.39 (3.92–5.52)	0.01
Iron	309–521 mg/l	382.55 ± 11.11 (360.07–405.03)	354.31 ± 7.46 (339.16–369.49)	0.04
Cadmium	0.3–0.9 μg/dl	0.38 ± 0.02 (0.53–0.65)	0.57 ± 0.03 (0.49–0.64)	-
Selenium	58–234 μg/dl	85.62 ± 5.17 (75.15–96.08)	52.88 ± 1.83 (49.15–56.61)	0.01
Nickel	1–50 μg/l	2.45 ± 0.21 (2.03–2.87)	4.85 ± 0.36 (4.11–5.61)	0.01
Manganese	1.6–75 μg/l	3.78 ± 0.37 (3.02–4.54)	4.81 ± 0.38 (4.01–5.59)	-
Iodine	5–12 μg/l	7.03 ± 0.28 (6.45–7.60)	4.90 ± 0.35 (4.17–5.63)	0.01
Arsenic	0.002–3 μg/dl	1.50 ± 0.14 (1.21–1.79)	0.10 ± 0.02 (0.05–0.15)	0.01
Chromium	0.7–2.8 μg/l	1.52 ± 0.09 (1.32–1.72)	1.30 ± 0.08 (1.13–1.47)	-

M – arithmetic means. m – standard errors. CI – confidence intervals 95%. p – significant difference in relation to control group by Student's t-test.

that these substances are capable of exhibiting mutagenic properties, often manifesting themselves as CAs [36], [37], [38], [39], [40], [41].

Considering the mechanisms of damage to chromosome structures, we may say that under the influence of various chemical agents, which include heavy metals, damage to the tertiary structure of chromosomes occurs, which leads to partial denaturation of DNA, when binding of divalent heavy metals to DNA, mutations such as transversions and transitions are possible. Hence, they can cause CAs, inducing point mutations, disrupt enzyme interactions, inhibiting individual enzymes.

A DNA molecule has several active centers that coordinate metal ions. First of all, these are oxygen ions of phosphate groups that carry negative charges. Some atoms of nitrogenous bases that enter grooves can also interact with metal ions. The seventh nitrogen atom of guanine is the most favorable position for the binding of positive ligands to DNA bases [38], [42].

The nature of the interaction of metal ions with various binding sites on the DNA molecule is determined by the charge of the ion and the structure of the electron shell of this ion. Ions of alkali (Na^+, Li^+, K^+) and alkaline-earth (Mg^{2+}, Ca^{2+}, Ba^{2+}) metals interact mainly with phosphate groups of DNA. Ions of transition metal (Ni^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+}) actively bind to phosphate groups and bases [38].

The fact of the interaction of nickel with nitrogenous DNA bases was revealed by fixing changes in the spectral properties of DNA [38].

The mechanisms of the effect of low concentrations of chemical factors on chromosomal structures have not been sufficiently studied. They can be direct and indirect [32], [38], [39], [43], [44].

Given all the above, there is practically no data about the mechanism of the formation of CAs under the action of heavy metal ions.

We assume that in the presence of some metal ions (Ni, Cd, Mn), the accuracy of DNA synthesis decreases; this is due to the ability of these elements to interact with the first nitrogen atoms through a donor-acceptor bond (nitrogen is a donor because it has an unshared electron pair), which can lead to transitions, base loss, and point mutations.

The ions of these metals are able to form an ionic bond with a phosphate group, which leads to a rupture of the phosphodiester bond between the phosphoric acid residue and deoxyribose; as a result, single fragments can form at the metaphase stage.

The pathways for the formation of chromosomal mutations, when exposed to a chemical factor, may be different. In addition to the direct genotoxic effect of chemical factors exerted on DNA, heavy metals also activate antioxidant defense system enzymes, which with prolonged chemical stress, can damage cellular structures by oxidizing the membrane components of the cell.

Conclusion

To sum up, long-term chemical load in the ecological disaster zone of the Aral Sea causes an increase in CA in population, in particular, in people of fertile age.

Obtained results indicate significant cytogenetic disorders in examined subjects during adaptation to a high chemical load.

The level of CAs in the examined persons living in the ecological disaster zone of the Aral Sea was 1.677 ± 0.149% and was in 40% significantly higher than in the control group (1.011 ± 0.119%).

Research findings recorded a significant increase of nickel by 97% in comparison with the control group and a decrease in vital essential trace elements (zinc, selenium, and iodine). Zinc level in blood was decreased by 40% in the main group in comparison with the control group.

The increased level of mutagenic load in the study group relative to the control is due to chemical mutagenesis, which is confirmed by the revealed chromatid-type aberration and correlation analysis.

Correlation and regression analysis determined that the nickel level in blood effects on the total level of chromosome aberration, the zinc level in blood and aberrations of chromatid type were in inverse relationships.

According to correlation analysis, an increase in trace elements (nickel, cadmium, and copper) in the blood can lead to undesirable consequences in the form of chromosomal abnormalities.

One of the mechanisms of the formation of CAs under the influence of a chemical factor may be newly formed atomic bonds between chemical elements and a DNA molecule, as a result of competition for an unshared pair of electrons of donor atoms of a DNA molecule

The current study supports the conclusion that at least in part some of these physiological relationships are potentially heightened in populations living in areas with significant ecological disturbances.

Any changes of trace elements and cytogenetic disorders reflect intoxication under long-term chemical load among the population in the ecological disaster zone of the Aral Sea region. As these changes characterize the toxicodynamic response of the body, they can be used as diagnostic parameters of high sensitivity, specificity, and prognostic significance.

Thereby, the revealed CAs can be a warning signal about the possible genetic consequences since they disrupt the balance of hereditary factors, they are the cause of a variety of abnormalities in the structure and life of the organism, manifested in the chromosomal abnormalities, diseases and syndromes.

Human heredity and the quality of its living conditions determine both the state of its health and society as a whole.

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