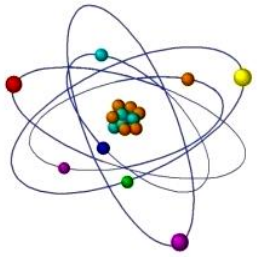
MICRONUCLACTED ERYTHROCYTES - AS A POTENTIAL NEW BIOMARKER OF LATE EFFECTS OF RADIATION IMPACT



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ABSTRACT*: The current task of modern biomedicine is to study the mechanisms of development of late effects of radiation-chemical impact, in order to develop simple and reliable criteria for predicting the severity of pathological processes and their consequences. The purpose of this paper was to determine the informativeness of current post-radiation changes in red blood system from the point of view of evaluating the severity of radiation pathology; In the groups of mice irradiated with sub- and semi-lethal doses, was studied the dependence of the probability of mice survival on the frequencies of micronuclear normochromic erythrocytes (MN-NCE) in the peripheral blood in the near and intermediate stages of the post-radiation period. The experiments were carried out on outbred mice (80 mice aged 8 week) and non-irradiated 10 mice. . Mice were randomly divided into groups irradiated at 3.5 Gy (group I, 30 mice), 5 Gy (group II, 30 mice), 6.5 Gy (group III, 20 mice), and sham-irradiated mice (group IV, 10 mice). Blood was collected from the tail vein of the mice. Blood smear was fixed into May-Grünwald' solution and dyed by Giemsa' s azur-eosin-methylene blue solution. Howell Jolly bodies in erythrocytes were counted for 1000 erythrocytes under a light microscope with inversion, lens magnification was 100x. In parallel with the blood examination, mice death caused by radiation were monitored daily and recorded to determine their survival. The data obtained by us shows that the increase in the frequencies of MN-NCE in the peripheral blood and the probability of mice death are time-correlated processes. The obtained results clearly indicate the high informative value of the frequencies of micronuclear erythrocytes in the peripheral blood in terms of assessing the depth of current pathological changes in erythropoiesis in the body's erythropoietic system (biological marker of the effect), however, when testing the genotoxic effects of various factors with the micronuclear test, the systemic factors of regulation of the frequencies of micronuclear erythrocytes should be taken into account.*

Key word: micronuclear normochromic erythrocyte, ionizing radiation, new marker.

INTRODUCTION

The current task of modern biomedicine is to study the mechanisms of development of late effects of radiation-chemical impact, in order to develop simple and reliable criteria for predicting the severity of pathological processes and their consequences. This is primarily related to the problem of prevention and minimization of the risk of near and late stochastic effects of radiotherapy [1,2]. The classical approach to assessment and prognosis of the severity of radiation pathology is based on the study of the morphological and genetic characteristics of the white blood cells [3-8], however, it should be noted that there are interesting perspectives to study the red blood cells as well - in particular, a significant difference between strains of mice in the delayed effect of irradiation on the frequency of micronuclear

reticulocytes was noted. The results show that the delayed genomic effects of irradiation on the hematopoietic system of mice can persist in vivo for long periods of time and that there are differences between mouse strains in terms of sensitivity to radiation-induced genomic instability. [9]. This approach allows taking into account so-called non-targeted effects [10-12] of ionizing radiation and, accordingly, impacts of complex effects of ionizing radiation and other health risk factors [13,14], mechanisms of individual and population radiosensitivity [9,15,16,17]. In our early studies, was studied the informativeness of cyto and molecular-genetic markers in terms of prognosis of near post-radiation complications [17]. The purpose of this paper was to determine the informativeness of current post-radiation changes of erythropoiesis in the erythrocytic system from the point of view of evaluating the severity of radiation pathology;

MATERIALS AND METHODS

The experiments were carried out on outbred mice (80 mice aged 8 week). Mice were randomly divided into groups irradiated at 3.5 Gy (group I, 30 mice), 5 Gy (group II, 30 mice), 6.5 Gy (group III, 20 mice), and sham-irradiated mice (group IV, 10 mice). The animals were kept under standard conditions on a standard diet and consumed water ad libitum. The protocol for conducting experiments and keeping mice was approved by the Ethical Committee for conducting experiments on animals of the Tbilisi State Medical University.

Mice were exposed via Gamma irradiation on a GUPOS-3M with a source of Cesium-137 (137Cs) in a dry chamber at a temperature of 25±10 ^{0}C (dose rate of 1.1 Gy/min). Falsely irradiated mice (group IV, 10 mice) were placed in a radiation chamber without an irradiation source. Subsequently, the mice were divided into cages and observed.

Blood was collected from the tail vein of the mice, for sampling were chosen 3 mice from each group, after gamma rays expose sampling was done at days – 2, 7 ,12, 20, 35, 42, 60, 75, 85 and 100. Blood smears were prepared for counting *Howell* Jolly bodies. For smear preparation drop of blood was applied to a clean, dry glass slide, then was pulled toward the entire slide and dried in the air. After smears where dried, for fixation they were placed into May-Grünwald's solution during 3 minutes and then dried again. Blood smears were dyed by Giemsa's azur-eosin-methylene blue solution (diluted 1:5 with distilled water) for 20 minutes and washed with water flow to remove excesses of dye and dried in the air. Howell Jolly bodies in erythrocytes were counted for 1000 erythrocytes under a light microscope with inversion, lens magnification was 100x. In parallel with the blood examination, mice death caused by radiation were monitored daily and recorded to determine their survival.

The causal relationship between frequency *Howel* [l] Jolly bodies in erythrocytes and the life span of laboratory mice was analyzed on the basis of the Cox proportional hazard model. Basic calculations and visualization of the results were carried out using a mathematical package "STATISTIC 12".

RESULTS

In the groups of mice irradiated with sub- and semi-lethal doses, was studied the dependence of the probability of mice survival on the frequencies of micro nucleated normochromic erythrocytes (MN-NCE) in the peripheral blood in the near and intermediate stages of the post-radiation period.

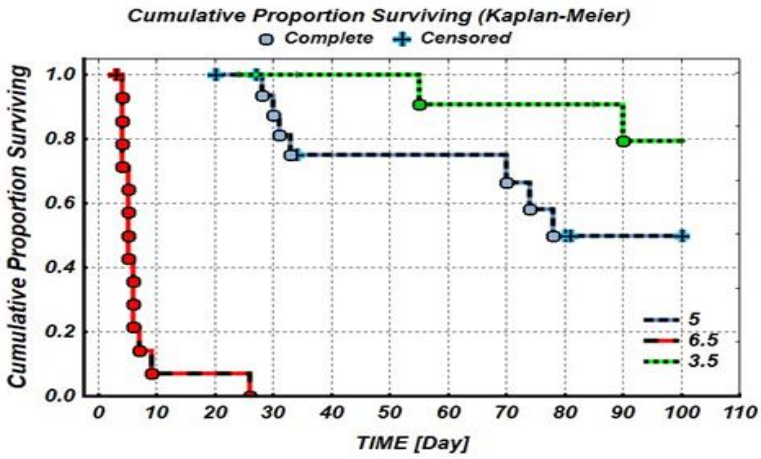

Fig.1. Survival curves of mice after total body irradiation (^{137}Cs γ-rays) with doses 6.5 Gy (circle), 5 (square) and 3.5Gy. (rhombuses). X-axis – Days after exposure, Y axis - part of animals at risk

Figure 1 shows dependence of the probability of survival of irradiated mice on the irradiation dose on post-radiation period in days. As expected, a sharp dose-dependence of the survival of animals is revealed, it is worth noting that the dynamics of the death of mice was not described by a smooth, but by a step function, this is especially notable at 5 Gy. dose-irradiated mice (range of 25-35 and 70-80 days), which should probably indicate a pronounced heterogeneity of mice in the study group in terms of radiosensitivity.

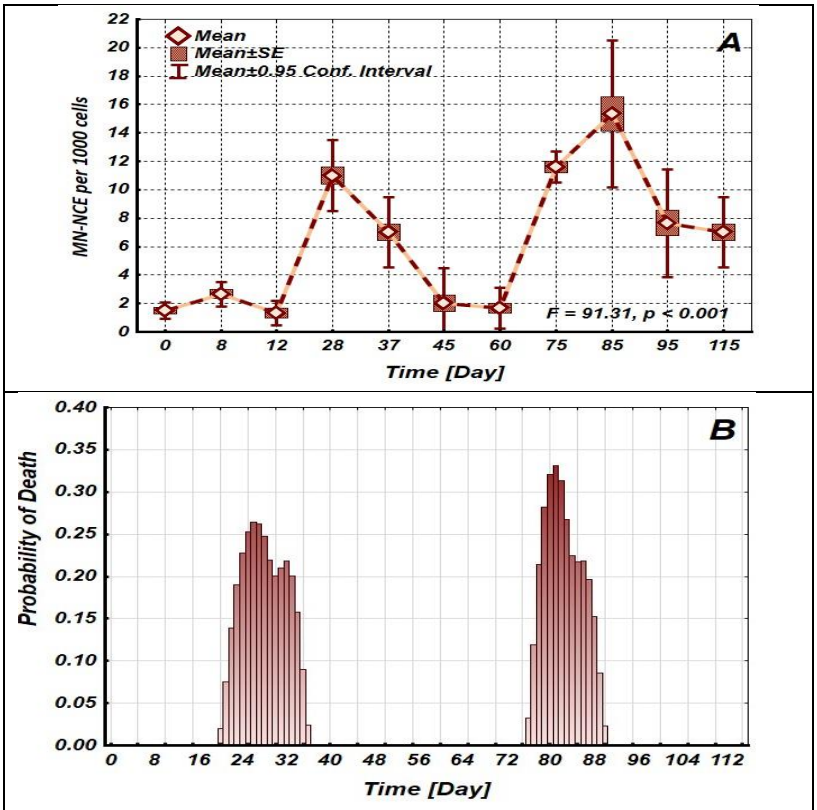

Figure 2. Mean number of micro nucleated normochromic erythrocytes (MN-NCE) per 1000 cells in group of animals irradiated with dose 5Gy (A) and probability density function (B) of the death of mice irradiated with a dose 5Gy.

In the dynamics of MN-NCE frequencies in the peripheral blood of 5 Gy. dose-irradiated mice, two clearly expressed peaks are recorded on days 20-30 and 75-85 of the post-radiation period (Fig. 2A). Visual analysis of the distribution functions shows that the increase in the frequencies of MN-NCE in the peripheral blood and the probability of mice death (Fig. 2B) are time-correlated processes. In order to quantitatively assess the credibility of this regularity, cross-correlation analysis of MN-NCE dynamics and animal death probability distribution functions were used (Fig. 3). Already by visual analysis it is clear that a kind of correlation exists between the intensity of animal death and the frequencies of micronuclear erythrocytes. which finds a quantitative reflection between the cross-correlations of these functions (Fig. 3)

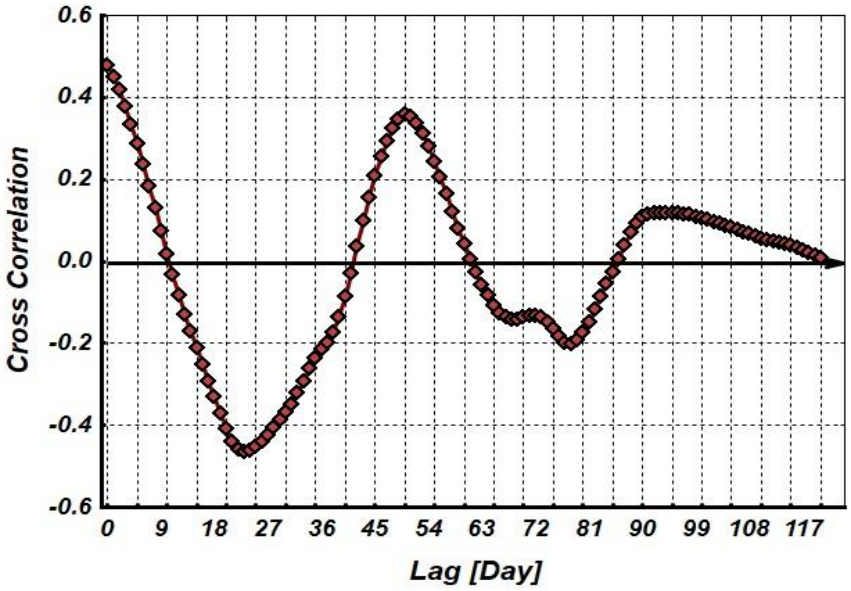

Fig.3. Cross-correlation function of the dynamics of MN-NCE frequencies in the peripheral blood of mice irradiated with a dose of 5 Gy. in the post-radiation period and the distribution of the probability of mice death

As can be seen from the graph, the maximum and minimum values of the function are approximately equal to 5, which indicates a high degree of correlation between the dynamics of MN-NCE levels and the distribution function of the probability of mice death.

DISCUSSION

As it is known, micro nucleus can be observed in cells of any proliferating tissue; however, they are most easily detected in cells without a nucleus in most mammalian species in erythrocytes (polychromatophilic - young and norm chromatophilic – mature). Micronuclei in erythrocytes are called Howell–Jolly body. A Howell–Jolly body is a cytopathological finding of basophilic nuclear remnants (clusters of DNA) in circulating erythrocytes. During maturation in the bone marrow, late erythroblasts normally expel their nuclei; but, in some cases, a small portion of DNA remains, which may be due to the genotoxic effects of various physical and chemical agents, or may be a manifestation of genomic instability associated with a number of internal and external factors [4,6].

The dynamics of Mn-NCE obtained from these positions in the initial stage of the post-radiation period should be associated with a high degree of reliability with the abortive rise of erythropoiesis in

irradiated mice (15-30 days after irradiation), which is followed by the gradual normalization of erythropoiesis in the later period (30-60 days). What concerns the second peak in the dynamics of Mn-NCE, it seemed less likely to us associate it only with the radio-induced instability of the genome (the biological mechanism of the nonlinear dynamics is unknown to us). It should be considered here that the production of Mn-NCE can be related not only to the damage of the genetic apparatus, but also to the intensification of erythropoiesis induced by the development of anemia; It is an experimentally proven fact that prior bleeding significantly increases the concentration of micronuclear reticulocytes in the peripheral blood of irradiated mice [11]. An increase in the concentration of Mn-NCE was detected under conditions of intensification of the proliferation-differentiation of erythroblasts induced by erythropoiesis [7], It should be noted here that even in early radiobiological studies, it was revealed that ionizing radiation causes not only damage to the genetic apparatus of the hematopoietic system, but also a reduction in the life span of circulating erythrocytes, which at a certain stage of the post-radiation period will become the reason for the intensification of erythropoiesis.

If we summarize the above, the superposition of the radio-induced instability of the genome and the intensification of erythropoiesis induced by radiation anemia can be considered as a hypothetical mechanism of the increase in the level of Mn-NCE on the 70-80th day of the post-radiation period, and from this point of view, the level of Mn-NCE in the peripheral blood is an integral part of the functional status of the erythrocyte system as a whole.

As for the close correlation of the probability of death of mice in the post-radiation period with the frequencies of Mn-NCE in the peripheral blood, the discussion of its specific biological mechanism is beyond the scope of this article, although the direct causal relationship of the radiation death of animals with the systemic stability of erythron to radiation exposure is clearly defined

CONCLUSION

The obtained results clearly indicate the high informative value of the frequencies of micronuclear erythrocytes in the peripheral blood in terms of assessing the depth of current pathological changes in erythropoiesis in the body's erythropoietic system (biological marker of the effect), however, when testing the genotoxic effects of various factors with the micronuclear test, the systemic factors of regulation of the level of micronuclear erythrocytes should be taken into account.

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