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EVALUATION OF THE ASSESSMENT DOSE WITH BIODOSIMETRY METHODS, APPLICABLE IN BULGARIA. USE OF DICENTRIC CHROMOSOMAL ASSAY (DCA) AND CYTOKINESIS-BLOCK MICRONUCLEUS ASSAY

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Abstract. *Radiation biodosimetry deals with the measurement of a biological response that serves as a surrogate for estimating the absorbed radiation dose in exposed humans. The biodosimetry methods include cytogenetic methods such as dicentric chromosomal assay (DCA), cytokinesis-block micronucleus assay (CBMN), Fluorescence in-situ hybridization (FISH) assay, Premature chromosome condensation (PCC), etc. All of them score the marking damages such dicentric chromosomes or centric rings to calculate the absorbed dose of ionizing radiation. As a part of the European union, Bulgarian radiobiology laboratories had to switch the direction of the mainly research activity to possibility for routine practice of analysis and diagnostic of the assessment dose after ionizing exposure. This possibility determines to use of more precise methods to diagnose cellular injuries accurately. For a short period of time Bulgarian laboratories had to choose method of analysis, to develop working protocols and their own calibration curves for them. The Research laboratory of Radiobiology and Radiation protection, Military Medical Academy-Sofia is in the process of integration of DCA as a main method of biodosimetry and CBMN as a supplementing method. The criteria to choose DCA as a main method is affordability and accuracy of the method. Next stage is to organize the whole process of integration as a routine diagnostic practice as additional source of information for the patients used by the clinical hematologists and oncologists. Aim of the study: The aim of the current study is to present and describe the selected biodosimetry methods, planned to be used in the Military Medical Academy-Sofia. Materials and methods: Dicentric chromosomal assay (DCA) and cytokinesis-block micronucleus assay (CBMN). Results: The review of the described methods, give the priority to the golden standard method (DCA). It is chosen as the most affordable, applicable and highly effective for the needs of the Scientific laboratory of Radiobiology and Radiation protection, Military Medical Academy-Sofia. Cytokinesis-block micronucleus assay (CBMN) is good supplementary method, but cannot be used as a main dosimetry method, because of its limitations. Conclusion: The biodosimetry assessment of the absorbed dose is a high skilled activity. It has involved team of professionals, correct selection of applicable methods and preliminary optimization of the process. Take into consideration of the advantages and disadvantages of the selected methods, the most affordable and effective method is DCA analysis.*

Keywords: *radiobiology, biodosimetry methods, DCA, CBMN*

1. INTRODUCTION

Exposure to ionizing radiation with over-threshold dose (≥ 1 Gy), could have serious and harmful effects on the entire body. All living organisms exist, reproduce, and develop in environment with natural radiation (radiation background). The human body can be exposed to radiation from external sources (outside the body) or internal sources (incorporated within the body).

Radiation biodosimetry deals with the measurement of a biological response that serves as a surrogate for estimating the absorbed radiation dose in exposed humans [1]. Assessing the dose of radiation exposure is crucial for understanding of its impact to the human body. When the dose exceeds the threshold (1 Gy) or when there is prolonged exposure to multiple low doses, there is an increased risk of radiation damage. The induced cellular damage could lead to cell death or changing of the cytogenetic information. These events can lead to adverse dose related tissue reactions (deterministic effects), or to the stochastic effects, when the risk of development increases with the dose [2].

In cases of over-threshold exposure in acute radiation damages (≥ 1 Gy), medical specialists anticipate the development of deterministic effects, such as acute radiation syndrome, radiation cataracts, and skin erythema. Stochastic effects, such as malignant tumors, leukemia, anemia, may develop over time, as a result of long-term exposure to repeated low doses [3].

Estimating the dose after an over-threshold exposure, such as during radiation incidents, is critical for diagnosis and timely treatment optimization due to the direct relationship between dose and effect [4,5]. As the estimated dose increases, so does the severity of the potential illness. Symptoms may develop within a time frame ranging from several hours to several weeks, depending on the dose. Accurate dose estimation provides detailed information to assess the risk of development of stochastic effects or the severe damages of the deterministic effects, that will support the medical treatment. The most precise and rapid way to estimate the dose is through biodosimetry methods [6,7].

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2. Aim

The Research laboratory of Radiobiology and Radiation protection, Military Medical Academy-Sofia is planned to integrate one main biodosimetry method (dicentric chromosomal assay, DCA) and one supplementary method (cytokinesis-block micronucleus assay, CBMN). The main routine work of the laboratory is measuring the dose by individual physical thermosluminescent dosimeters (TLD). The biodosimetry methods are going to be used additionally. The aim of the current study is to present and describe selected biodoismetry methods with their advantages and disadvantages.

3. Materials and methods

In the current study is made a comparison of the advantages and disadvantages of the both methods Dicentric chromosomal assay (DCA) and Cytokinesis-block micronucleus assay (CBMN). The chosen protocol of DCA follow the recommended guidelines of International Atomic Energy Agency and the criteria of ISO 19238:2014 for quality assurance, quality control and evaluation of the performance of biological dosimetry by cytogenetic DCA [8,9]. The metaphases that are scored follow three main criteria: good morphology and spread, scoring only first division cells and 46 chromosomes metaphases. It is essential to score the number of dicentrics in the scored cells (one, two, three dicentrics in one metaphase) [10,11]. Traditionally, the process involves manually counting of 1000 metaphases or 100 total amounts of dicentrics, which is time-consuming [7]. However, in radiation emergencies and quick DCA-triage is using QuickScan method described by described by Flegal et al. [12]. In that method the number of counted methaphases is reduced to 50 or 30 dicentrics total to speed up the process. The use of automated equipment in laboratories across the EU countries, including Bulgaria, has greatly accelerated this process, allowing to perform the analysis for 1-2.5 hours [5].

The CBMN is a routine method based on the formation of small membrane-bound DNA fragments (micronuclei) in nucleated cells that have undergone only one nuclear division [13]. Micronuclei could be acentric (chromosomal fragments without a centromere) or from whole chromosomes unable to migrate with the rest of the chromosomes, during anaphase of cell division [6]. Micronuclei formation result of toxic activity and is not specific for radiation exposure [14]. For routine biodosimetry is recommended to score minimum 1000 binuclear cells [11,15]. CBMN is used to measure over limit dose (≥1Gy) and the recommended range is 1-5 Gy [6, 15, 20]. The intensity of staining with 4% Giemsa is relatively the same as the main nucleus, but could be greater or less [16]. Optimizing the work protocol complies with all IAEA requirements for reporting, selection and analysis of micronuclei formed in binucleated cells [8].

4. Results

The current study compared the two methods, Dicentric chromosomal assay (DCA) and Cytokinesis-block micronucleus assay (CBMN), for their advantages and limitations.

Ionizing radiation damage DNA in several ways, including causing single-strand breaks (SSB), double-strand breaks (DSB), changes to the nitrogenous bases, and breaking the DNA backbone. DSBs are especially harmful and can be fatal to the cell. DSBs are a typical form of DNA damage caused by ionizing radiation [17]. Ionizing radiation can directly affect the cell by DNA damage, chromosomal aberrations, protein damages, and metabolite modification [18].

Chromosomal aberrations, like dicentric chromosomes (chromosome with two centromeres) and centric ring chromosomes (chromosome with both linked edges and centromere), are key indicators of genetic damage, caused by radiation. Dicentric chromosomes are the most common indicator of radiation exposure, while centric ring chromosomes, though less frequent, indicate very high levels of radiation.

The Dicentric Chromosome Assay (DCA) is considered the most reliable method in radiobiology for detecting DNA damage caused by radiation. This biodosimetry technique specifically detects the effects of ionizing radiation by using cytogenetic karyotyping to find dicentric chromosomes [19]. These structures can be easily seen during the metaphase stage of cell division. Higher radiation doses lead to the formation of more dicentric chromosomes and other abnormal chromosomal structures, as well as the release of acentric fragments. The chromosomal aberrations are usually lost in subsequent cell divisions [20].

To precisely estimate the radiation dose, each laboratory must create its own dose response curve. The number of dicentric chromosomes correlates with the radiation dose, which makes it possible to detect low-dose radiation exposure [10]. The DCA method is effective for assessing radiation doses in the range of 0.1 – 5 Gy, as higher doses cause significant damage, making it difficult to establish a clear dose-effect relationship [7, 21]. That method is suitable to estimate Figure 1: Giemsa staining of metaphase chromosome and performed dicentric chromosome analysis (DCA); the risk assessment of late effect's development, after chronical irradiation with low-doses. In that case should be performed multiple counts of samples with a larger number of cells over time [3].

DCA method is the primary biodosimetry technique that is planned to be used at the Military Medical Academy in Sofia. Figure 1) shown the metaphasis chromosomes that are used to count for the aberrations.

Cytokinesis-block micronucleus assay (CBMN) is used as a supplementary method of DCA, due to its limitations such as non-specificity and inter-individual variations of response [22]. It has been used mostly in the research work. For statistical

processing of the results, it is necessary to enumerate a minimum of 1000 binuclear cells [23, 24]. That

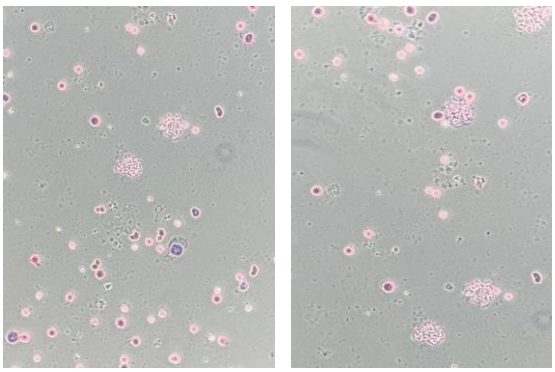

Figure 1: Giemsa staining of metaphase chromosome and performed dicentric chromosome analysis (DCA);

method is planned to be performed in the Military Medical Academy-Sofia, as a supplementary method of DCA (Fig.2). Conventional scoring, comprised of micronucleus (MN) frequency per 1,000 binucleated (BN) cells (MN/1,000 BN cells) for dose estimation [17]. Micronuclei, detected through the cytokinesis-block micronucleus assay, are valuable indicators of ionizing radiation exposure, especially in short-term lymphocyte cultures [24] . The blind samples experiments show that the accuracy of the conventional CBMN is up to 4 Gy [17]. The dose detection limitation of the CBMN for individual dose assessment is restricted to 0.2 Gy [25]. That analysis is applied for a biomonitoring of hospital workers. The assay is applicable for biodosimetry of radiation accidents [13]. Those advantages lead us to use it as a supplementary method of the DCA [26].

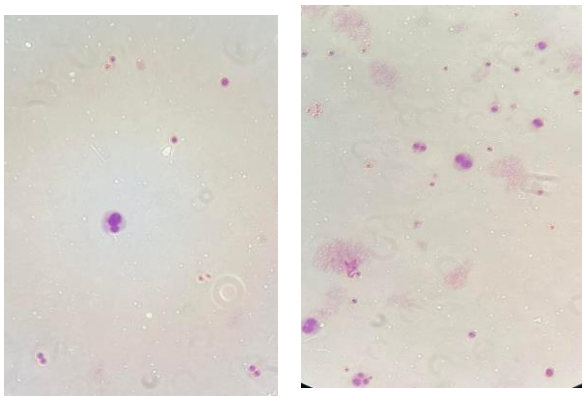

Figure 2: Giemsa staining of anaphase cells (binuclear cells) for CBMN

Biodosimetry methods help to assess biological dose for regulatory needs and timely medical management of radiation exposed individuals. For acute significant exposures, availability of timely dose information can help the medical team to be prepared with interventional procedures such as isolation, bone marrow colony growth factors and bone marrow transplantation [27].

5. Conclusion

The biodosimetry assessment of the absorbed dose is a high skilled activity that involves team of professionals, working in the laboratory, correct selection of a few appropriate methods and preliminary optimization of the process. Take into consideration of the advantages and disadvantages of the selected methods, the most affordable and effective method is DCA.

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