Evaluation of cadaveric bone marrow cells of children from different regions of Kyrgyzstan

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ABSTRACT

Introduction and Aim: The bone marrow is responsible for the supply of oxygen and restores the cells that form the blood of a tissue. The objective is to study the cadaveric bone marrow cells from dead children of different regions of Kyrgyzstan.

Materials and Methods: Bone marrow examination was performed on 38 cadavers (Bishkek—11, Kara-Balta—9, Cholpon-Ata—10, and Naryn—8) of children dead due to causes unrelated to immunodeficiency. The cadaveric bone marrow cells from dead children were collected within a day after death.

Results: Studies of cadaveric bone marrow cells from dead children in Kara-Balta showed an increase in basophils, eosinophils, lymphocytes, leukoerythroblastosis, and a decrease in myelocytes. Erythroid growth according to the normoblast hematopoiesis was slightly decreased. Megakaryocytes were single or absent (no function) and contained small number of platelets.

Conclusion: Cadaveric bone marrow cells taken from dead children who lived near the uranium tailings dump of Kara-Balta, demonstrated an anomaly with the hematopoietic function of the bone marrow, the state of the stroma, the ratio of hematopoietic and adipose tissue, cellular composition, different pathological processes as indicated by myelocytes in comparison to dead children from other regions (Bishkek, Cholpon-Ata, and Naryn).

Keywords: Bone marrow, hematopoiesis, megakaryocytes, reticulocytes, myelocytes, cadaveric bone marrow cells.

INTRODUCTION

Radioactive contamination in the Kyrgyz Republic happened because of the activities of mining and ore processing enterprises of the uranium industry. As a result of this, the total area of territories exposed to varying degrees of radioactive contamination is about 6 thousand hectares, on which there are 145 million tons of radioactive waste represented by dumps of ores, rocks, and tailings of hydrometallurgical Processing (1). When designing tailings and mining dumps, they generally adhered to the requirements imposed on most such facilities. Therefore, tailings dumps were constructed in close proximity to settlements, and waterways that are sources of drinking and irrigation water. In Kyrgyzstan, because of the exploitation of uranium deposits, the enrichment of uranium raw materials, sedimentation tanks, and tailings with a high content of uranium, thorium, and other radioactive elements have increased (2, 3). Therefore, radiation safety issues are particularly relevant for Kyrgyzstan since the country was previously the main supplier of uranium raw materials in the form of uranium and molybdenum oxides (4, 5). The constant intake of relatively small doses of radionuclides and heavy metals into the body doesn’t lead to classical radiation diseases but affects the immune system. As a result, the body becomes more susceptible to diseases such as acute respiratory infections, and gastritis for a long time. Humans are more vulnerable, as their hematopoiesis system affects, and the level of exposure to the same doses of radiation in children is 10-20 times higher than in adults (6-11).

The bone marrow functions as a biological shield of the body and bone formation. It is responsible for the supply of oxygen and restores the cells that form the blood of a tissue (red blood cells, leukocytes, and platelets). In humans, bone marrow is first appearing in the 2nd month of embryogenesis, in the 3rd month in the scapula, ribs, sternum, vertebrae, and others. In the 5th month of the embryonic bone marrow functions as the primary blood-forming organ, providing differentiated bone marrow hematopoiesis with elements of granulocytes, erythrocytes, and megakaryocytes. The objective is to study the cadaveric bone marrow cells from dead children of different regions of Kyrgyzstan.

MATERIALS AND METHODS

The present study assessed cadavers of fetuses from 2015 to 2020. Bone marrow examination was performed on 38 cadavers (Bishkek – 11, Kara-Balta – 9, Cholpon-Ata – 10, and Naryn – 8) of dead children (7–12 years) with causes unrelated to immunodeficiency. The cadaveric bone marrow cells as
from dead children were collected within a day after death. The sternal puncture was performed, fixed, and stained red bone marrow specimen collection and preparations were examined under x20-fold magnification to assess the cells of the bone marrow. The number of megakaryocytes and reticulocytes was counted on the preparations, and myelocytes were counted in smears. Materials from Bishkek city were taken for the control group. The hemopoietic changes were compared with the other specimens obtained in the other 3 regions.

Mostly the deaths of children were due to various reasons such as traumatic brain injury, drowning, car accidents, falling from a height, frostbite, etc. The causes of death and the main diseases were determined by the conclusion of a forensic medical examination of corpses and histological and anatomical studies. The material was taken within a day after death. The inclusion criteria include the age and duration of stay in that region. The exclusion criteria include people with immunodeficiency, hemorrhagic syndrome, tumors of the blood and lymphoid systems, and any chronic diseases that lead to hemopoietic changes.

Then the preparations were examined with a solid immersion lens. At the same time, differentiated counting of megakaryocytes is carried out. Count all the cells in different parts of the smear in a row with a total of at least 500, and then calculate the output percentage of cells. The cells of the red bone marrow are subjected to significant quantitative and qualitative methods, therefore, for an objective assessment of the red bone marrow punctate, in addition to counting the myelocytes, it is necessary to determine the appropriate bone marrow indices. Histological sections were stained using hematoxylin and eosin (H & E) and subjected to immunohistochemical examination. Confidentiality was maintained concerning the data collected and this study was approved by the I.K. Akhunbaev Kyrgyz State Medical Academy Bioethics Committee (Protocol No. 1 dated March 25, 2019).

The obtained data are presented as the mean ± standard deviation. Statistical analysis was performed using Excel. XLSTAT v2020.1 (Microsoft, Addinsoft, Paris, France) and Statistica v8.0 (StatSoft Inc., Tulsa, USA). A two-sided p<0.05 was considered statistically significant.

RESULTS

In Bishkek, basophil and eosinophil counts are slightly increased compared to other regions. The proerythroblast is represented by lymphocytes (Fig. 1). The reticulocyte production index is in the normal range (0.7 ± 0.03).

The study of cadaveric bone marrow cells from dead children in the city of Kara-Balta showed that the white bone marrow cells differ from the counts in children from Bishkek. There is a decrease in the level of cells compared to Bishkek (69.2%), at the same time values correspond to the increased level.

![Lymphocytes](Fig. 1: Lymphocytes in cadaveric bone marrow cells from dead children)

The level of myelocytes tends to decrease by 38.6% (Fig. 2). There is also an increase in basophils (60.9%) and, eosinophils (31.9%). There is an increase in lymphocyte counts by 44.1% and there are adipocytes present in some areas.

![Myelocytes](Fig. 2: Myelocytes in cadaveric bone marrow cells from dead children)

Cadaveric bone marrow cells from dead children of Kara-Balta are characterized by a decrease in erythroblasts compared to Bishkek (5.0%; Fig. 3). The mitotic index (MI) of bone marrow specimens in Kara-Balta differs from Bishkek by 30.0%, while the leukoerythroblastosis increases almost twice.

![Erythroblasts](Fig. 3: Erythroblasts in cadaveric bone marrow cells from dead children)

The red bone marrow of former residents of Kara-Balta is characterized by a decrease in the level of erythroblasts (5.0%) compared to Bishkek. At the same time, there is an increase in subsequent generations except for basophils (Fig. 4).
The indicators of erythroid growth significantly differ from Bishkek by 35.4%.

The study of cadaveric bone marrow cells from dead children in the city of Kara-Balta showed that the white bone marrow cells differ from the counts in children from Bishkek. There is a decrease in the level of cells compared to Bishkek (69.2%), at the same time values correspond to the increased level.

When analyzing the cadaveric bone marrow cells from dead children in Cholpon-Ata, an increase in blast cells is noted from the side of the granulocytes compared to Bishkek. At the same time, there is an increase in myelocytes (38.2%).

Basophils are significantly decreased compared to the other two groups (Fig. 5). There is an increase in the number of oxyphils (43.4%), and granulocytes (10.7%). An increase in normoblasts (57.1%), oxyphils (113.2%) with a simultaneous decrease in basophils was seen (Fig.6). The erythroid growth increases by 48.8%.

Cadaveric bone marrow cells from dead children in Naryn are characterized by a significant increase in blasts and myelocytes by an average of 50%. The level of myelocytes increases by 56.2%, segmented (25.9%), basophils (11.1%), and eosinophils (23.7%) (Fig. 7). Granulocyte growth (22.6%) is higher than in Bishkek. At the same time, there is an increase in the level of lymphocytes (39.2%) and a decrease in monocytes (13.0%). The analysis shows that compared to Bishkek, there was a slight increase in the delta neutrophil index and the reticulocyte production index with a simultaneous decrease in the leukoerythroblastosis (1.8%; Fig. 8).

**Fig. 4:** Monocytes in cadaveric bone marrow cells from dead children

**Fig. 5:** Basophils in cadaveric bone marrow cells from dead children

**Fig. 6:** Oxyphils in cadaveric bone marrow cells from dead children
The histological examination showed sinusoidal capillaries of hematopoietic stem cells collected from cadaveric bone marrow cells of dead children from Bishkek and Kara-Balta (Fig. 9 and 10).
As a result of the study, it was found that the immune status of the lymphocyte subpopulation in all regions is within the normal range. The indicators of the humoral immunity in Kara-Balta city are much reduced, as well as the results of the macrophage-phagocytic link of immunity are reduced from the normal (Table 1).

### Table 1: Data of indicators/reference values of cadaveric bone marrow cells from dead children of different cities

| Indicators/reference values | Normal values | Bishkek M ± SD | Kara-Balta M ± SD | Cholpon-Ata M ± SD | Naryn M ± SD |
|-----------------------------|---------------|----------------|-------------------|-------------------|-------------|
| Immune lymphocyte subpopulation |               |                |                   |                   |             |
| T-lymphocytes (CD3+), %     | 60-80         | 48.3 ±3.2 *    | 43.3 ±1.2         | 40.7 ±1.3         | 49.5 ±0.5 * |
| B-lymphocytes (CD 19+), %   | 10-23         | 21.3 ± 2.2     | 18.3 ± 2.3        | 19.7 ± 3.0        | 19.0 ± 0.01|
| T-helpers (CD4+), %         | 30-50         | 31.0 ± 4.0     | 27.7 ± 2.3        | 24.7 ± 2.7        | 31.0 ± 0.01 *|
| Cytotoxic lymphocytes (CD8+), % | 20-25 | 21.3 ± 0.9     | 18.0 ± 2.1        | 16.3 ± 2.6        | 22.5 ± 2.5 |
| NK-cells (CD 16+), %        | 6-26          | 18.0 ± 2.6 *   | 13.0 ± 2.1        | 11.3 ± 1.5        | 15.3 ± 0.5 * |
| Immune regulatory index      | 1.2-2.5       | 1.24 ± 0.2     | 1.7 ± 0.2         | 1.57 ± 0.2        | 1.4 ± 0.2  |
| Humoral immunity             |               |                |                   |                   |             |
| Immune complexes, g/l        | 120           | 108.0 ± 8.5    | 89.7 ± 7.4        | 106.0 ± 7.2       | 116.0 ± 3.0 |
| Innate immunity cells        |               |                |                   |                   |             |
| Phagocytic indicator         | 65-80         | 45.7 ± 1.7     | 36.3 ± 2.9        | 40.3 ± 3.7        | 44.0 ± 4.0 |
| (neutrophils %),             |               |                |                   |                   |             |
| Phagocytic number            | 3.7-5.4       | 2.4 ± 0.3      | 1.7 ± 0.1 *       | 2.1 ± 0.1         | 2.3 ± 0.09 |
| (neutrophils %),             |               |                |                   |                   |             |
| Integral phagocytic index, % | 1.2-3.2       | 1.1 ± 0.02 *   | 0.6 ± 0.05 *      | 0.85 ± 0.1        | 1.1 ± 0.08 |

Data given as mean ± standard deviation. *p<0.05 (significant relative to Bishkek).

### DISCUSSION

The results of the Bishkek study showed that the bone marrow punctate had cellular elements like erythroblasts according to the normoblastic hematopoiesis. The lymphoid growth is represented by lymphocytes. T-lymphocytes of secondary lymphoid organs have a different structure and cellular composition, which is a prerequisite for primary T-lymphocytes responses (12). Megakaryocytes were within normal limits and platelets were slightly increased. In the studied cadaveric bone marrow cells from dead children in Cholpon-Ata, the myelocytes showed hematopoiesis which was within normal limits. There is a tendency to increase basophils (3.7%) and granulocyte growth (10.7%). Megakaryocytes are within normal limits. Most of the neutrophils are present in the bone marrow and mobilized rapidly during infections as bone marrow plays a key role in host defense (13).

The results of the study in Naryn showed that basophils, and erythroid growth, compared to Bishkek, the values are similar. Basophil and oxyphil counts were slightly increased. Bone marrow basophilia is a specific but not sensitive, marker of disruption of the normal marrow maturation controls (14).

Studies of cadaveric bone marrow cells from dead children in Kara-Balta showed a tendency to increase basophils, eosinophils, lymphocytes, leukoerythroblastosis, and a decrease in myelocytes. Erythroid growth according to the normoblastic hematopoiesis is slightly decreased.

Megakaryocytes are single or absent (no function), and have a small number of platelets. A similar study in older adults of Kara-Balta showed an increased number of lymphocytes, decreased erythroblasts, and granulocyte growth. These changes are due to the influence of exogenous, endogenous, and demographic factors (15).

The health of the next generations depends less on the influence of material living conditions and is mostly determined by the health of parents, the influence of industrial, professional, psychosocial, and medical factors, and the environment where people reside. Therefore, the population policy provides the consolidation of the family as the most important in socialist society, to create better conditions for combining motherhood with the active participation of women in work and general life activities, the implementation of a system of measures to increase life expectancy and working capacity of people is not feasible without strengthening the population health (16).

The limitation of this study refers to further investigation for a long time on a larger sample size of cadaveric material.
CONCLUSION

Thus, cadaveric bone marrow cells from dead children who lived in Kara-Balta, located near the uranium tailings dump, there is a violation of the hematopoietic function of the bone marrow, the state of the stroma, the ratio of hematopoietic and adipose tissue, cellular composition, various typical pathological processes, as indicated by myelocytes in Kara-Balta compared with counts in other regions (Bishkek, Cholpon-Ata and Naryn).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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