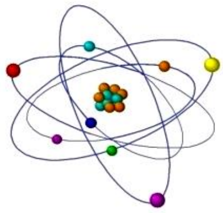

INTRODUCTION OF MODERN METHODS OF RADIATION GENETICS INTO THE MEDICINE IN GEORGIA

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ABSTRACT

The article provides an overview of the development of radiation genetics in the Georgian medicine. It considers stages of introduction of genetic methods in the examination of persons exposed to different doses of ionizing radiation under different conditions. The results of chromosomal biodosimetry, introduced since 2000, have been summarized in persons who had received high and low doses as a result of accidents due to contact with abandoned, orphan radiation sources, as well as during professional contacts and in the process of radiation therapy. In addition to the results of chromosomal research, both in total irradiation in accidents as well as during radiotherapy, the importance of using methods for determination of micronuclei level *in lymphocytes, buccal and erythroid cells, as well as, using the comet method, DNA damage for the purpose to assess the clinical state of irradiated subjects is being analyzed.*

Key words: chromosomal biodosimetry, micronuclei, DNA damage medical management

The estimation of biologic effects of Ionizing Radiation (IR) started immediately after their discovery.The first scientific articles were written at the end of the 19th century, and then it was concluded that this type of radiation is harmful to biological organisms, including humans. Meanwhile, humanity cannot avoid the use of ionizing radiation, because for the delivery of energy, the transfer of energy and other possibilities, especially in medical diagnostics and treatment, this is the best method. The use of ionizing radiation is constantly increasing, but, in addition to positive results, its destructive effect on the human body has been proven[2].At the end of the 19th century a famous Georgian scientist, Tarkhnishvili, discovered the effect of theinfluence of X-rays on the central nervous system, animal behavior, the heart and circulation, and embryonic development Indeed, these works have given rise to a new field in science as Radiobiology. Ionizing radiation is one of the most powerful physical mutagens among harmful environmental factors. Mutagen is an agent that affects the genetic apparatus and changes the DNA of a cell. It is assumed that DNA damage in the nucleus of single individual cell can initiate carcinogenesis[9]. Among the various types of lesions induced double-stranded DNA breaks are considered to the most relevant of the deleterious effects of IR.All systems of living organisms respond to the impact of mutagens. Radiation can cause and heal the most complicated diseases.The key moment in the development of tumor cells is a mutation. Being a strong mutagen, ionizing radiation primarily causes changes in the genetic apparatus and induces genome instability. Genetic instability plays a central role in carcinogenesis. During tumor development, diploid cells show mutations in genes responsible for maintaining genome integrity (Caretaker genes) and in genes that directly control cell proliferation (Gatekeeper genes). That’s why cytogenetic indexes are excellent biomarkers for detection the effect of irradiation [1,6].Changes in organisms caused by total body irradiation in accidents and radiation catastrophes have been well studied.

General and local irradiation, even in the identical doses, causes significantly different effects. Although the general radiobiological principles underlying external beam and radionuclide therapy are the same,there are significant differences in the biophysical and radiobiological effects of irradiation [3].

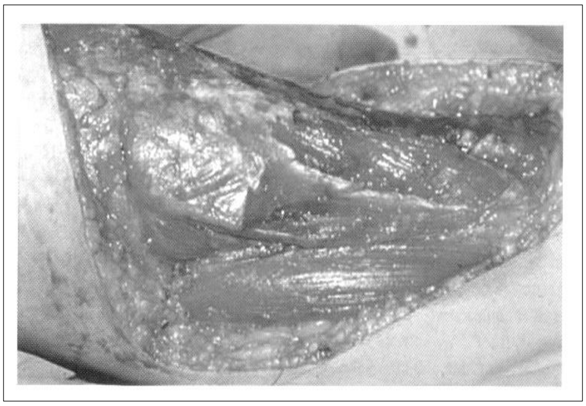

Fig.1. Radioactive sourcesin 1997 in 11 military servicemen developed skin reaction of different stageswas suspected.

Today the impact of ionizing radiation of various doses is intensively studied. Most works are focused on low doses; this effect on organism does not immediately cause clinical changes and appears only after long time and more often by development of cancer associateddiseases.

The main contingent for investigation of low doses radiation impact are professionals, being influenced due to their occupation and inhabitants of regions with increased radiation background.Although, the general radiobiologic principles underlying external beam and radionuclide therapy are the same, there are significant differences in biophysical and radiobiological effects. Dosimetry is a base of radiation safety. The main methods of this are physical dosimetry methods, but this methodsprovide only extrapolation information about the dose absorbed by the human organism and its biological effects. In contrast, biodosimetry methods give us opportunity to register the real biological damage to organism caused by ionizing radiation. Biodosimetry plays an important role in the triage and medical management of radiological casualties. Biological dosimetry using the levelof chromosome damage, is very important, because unlike physical dose measurement, it takes into account interindividual variation in susceptibility.In Georgiathe necessity of biodosimetry studies arose after several accidents, which happened in Georgia at the end of 90^{th}, especially after the incident in the Lilo training center after irradiation 11 military servicemen.During withdrawal of Soviet troops from Georgia, because of bilateral violation of the environmental safety regulations for radioactivity-containing ammunition transfer, Georgia became the range for investigation of low dose radioactive impact on living organisms.Radiation impacts directly could have influenced the health state of the population, the density of which is quiet high around the regions of some military lands.The personnel of Georgian military bases and the population from the adjacent territories could have been affected by radioactive sources of different intensity. During the 10 years 18 individuals got serious radiation trauma, and quite big contingent was exposed to chronic impact of low doses irradiation.After withdrawal of Soviet military troops from Georgia many radioactive sources were left without appropriate supervision.

Fig.2. Poorly healing skin wounds

In cytogenetic laboratoryof Georgian Institute of Hematology and Transfusiology were detected specific disorders –dicentrics and ring chromosomes. In 2000 y. employees of these laboratory with support of IAEA, were sent to the IPSN of French Atomic Centre. In the laboratoryof multi-parametric dosimetry the owncalibration curves for dicentric chromosomes' yield were drawn.

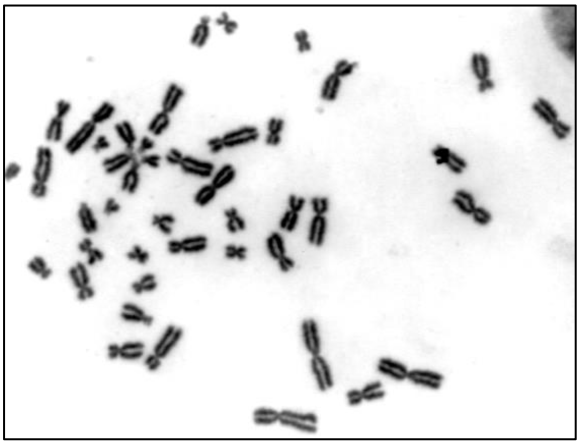

Fig. 3.Dicentric chromosome and acentric fragments

During following years were investigated more than 300 persons. The aim of the work was to determine the received dose for carrying out medical and preventive actions timely. The work was supported by ISTC (International Science and Technology Centre) in frame work of Project G-564) and Georgian State Radiation Program.Biodosimetry was carried out with conventional dicentric chromosomal assay. Parallel application of two cytogenetic methods – quantity calculation of dicentrics and definition of level of micronuclei promoted more exact definition of the absorbed dose (2,4). Before detection of absorbed individual dose upon quantity of unstable chromosome aberration on upon micronuclei yield were established the background data. The first doseswere established in11 military serviceman fromLilodata.

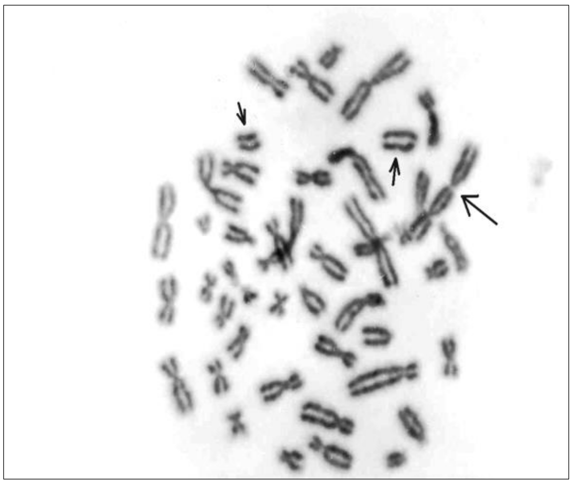

Fig. 4. Metaphase with chromosomes association, composed by 5 chromosomes

Investigations were carried out in dynamics. In all of them were revealed unstable chromosome aberrations-dicentrics, centric rings and fragments (slides) and 1 and more micronuclei in binuclear lymphocytes. The doses 0.5 - 1.6 Gy were detected [11].

Patie showed a decrease in humoral immunity, oligo- or azoospermia. Severe hematological abnormalities were not observed. Later developedlymphocytosis. The next accident happened in mountain region – village Lia. Cytogenetic analyses were carried out on three men, who had serious radiation trauma in result of contact with uncovered, highly active ($1.3{\cdot}10^{15}$Bq) thermo generator (^{90}Sr).As it was determined by IAEA special commission, X-rays dose on 1m distance was 1Gy, and in close contact – 240 Gy. In each person > 500 metaphases were analyzed. The following doses were determined. The absorbed doses. 2.8 Gy, 3.3 Gy and 1.2 Gy respectively were determined. Besides general dose, the dose was established with the method of Dolphin to establish the exact dose on the irradiation region. They were 3.0Gy, 4.3 Gy and 1,9 Gy respectively. All three had severe radiation trauma and they died at different times.

Determination of the received dose also was carried out in professional group-64people(liquidators, radiologists and radiation therapists) and in the individuals who leave or work on the territory, where radiation source was found (132 persons),Besides dicentrics and micronuclei the number of acrocentric chromosomal associations per cell (association index) was scored. Statistical confirmation of significance of acrocentric chromosome associations' phenomenon under low dose radiation impact was obtained. (12) The number of associations increased to 1 Gy and then decreased gradually. As a rule, associations were composed from 4 and more crocentric chromosomes.

For determination of acrocentrics' nucleolar organizing regions' (NOR) transcriptional activity the part of preparations were $AgNO_3$-stained by Bloom and Goodpasturemethod (7).

The extension of satellite filaments of acrocentric chromosomes was noticeable, what was the result of their deheterochromatinisation. It is considered that frequency of associations depends from satellite condensation extent and chromosomes with elongated filaments have tendency towards associations' formation.$AgNO_3$ stainingof nucleolar origanizing regions (NOR) revealed positive reaction, what confirms the amplification of transcriptional activity of these regions.

The assumption was madethat this phenomenon could be considered as a first response (indicative activity or stage) of cell on radiation impact of low intensity.

In order to carry out preventive measures, when examining persons exposed to radiation even in the cases, when on suspicion of low doses irradiation revealed number of dicentrics does not allow dose establishing, ascertaining increased number of acrocentric chromosomes' associationmakes ground for suspectIn 2002-2004 in two regions of Georgia – (Daba Vaziani and Dedoplis Tskaro) by was established that 19 individuals had received radiation doses exceeding 0.2 Gy and in 30 persons were revealed dicentrics, which quantity exceeded the background data, but didn't give us an opportunity to establish the received dose as it could be less than 02, Gy.

After 14 years re-examination was made to the inhabitants from risk group of both regions.- 37persons. (The work was supported by the IAEA Projects 17099/RO and 18791/RO).This examined group included individuals who had received an estimated dose 0.2–0.7 Gy or had increased number of chromosomal aberrations, though insufficient to determine a dose. Out of 19 people subjected to dose estimation in 2004, eight (estimated doses 0.3, −0.5 Gy) died from various cancers.

We could reexamine nine persons who were subjected to dose estimation and also 28 inhabitants from the risk group. .In one man, whose previous established dose was 0.3 Gy, we/ found 3 dicentrics (0.01 per cell) in a 300 analyzed metaphases and stable chromosome aberrations (marker chromosomes). According to our calibration curve, the possible dose of exposure should be <0.2 Gy but more than our control.

We were interested in one patient with previously established dose of 0.7 Gy. This patient suffered from the first degree of disability. Clinical examination of this patient has revealed slight enlargement of lymph nodes, gastritis, colitis, and lymphocytosis. Two dicentric chromosomes without acentric fragments were detected in 200 metaphases. No stable aberrations were observed.In individuals who have been examined this year, no significant cytogenetic, clinical, or hematological disorders have been observed. We have also investigated DNA damage by comet assay. We did not observe any significant difference in the DNA damage produced in the exposed residents compared to the unexposed individuals (8%–12%). Since the comet assay detects the DNA break breaks, it is possible that the breaks formed due to initial irradiation might have been eliminated from the system. All investigated individuals were examined by physician-oncologist, and peripheral blood tests were conducted. In some of them, anemia and lymphocytosis were detected. Other disorders in hemogram were not identified. Comparing the chronic irradiation of 10–12 years ago (0.2–0.7 Gy) and clinical outcome, differential response was seen in patients who received similar amounts of radiation wholebody irradiation with identical doses is causing very heterogeneous response in different individuals. This might depend on different factors, such as immunological stage, age, and sex. There are several data that organism response on IR can depend also on genetic polymorphism. [6, 8, 15].

Many scientists have studied the effect of total body irradiation, but only in recent decades work has begun on the problem of the effect of local irradiation on the whole human body.

The next stage of our research was to study the state of cancer patients during radiotherapy.The goal of radiation therapy (RT) is to deliver a therapeutic dose to target tissues, while minimizing the risk of complications for normal tissues. Nowadays, technological advances in radiation delivery and the introduction of particle therapies have strongly limited the amount of dose distributed to normal tissues and eenhanced the tumor killing capacity.During radiation therapy, there is a risk associated with irradiation of normal healthy tissue and the development of radiation-induced complications. (5). To assess the biological effect, it is essential to use a reliable test systems, which will also include individual radiosensitivity of the organism. (13). The effect of local irradiation was studied in 30 cancer patients with tumors of the same anatomical localization undergoing the radiotherapy taking into account the stage of the disease.These studies were carried out by employees of the laboratory of radiation safety problems of .I,.Beritashvili Center of Experimental Biomedicine.All patients were treated with a fractional radiotherapy. The irradiation was carried out on a linear accelerator with the following level: 2Gy/fraction, total dose (65.25-70 Gy). Age of the patients was varied between 55-72 years. The stage of the disease, age and gender of the patient were registerered in all cases. Hematologic and genetic analyses were carried out in dynamic. The study of chromosomal abnormalities, the DNA damages by the comet assay, and the micronuclei in lymphocytes, erythroid and buccal cells revealed a statistically significant correlation between the initial cytogenetic indices in cancer patients and their dynamic changes during and after the radiation exposure.Considering the heterogeneity in the response of patients as well as individuals to IR, caution should be exercised and appropriate treatment regimen should be planned for effective therapeutic outcome (14).

During radiation therapy should be taken into consideration patient's individual sensitivity to irradiation and different radiosensitivity of various tissues. Extrinsic factors, including dose, age, additional treatment, and comorbidities can also modify individual response. Au WW. Mutagen sensitivity assays in population studies.Mutat Res.2003 Nov;544 (2-3):273-7.

However, excluding these extrinsic factors, about the 80% of individual response to RT remains unexplained, raising the possibility of underlying genetic differences as a cause for these variations (9).

The heterogeneity of the level of damage correlating with the efficiency of the flow after radiotherapy confirms the informativity of investigated biomarkers during local irradiation.The received data revealed the appropriateness of registering genetic parameters for predicting of post-radiation complications.The development of the use of modern methods of radiation genetics in the clinical management of persons irradiated in different situations necessitates the further development of this area.

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