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BIOLOGICAL EFFECTS OF MEDICAL RADIATION ON HUMAN CELLS: A FOCUS ON LYMPHOCYTE RESPONSES

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Abstract: The purpose of this research was to examine the effects of medical radiation on human cells, namely lymphocytes.

We analyzed the frequency of chromosomal abnormalities in cells exposed to X-ray photon irradiation at 6 and 10 MV, with dose rates ranging from 5.50 to 23.08 Gy/min and absorbed doses ranging from 0.5 to 8 Gy. For the sake of comparison, a 60Co curve was used. Standard cytogenetic procedures were used to produce metaphases from the cell cultures, and chromosomal analysis was carried out. Based on our findings, bio dosimetry may be carried out using doses and energy that are greater than those of the reference dose presently in use. We found that the aberration frequencies varied significantly across the various irradiation methods. The radiobiological impact is stronger in FFF mode compared to FF mode. A linear quadratic dose response calibration curve was built to determine the relative biological effectiveness (RBE). The average RBE values for 60Co γ irradiation were 1.28, for 6 FFF they were 1.11, and for 10 FFF they ranged from 0.79 to 0.92, all caused by the reference radiation, which is 6MV (5.50 Gy/min). These findings might be even more significant in a therapeutic context due to the significant variations across radiation modalities in hypofractionation situations. In the event of a patient's unintentional overdose, it is crucial to quantify the overdose using the proper calibration curves for bio dosimetry.

Keywords: Radiation, Biological, Cell, Lymphocyte

Introduction

When it comes to investigating radiation accidents, biological dosimetry plays a crucial role, and it may also shed light on treatment approaches [1]. Dicentrics plus rings generated by 137Cs, 60Co gamma rays, and 250 kVp X-rays have been extensively studied in terms of dose response correlations [2, 3]. However, LINACs have recently supplanted a number of 60Co (1.25 MV) sources, there is a growing need for updated biodosimetry calibration

curves that are better suited to energy levels >4 MV. This is necessary to ensure readiness for dosimetric mishaps [4-6].

Ionizing radiation's impact on living organisms is dose-dependent, although energy and other factors including dosage rate and filter efficiency also play a role [7]. The photon beams that are flattening filter-free (FFF) and those that are flattening filter (FF) are both generated from the same electron beam that has the same nominal energy. While FF removal increases output, it reduces photon beam penetration quality due to reduced beam hardening; hence, in all scenarios, the energy of the electrons at the target is unchanged. The photon energy spectrum and beam profile of the FFF beams are distinct. When it comes to high-dose stereotactic radiotherapy or radiosurgery, the FFF mode is ideal since it boosts beam intensity while decreasing treatment duration.

There is experimental information on the radiobiological consequences of large doses (5-20 Gy) described in [8-10], although there is a lack of knowledge on the high dosage range. In dealing with situations involving radiation exposures that pose a danger to health or even life, this dosage range becomes very crucial. The development of more precise LINAC-based medical treatment methods has highlighted the critical need to expand the dicentric assay's calibrated range of radiation quality. The majority of these studies, however, used a cobalt (gamma) source to irradiate blood donors at dose rates ranging from half a Gy/min to one and a half Gy/min.

The fast pulse width (FFF) beam's high dose rates have reignited worries about the radiobiological consequences on both healthy tissues and cancer [11]. As an example, Lohse et al. [12] used 2 cell lines glioblastoma that had been irradiated with 5 or 10 Gy at various dosage rates to study the radiobiological effects of FF and FFF beams. Assays for colony development were conducted and dose verification was carried out. Findings showed that compared to the conventional flattened beam, cell lines glioblastoma irradiated with the FFF beam had a lower rate of clonogenic cell survival. While Verbakel et al. discovered no difference when comparing the two approaches, three distinct human cancer cell lines demonstrated an increase in cell survival following FF beam irradiation up to a fraction dosage of 12 Gy [13]. To summarize, evidence about normal, non-cancerous cells is still lacking, and these uncommon and contentious investigations relied on colony formation tests.

Because of their ubiquitous circulation and suitability as a model for biodosimetric tests, we opted to employ peripheral blood cells [14, 15].

The purpose of this research was to examine the effects of medical radiation on human cells, namely lymphocytes.

Methodology:

To produce uniform dosages, 2 mL cryotubes containing blood samples were irradiated in a plastic phantom filled with water, with the tubes kept at ambient temperature and their bases set 4 centimeters below the water's surface. Eclipse 13.6 treatment planning software and a TrueBeam linear accelerator were used to administer the radiation. Following the IAEA TRS398 protocol, the apparatus was adjusted in a water-based calibration procedure. At dosage maximum, with a source surface distance (SSD) of 100 cm, 1 MU is equivalent to 1 cGy. For the absolute calibration, we used a Farmer chamber from Freiburg, Germany, model PTW 30010. At the isocenter, with a 95 cm SSD, the samples were placed. At the isocenter, the field size was 8 x 8 cm and the gantry angle was 90 degrees. The various dosage rates, shown as Gy/min in Table 1, are shown. For our measurements, we used these

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parameters since these are the only accessible dosage rates. In our research, we used these dosage rates since they are preprogrammed parameters of the linear accelerator. Doses varied between half a Gy and eight Gy.

Table 1. Linear accelerator applied energy, nominal dose rates, and real blood sample dose rates

Energy / Type Of energy	6 MV	10 MV	6 FFF	6 FFF	10 FFF	10 FFF	10 FFF
Nominal MU/min	600	600	600	1400	400	1600	2400
Actual Gy/min	5.50	5.88	5.36	12.5	3.85	15.4	23.08

Lymphocyte cultures

Venipuncture was used to gather venous blood samples from 20 healthy, non-smoking participants (mean age: 37.8 ± 8.1 years; 13 females and 7 males) and these samples were then put into vacutainers that had been heparinized. The dosage rate curves needed to be prepared; thus this was done. The dose-response curves were checked by exposing five patients' blood samples to radiation at levels of 3 as well as 6 Gy (6 FFF, 12.5 Gy/min). The patients' ages ranged from 70 to 5.5 years, and there were four males and one female. Shortly after exposure, the cells were grown according to conventional cytogenetic protocols: Blood was combined with 0.8 mL of cell culture medium RPMI1640, which includes 15% BSA and 0.5 mL each of penicillin and streptomycin. The volume of the combination was 9 mL. Phytohaemagglutinin M (0.2%) was used to stimulate the proliferation of lymphocytes. For 52 hours at 37°C, the samples were left to incubate. During the last two hours of culture, 0.1 µg/ml Colcemid (Gibco) was added to suppress cell growth. Centrifugation was followed by hypotonization of the cell cultures with 0.075 M KCl at 37°C for 15 minutes, followed by fixation with a cold methanolacetic acid 3:1 mixture. The cells were cultured in a small amount of fixative after many washes, and then mounted on glass slides. After drying, the slides were stained with 3% Giemsa.

Study of chromosomal aberrations

An Olympus BX51 light microscope was used to examine over 200 metaphases at each experimental point. Analysis of the chromosomes was carried out during the 1st cell division. Metaphase cells that were clean and oval were the only ones we counted. Chromosome abnormalities (CAs) were defined as the existence of two pieces, one of which was dicentric or ring-shaped and the other acentric, in the tested cell. The location of the chromatin loss did not differentiate the excess pieces as interstitial or terminal deletions. Any acentric pieces that were not part of any dicentric or ring abnormalities were considered to be extra pieces. Two dicentric equivalents were considered to be one tricentric chromosome. The standards set down by ICPEMC [16] were followed throughout the examination. Four seasoned researchers coded the slides and examined the metaphases. To reduce the impact of any biases introduced by individual scorers and slides, each person scored a subset of the slides. Statistical analysis was done by using SPSS version 23.

Results:

As can be seen from Table 1, all of the donors' blood samples were subjected to radiation at dose rates varying from 3.85 to 23.08 Gy/min, with energies spanning from 6 to 10 MV and doses ranging from 0.5 to 8 Gy. Four

separate people's blood samples were used to create all of the dose-response curves. As a control sample, one non-irradiated aliquot was used every time. To rule out the potential of individual sensitivity, it was required to irradiate the blood of the same donor in order to differentiate between 6 MV in FFF and FF modes. A blind count of 100-200 complete metaphase cells was used to score each condition at each dosage point and donor.

There were statistically significant variations between the dicentric and ring frequencies at 2-8 Gy absorbed doses (Fig. 1).

Furthermore, a comparison was made between the impacts of standard irradiation treatments on blood samples obtained from the identical donor. Dosage rates of 3.85, 15.40, and 23.08 Gy/min in the 10 MV FF mode, in addition to 5.88 Gy/min in the FMF mode, were utilized (see Figure 2). When compared to 10 MV FFF 5.88 Gy/min, the aberration frequencies (dicentrics + rings) were significantly greater for 10 MV FFF 3.85, 15.40, and 23.08 Gy/min. The total aberrations were significantly different by a significant margin between 3 and 8 Gy ($P < 0.0001$). A statistically significant difference ($P < 0.05$) was also seen between the differences between 2 Gy and 8 Gy. At 6 MV, total aberrations, dicentrics plus rings, and FFF were significantly greater than they were at 10 MV (Fig. 1 and 2). This was the case for samples with 2 Gy ($P = 0.001$) and 8 Gy ($P = 0.011$).

There was no statistically significant difference in the frequency of chromatid breaks, chromosomal fragments, exchanges, or translocations when the experiment was performed in FF mode as opposed to FFF mode (Fig. 1 and Fig. 2). This was the case regardless of the energy used. The percentage of aberrations that were dicentrics and rings was 22.2% when the 6. MV FFF was 5.36 Gy/min at 0.5 Gy; this figure climbed to 70.1% when the FFF was 8 Gy; and when the 10 MV FFF was 3.85 Gy/min, the similar statistics were 33.3% at 0.5 Gy and 61.7% at 8 Gy. Regarding the ratio of dicentric to centric ring yields, the ratio was around 5-10:1 regardless of the dose. Approximately the same number of dicentrics were found as extra fragments when the dosage was 2 Gy or lower; however, when the dosage was 3 Gy or higher, the frequency of dicentrics was approximately twice as high.

At dosages between 0.5 and 1 Gy, chromatid fractures and acentrics (which are different from dicentric or ring linked fragments) outnumbered dicentrics and rings. However, at doses more than 2 Gy, the tables turned, and dicentrics and rings were in the majority. At 8 Gy, the ratio of excess fragments to dicentrics varies with energy and ranges from 40.5% to 60.4%. Traditional Giemsa staining can still detect translocations, although their frequency increased quadratically with dosage, and compared to dicentrics + rings (0-38/100 cells), the rate is ten times lower. Translocation findings obtained via the use of FISH cannot be directly compared to those obtained using our approach due to the fact that Giemsa-stained translocations are not always evident in ten percent of the cases. However, the Giemsa technique allows for the analysis of a greater number of metaphases. Chromatin breaks were shown to grow linearly with dosage in our study, reaching a maximum of 14/100 cells at 6 Gy. Regardless of the irradiation method, the frequency of chromatid fractures remains rather constant. Nevertheless, chromatid fractures do not exhibit specificity and have a lesser impact on irradiation.

While chromatid breaks were the most common kind of chromatid aberration, the other type, exchanges, were less common. With 6 FFF, the maximum number of cells that could be exchanged was 3/100.

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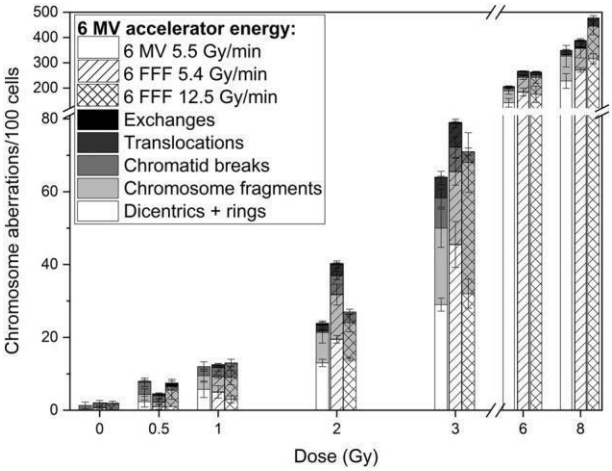

Figure 1. Human blood cells from two donors were exposed to a 6 MV/FFF photon beam at several dosage rates; it shows the frequencies of dicentrics + rings, chromosomal fragments, translocations, and overall aberrations**.**

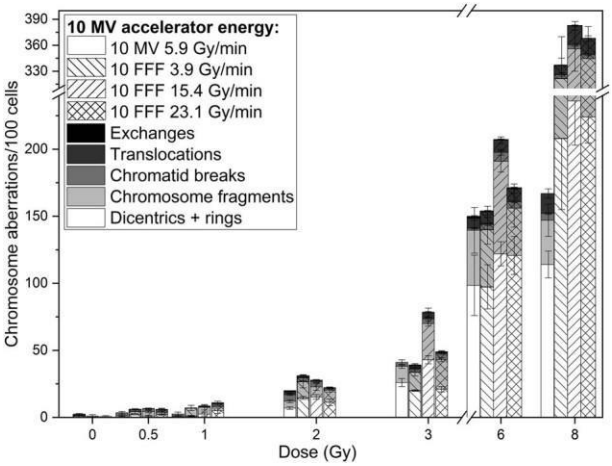

Figure 2. Dicentrics + rings, chromosomal fragments, translocations, and overall aberration frequencies in human blood cells from two donors exposed to varying dosage rates of a 10 MV/FFF photon beam.

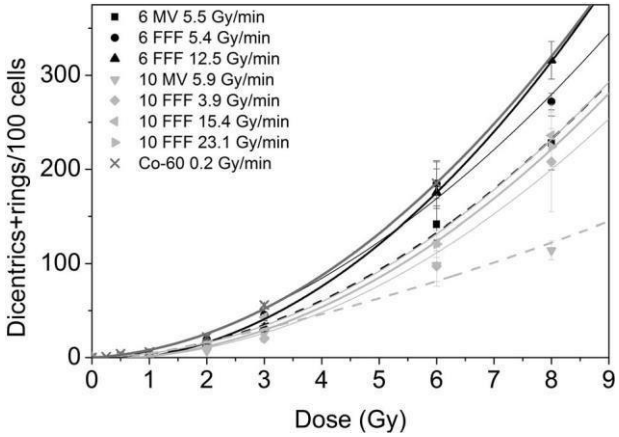

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Figure 3. Calibration curves for dicentrics + rings caused by LINAC X-ray irradiation, regarding dose and response. Lines depict the predicted curves of the linear quadratic model.

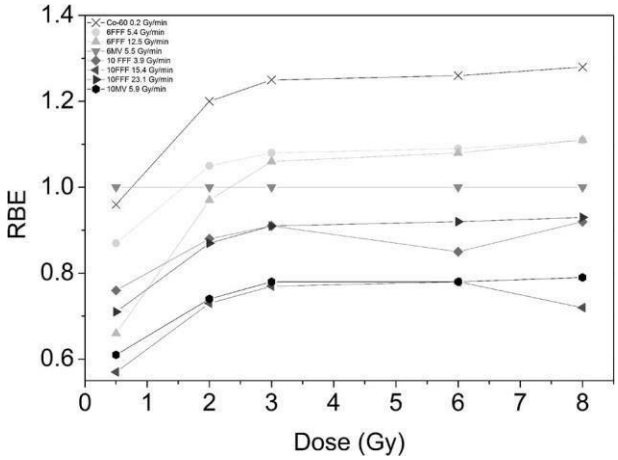

Figure 4. The following examples show average RBE values for different photon energies as a function of dose: 60 Co γ 0.2 Gy/min, 6 FFF (5.4-12.5 Gy/min), 10 MV (5.9 Gy/min), and 10 FFF (3.9-23.1 Gy/min).

Using the linear-quadratic model to formally fit dose-response data:

When evaluating dosage estimates, the baseline degree of chromosomal abnormality is crucial. Nineteen individuals in good health provided us with data for the dicentric method. Six dicentrics and rings per six thousand cells (0.001 dicentrics and rings/cell) was the mean background level. Our prior population survey found the same thing [20]. It was determined that 28,000 metaphase spreads were detected after in vitro irradiation with either 6 or 10 MV FF and FFF. This was done in order to document all stable and unstable chromosomal abnormalities.

Averaging 0.119, the dicentrics plus rings distributions followed the Poisson model. Figure 4 presents dose-response calibration curves made of eccentrics plus rings generated by LINAC X-ray irradiation. The data is analyzed using CABAS. Direct influences on the β values were the dose rate and the energy consumed. Higher β values were recorded for the 6 MV FF and FFF modes (0.037 and 0.045 Gy−2, respectively) than for the 10 MV FF and FFF modes (0.023 and 0.036 Gy−2, respectively). The values of β were found to be 0.044 ± 0.001 Gy−2 and 0.023 ± 0.002 Gy−2, respectively, with the greatest values occurring in the 6 FFF mode with 12.5 Gy/min and the lowest values occurring at 10 MV FF with 5.88 Gy/min on average. When compared to the β values, the α component exhibited lower values; the lowest was 0.0009 Gy−1 and the highest was 0.020 Gy−1. As the photon energy increased, the linear coefficients shrank. Additionally, we used dose estimate calculations that were derived from all of the observed curves. These calculations were found to align with the dosage range indicated by the measured data. At a dose of 0.5 Gy, the difference between the curves that are steeper and those that are flatter is around 0.2 Gy. However, when the dosage range is between 6-7 Gy, this figure may reach as high as 1 Gy. larger dosages often have a larger degree of projected dose variability than lower doses. Our results for α and β would be different from those in the 0-8 Gy dosage range if we limited our computations to only the 0-3 Gy range. As the energy level rises, the linear coefficients become less significant. Our observed dose rate curves also depart from expectations; i.e., the yield of dicentrics plus rings was dose-dependently increasing as radiation dosage did.

For this reason, we compared our findings to information found in the literature [21, 22]. We also utilized the anticipated doses as a benchmark to supplement the tables with information from 100 and 200 dicentrics. We used CABAS software to determine dosages for both our data and the other papers cited. When comparing our dose rate curves to those of other authors at a frequency of 2 dicentrics plus rings per 100 cells and with differing applied energies, we found that there was no statistically significant difference between the two sets of curves. There are notable variations, nevertheless, when dealing with 20–200 dicentrics in addition to rings/100 cells. According to the findings of our research, the dosage is 5.58 Gy/min when 20 dicentrics plus rings are applied to 100 cells. This is much greater than the 6 MV FF dose of 0.54 Gy/min that was anticipated [4]. With FF and FFF modes operating at 6 and 10 MV energies, our experimentally determined dosages differed significantly from those reported by Lemos-Pinto et al. [4]. We found that earlier works [2, 8, 21, 23] predicted lower doses by employing cobalt gamma sources or 6 MV LINAC [4]. This was established by applying our own dose rate curves, which were made up of 6 MV FFF and 10 MV FFF.

By modifying the irradiation's energy and dose rate, hypofractionated radiotherapy is able to regulate the treatment's biological effect. In order to treat one hundred cells that have two hundred dicentric plus ring abnormalities, either an 8.0 Gy therapy at ten MV FFF (23.08 Gy/min) or a 6.7 Gy dose at six MV FFF (12.5 Gy/min) would be required. When compared to the 6 FFF, which is 12.5 Gy/min, the 10 FFF, which contains 23.08 Gy/min, has a huge biological benefit.

For the purpose of determining the RBE value, the dose-response data was used in order to examine the influence of various photon energy and dosing rates. The relative biological efficacy (RBE) of two radiation sources is the ratio of their respective doses that have the same effect, as measured in relation to a reference radiation source. The reference radiation was 6 MV FF, which has a flux of 5.50 Gy/min. At doses ranging from 0 to 8 Gy, In the 6 MV FFF range (5.36-12.50 Gy/min), During the 10 MV FFF range (3.85-23.08 Gy/min), the RBE value averaged out at 1.11, which was measured, it was 0.72, and in the 60Co γ range (0.2 Gy/min), it was 1.28 (Fig. 4).

DISCUSSION

Consistent with our previous results, our investigation found spontaneous aberration frequencies [20]. Consistent with previous reports of 12-49/100 cells [24-26], At a 2 Gy irradiation dose, our dicentrics plus rings produced cell yields ranging from 7.50– 20.50/100. While the dicentric plus ring frequencies ranged from 80.5 to 199.7/100 cells after 6 Gy

irradiation and 104 to 336/100 cells after 8 Gy, they were 19-52/100 cells after 3 Gy. How these abnormalities manifest is quite dosage and energy dependent.

We used 3 and 6 Gy radiation to confirm our dose-response curves in five more subjects. Radiotherapy patients' radiosensitivity may also be partially assessed using this approach [27]. Radiotherapy was administered to the blood samples of the patients at doses of 3 and 6 Gy at a frequency of 12.5 Gy/min at a 6 MV FFF concentration. After counting the frequency of dicentrics and rings, the appropriate dose rate curve was used to estimate the dosage. Different individuals have dicentric plus ring aberrations at different rates; for example, at 3 Gy, the average deviation from the calibration curve was 6.3%, while at 6 Gy, it was 2.7%. Likewise, a 60Co γ dose rate

curve (0.2 Gy/min) was used for the purpose of dose estimation. The average deviation at 3 Gy was 22.3%, whereas at 6 Gy it was 18.5%.

Using several irradiation procedures resulted in noticeably varying aberration frequencies. Radiobiological effects are more pronounced in the FFF mode than to the FF mode. We have also shown that reduced energy created greater dicentric plus ring aberrations. The result is that the effective energy is lower in the FFF mode compared to the FF mode, and the energy spectra are also heterogeneous. The studies of [28, 29] demonstrated this using physical measures, despite the lack of biological data. It is the similarities between the depth dosage characteristics of the 6 MV FFF beam and those of the 4 MV FF beam, as well as the improved absorption of lower energy by the FF, that are responsible for these occurrences. With a 6 MV FF radiation technique emitting 5.50 Gy/min and a 6 MV FFF radiation method emitting 12.5 Gy/min, we found that the FF and FFF radiation methods had a considerable difference in the potential to trigger the creation of chromosomal fragments. Specifically, A total of 98-128/100 dicentrics and rings were created by the FF method when it was applied at 8 Gy, whereas the FFF technique produced 228-316/100 cells. We propose that more cells should be assessed in the low dosage range for a smaller number of aberrations, even if the standard error grows with dose for any given approach when it comes to calibration curve measurements. Since variations in response from one person to another could affect the outcomes, it is crucial to compare several methods using the same irradiated blood from the same donor.

Using photon beams with 6 and 10 MV FF energies, we exposed blood samples to varying dosage rates in our tests. Chromosomal aberration frequencies did vary among cells treated to the same amount of radiation at various dosage rates, however these variations were not always statistically significant. During irradiation, DNA repair was possible when the dose rate was about 10 mGy-1 Gy/min, according to the measurement of [30]. Since administering the dosage required far less time than cells needed to repair DNA, dose rates exceeding about 1 Gy/min had no appreciable impact [30].

So, DNA repair couldn't have been that important. We ran all of our experiments at rates greater than 1 Gy/min.

Our results corroborate those of earlier research showing that the dose-effect curves for various photon energy vary significantly. Hill [31] found, for instance, that the linear energy transfer value of lower-energy photons is larger, suggesting that they should be more effective medically. Due to a decrease in the energy of the secondary electrons released, microdosimetric energy depositions reveal a marked change towards the higher energy deposition patterns. In terms of quantification as well as cell survival data [30] showed that 200 kV X-rays had an RBE value of 10% more than 6 MV photon

beams. We discovered a 28% reduction in the RBE when comparing energies of 10 MV and 6 MV.

Regardless of energy or dosage rate, the frequency of chromatid breaking cases increased in a dose-dependent manner, according to our findings. Many authors have postulated that endogenous reactive oxygen species (ROS) are the probable culprits of spontaneous chromosomal rearrangements, which manifest as chromatid-type anomalies [32, 33]. A large output of chromatid-type chromatid breaks, aberrations, as well as exchanges may be caused by an increase in ROS generation in mitochondria, which is stimulated by an elevated dosage. The effects of endogenously generated chromatid fractures are similar to those of low-dose ionizing radiation at low-irradiation doses (between 0 and 0.5 Gy) [34].

At a dosage of 0.5 Gy, the dicentrics and rings account for 13.3-33.3% of the overall aberrations, whereas at a dose of 1.0 Gy, they account for 14.3-45.5%, and at a dose of 2.0 Gy, they account for 35-54.9% of the irregularities. Based on the energy, this figure rises to 45– 70.2% in the 3–8 Gy dosage range. Doses of 6 Gy and higher are only mentioned in a few numbers of publications [8]. But we still think the approach worked even under these circumstances; the only real problem was scoring too many pieces.

After looking at larger dosages (10, 16 and 20 Gy), Vinnikov and Maznyk [8] found that the primary technical issues are associated with low metaphase quality and an excessive number of chromosomal rearrangements. Low doses seem to amplify additional abnormalities that are not radiation specific. Consequently, overall aberration value and other forms of aberration may also serve as crucial markers for the biological impact of radiation. When doing biological dosimetry below 2 Gy, total aberrations should be considered in lieu of dicentrics plus rings.

The latest research also reveals that acentric fragments may enter the cytosol and trigger an immunological response at levels as high as 15–18 Gy. Cytosolic DNA concentration drops, nonetheless, with increasing dosages. One important aspect of choosing the best radiation protocols for immunotherapy is determining the ideal dosage to induce enough double-stranded DNA breaks; the chromosomal aberration technique may assist with this [34, 35].

CONCLUSION

According to the results of our research, the relative biological impact of 6 MV FFF is more than that of 10 MV FF and 10 MV FFF, respectively, by 11%; nevertheless, the biological damage caused by 6 MV FFF is less severe because of its lower concentration.

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